University of Reading

Linking biochemistry and genetics in celery with taste and flavour perceived by the consumer: creating a more acceptable product

Department of Food and Nutritional Sciences

School of Chemistry, Food and Pharmacy

Lucy Turner

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Appendix XIII - Consumer acceptability and sensory profile of three new celery *(Apium graveolens)* hybrids and their parental genotypes (As submitted to the International Journal of Molecular Sciences, 2021

Abstract

Celery *(Apium graveolens)* is a biennial crop grown across the globe for its health benefits and distinct flavours. Consumed either raw, in salads or forming the base of many soups, stocks and sauces, celery is a major constituent of the "holy trinity" in many cuisines. Current research investigating the aroma profile of celery excludes information about the cultivar, origin, geographical location of growth and other variables producing misinterpreted and unrepeatable data. All these factors have been marked as drivers of horticultural crop performance in the literature.

Using celery genotypes sourced from a breeding population in multi-year (2018 and 2020) and multi-site (UK and Spain) experiments, factors including cultivar, maturity, geographical location and harvest year and their influence over the aroma composition of celery were investigated. By combining solid-phase microextraction gas chromatography-mass spectrometry, the aroma profile of celery has been elucidated. Volatile composition variations and their impact on sensory perception has been examined through sensory profiling with a trained panel (n=11).

Significant differences in the volatile composition were observed to be influenced by genotype, maturity, harvest year and geographical location, thus leading to significant differences in the sensory profile. Warmer temperatures resulted in celery with higher proportions of sesquiterpenes and phthalides whereas in cooler temperatures, higher proportions of monoterpenes were observed. Three genotypes exhibited similar volatile compositions and sensory profiles regardless of these factors and were genetically crossed prior to presentation to a consumer panel (n=118) identifying the consumer acceptability and attribute preference of three celery hybrids and their parental genotypes. Studying the relationship between genotype and environment will provide clear information to guide growers in how to consistently produce a higher quality crop. Consumer segmentation identified three groups of consumers exhibiting differences in the hedonic reaction to the celery samples. Moist mouthfeel and sweet taste were identified as drivers of liking.

Declaration of original authorship

Declaration: I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Signed: Lucy Turner

Personal acknowledgements

I would like to give thanks to all my friends for their constant love and support throughout the completion of my PhD. I would like to thank my Mother, Father and Tally for their consistent motivation, support and on-demand proof-reading services. In addition to all the staff, Post-doctoral Researchers and PhD students at the University of Reading.

I would especially like to thank my supervisors; Professor Carol Wagstaff who initially inspired me to complete the PhD and inspired me throughout my project, Dr Stella Lignou, I would not be where I am today without you, thank you for providing me project support as well as mental and emotional support. Finally, Dr Frances Gawthrop, for opening my eyes to celery and teaching me skills that I never thought I would learn. Thank you all for the support, time, patience and for reading every draft, abstract, presentation and poster I sent.

Thank you, Dan for your love and support, you kept me smiling, laughing and strong during my most stressful moments.

Contribution to Papers and Formal Acknowledgements

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Chapter 1: Investigating the factors that influence the aroma profile of *Apium graveolens*: A review: All text, tables and figures was produced by Lucy Turner, comments and guidance provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd.

Chapter 2 Determining the most abundant volatile compounds present in celery using 24 genotypes**:**Text, tables and figures were produced by Lucy Turner. Comments and guidance were provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd. All experimental material (seeds) was provided by Tozer Seeds Ltd, grown by G's Fresh Ltd and harvested by Lucy Turner, with help from Dr Sara Jennings (Tozer Seeds Ltd). Laboratory extractions, analysis (GCMS) and statistical analysis (PCA and ANOVA) was completed by Lucy Turner with guidance from Dr Stella Lignou. Sensory vocabulary, training and scoring sessions were led by Lucy Turner with guidance from Dr Stella Lignou. Environmental data was provided by G's Fresh Ltd.

Chapter 3: Investigating the relationship of genotype and climate conditions on the volatile composition and sensory profile of celery *(Apium graveolens)*: Text, tables and figures were produced by Lucy Turner. Comments and guidance were provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd. All experimental material (seeds) was provided by Tozer Seeds Ltd, grown by G's Fresh Ltd and harvested by Lucy Turner with help from Dr Sara Jennings. Laboratory extractions, analysis (GCMS) and statistical analysis (PCA and ANOVA) was

completed by Lucy Turner. Sensory vocabulary, training and scoring sessions were led by Lucy Turner. Environmental data was provided by G's Fresh Ltd.

Chapter 4: Investigating the relationship of genotype and geographical location on volatile composition and sensory profile of celery *(Apium graveolens)*: Text, tables and figures were produced by Lucy Turner. Comments and guidance were provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd. All experimental material (seeds) was provided by Tozer Seeds Ltd, grown by Grupo G's España and harvested by Lucy Turner with help from Alan Badura. Laboratory extractions, analysis (GCMS) and statistical analysis (PCA and ANOVA) was completed by Lucy Turner. Sensory vocabulary, training and scoring sessions were led by Lucy Turner. Environmental data was provided by G's Fresh Ltd and Grupo G's España.

Chapter 5: Examining the compositional differences of eight celery genotypes grown in two different locations in Spain: Text, tables and figures were produced by Lucy Turner. Comments and guidance were provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd. All experimental material (seeds) was provided by Tozer Seeds Ltd, grown by Grupo G's España and harvested by Lucy Turner with help from Alan Badura. Laboratory extractions, analysis (GCMS) and statistical analysis (PCA and ANOVA) was completed by Lucy Turner. Environmental data was provided by Grupo G's España.

Chapter 6: Influence of harvest maturity on the aroma quality of two celery *(Apium graveolens)* genotypes: Text, tables and figures were produced by Lucy Turner. Comments and guidance were provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd. All experimental material (seeds) was provided by Tozer Seeds Ltd, grown by G's Fresh Ltd and harvested by Lucy Turner, with help from Dr Sara Jennings (Tozer Seeds Ltd). Laboratory extractions, analysis (GCMS and GCO) and statistical analysis (PCA and ANOVA) was completed by Lucy Turner and Dhriti Dawda (University of Reading) with guidance from Dr Stella Lignou and Prof Carol Wagstaff.

Chapter 7: Consumer acceptability and sensory profile of three new celery (Apium graveolens) hybrids and their parental genotypes: Text, tables and figures were produced by Lucy Turner. Comments and guidance were provided by Prof. Carol Wagstaff and Dr Stella Lignou from the University of Reading and Dr Frances Gawthrop from Tozer Seeds Ltd. All experimental material (seeds) was provided by Tozer Seeds Ltd, grown by Grupo G's España and, due to Brexit and COVID-19, harvested by Tozer seeds Iberica. Laboratory extractions and analysis (GCMS) was completed by Lucy Turner. Sensory vocabulary, training and scoring sessions were led by Lucy Turner. Consumer preference trial was completed by Lucy Turner with guidance from Dr Stella Lignou. Statistical analysis (ANOVA, PCA, penalty analysis, Friedman's, AHC) was completed by Lucy Turner with guidance from Stella Lignou.

List of Abbreviations

A – Alcohol

AEDA – Aroma extraction dilution analysis

AHC – Agglomerative hierarchical cluster

AL – Aldehyde

ALK - Alkane

BAE – Bitter after-effect

BT – Bitter taste

CA – Colour

CAE – Celery residue in the mouth

CM – Crunchy mouthfeel

DMAPP – Dimethylallyl diphosphate

DXP – 1-deoxy-D-erythritol

E - Ester

FCA - Fresh coriander aroma

FCF – Fresh coriander flavour

FD - Flavour dilution

FFA – Fresh fennel aroma

FFF – Fresh fennel flavour

FM – Firmness of first bite

FPA - Fresh parsley aroma

FPF – Fresh parsley flavour

GPP – Geranyl pyrophosphate

GC/MS – Gas chromatography mass spectrometry

GC/O – Gas chromatography olfactometry

GC/GC/FID – Gas chromatography/gas chromatography/flame ionisation detector

GGA - Grassy/green aroma

GGAE- Grassy/green after-effect

GGD – Grassy/green flavour

IPP – Isopentenyl diphosphate

JAR – Just-About-Right

K – Ketone

L - Lactones

LOX - lipoxygenase

LRI – Linear Retention Index

M1 – Premature

M2 – Commercially mature

M3 – Postmature

M – Monoterpene

MA – Monoterpenoid Alcohol

MEP/DOXP – Non-mevalonate pathway

MIAPAE – Minimum Information about a Plant Aroma Experiment

MM – Moist mouthfeel

MVA-pathway – Mevalonic acid pathway

MVA-PP – Mevalonic acid pyrophosphate

NAE – Numbness after-effect

nd – Not detected

ns – Not significant

O - oxide

PCA – Principal Component Analysis

RA- Ribbed appearance

RF – Rocket flavour

S - Sesquiterpene

SAE – Salty after-effect

SAFE – Solvent assisted flavour extraction

SAT – Salt taste

SF – Soap flavour

SM – Stringy mouthfeel

SOAE – Soapy after-effect

SPME GC/MS – Solid phase microextraction gas chromatography mass spectrometry

STA – Stalk thickness

ST - Sweet taste

P – Phthalide

U - Unknown

UAE – Umami after-effect

UT – Umami taste

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475 **Glossary of chemical compounds**

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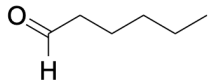
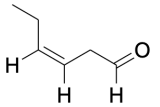
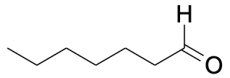
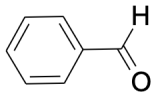
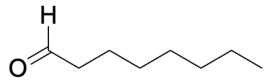
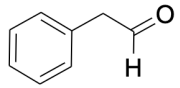
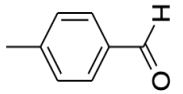
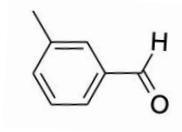
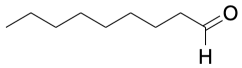
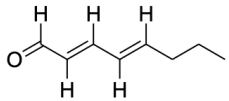
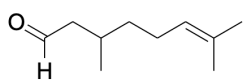
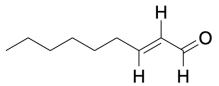
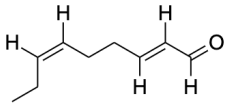
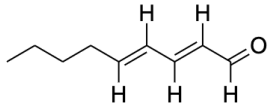
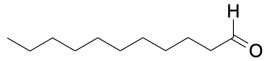
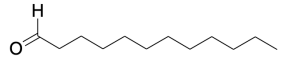
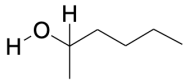
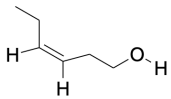
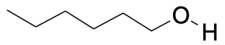
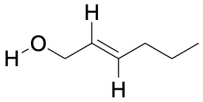
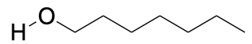
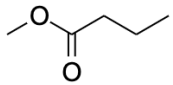
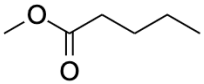
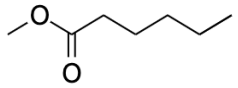
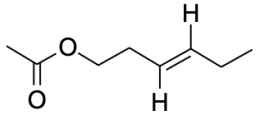
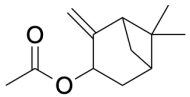
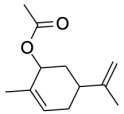
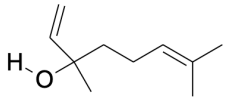
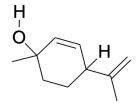
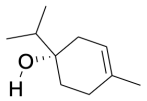
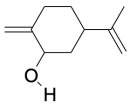
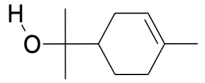
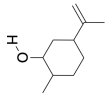
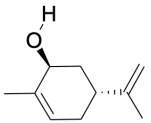
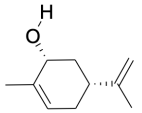
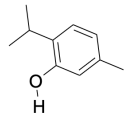
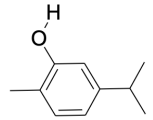
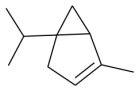
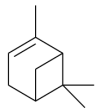
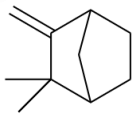
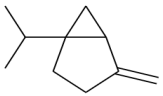
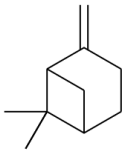
Compound Name	Synonyms	Aroma descriptor	Formula	Configuration	LRI*
Aldehydes					
hexanal	caproaldehyde, hexaldehyde, 1-hexanal, n-hexanal	green, fatty, leafy	C6H12O		800
(*Z*)-3-hexenal	cis-3-hexenal, (Z)-hex-3-enal, hex-3-enal	green	C6H10O		804
heptanal	heptaldehyde, enanthaldehyde, 1-heptanal	green, herbal, fatty	C7H14O		901
benzaldehyde	benzoic aldehyde, phenylmethanal, benzecarbonal		C7H6O		961
octanal	capryaldehyde, caprylic aldehyde, octanaldehyde	citrus, orange peel, green	C8H16O		1004

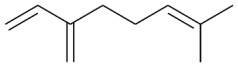
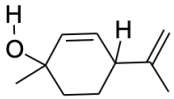
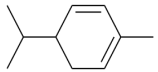
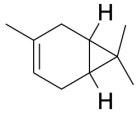
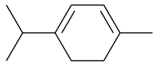
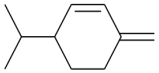
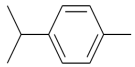
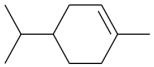
phenylacetaldehyde	2-phenylacetaldehyde, benzeneacetaldehyde, hyacinthin	honey, floral rose, sweet	C8H8O		1049
p-tolualdehyde	p-methylbenzaldehyde, p-formyltoluene, benzaldehyde 4-methyl-, p-cresyl aldehyde	fruity, cherry, phenolic	C8H8O		1070
m-tolualdehyde	3-methylbenzaldehyde, 3-tolualdehyde, m-methylbenzaldehyde, 2-formyltoluene	sweet, fruity, phenolic	C8H8O		1086
nonanal	pelargonaldehyde, nonanaldehyde, nonylic aldehyde	waxy, aldehydic, fresh	C9H18O		1101
(E,E)-2,4-octadienal	(2E,4E)-octa-2,4-dienal, 2,4-octadienal, trans,trans-octa-2,4-dienal	green, fruity, melon	C8H12O		1115
citronellal	3,7-dimethyloct-6-enal, rhodinal, β-citronellal, 2,3-dihydrocitral	waxy, floral, herbal	C10H18O		1158
2-nonenal	trans-2-nonenal, (E)-2-nonenal, 3-hexyl-2-propenal	green cucumber, aldehydic	C9H16O		1160

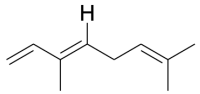
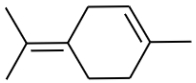
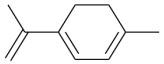
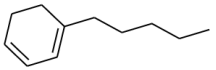
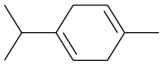
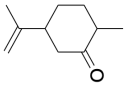
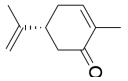
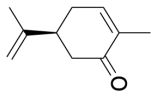
(*E*,Z)-2,6-nonadienal	nonadien-2(trans)-6-(cis)-al, 2-(trans)-6-(cis)-nonadienal, cucumber aldehyde	green, cucumber, fatty	C9H14O		1160
(2E, 4E)-nonadienal	trans,trans-2,4-nonadienal, 2,4-nonadienal, (2E,4E)-, 2,4-nonadien-1-al	green, fatty, melon	C9H14O		1210
undecanal	undecanaldehyde, undecyl aldehyde, hendecanal	waxy, soapy, floral	C11H22O		1308
dodecanal	dodecyl aldehyde, lauric aldehyde, lauryl aldehyde	waxy, soapy, citrus	C12H24O		1410
Alcohols					
2-hexanol	hexan-2-ol, 2-hexyl alcohol, methylamyl alcohol	fruity, fatty, terpenic	C6H14O		803
(*Z*)-3-hexenol	cis-3-hexen-1-ol, leaf alcohol, (Z)-hex-3-en-1-ol	green	C6H12O		855
hexanol	hexyl alcohol, caproyl alcohol, caproic alcohol, hexan-1-ol	green, fruity, apple	C6H14O		865
2-hexenol	trans-2-hexen-1-ol, (E)-hex-2-en-1-ol, hex-2-en-1-ol	green, leafy, fresh, grassy	C6H12O		887

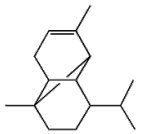
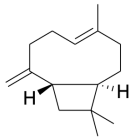
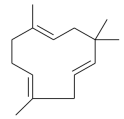
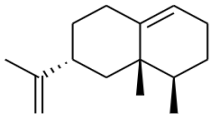
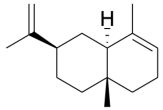
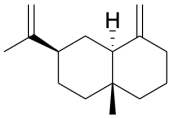
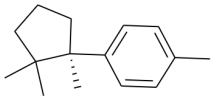
heptanol	heptan-1-ol, heptyl alohcol, enanthic alcohol, gentanol	musty, leafy, herbal	C7H16O		935
Esters					
methyl butanoate	methyl butyrate, butyric acid, methyl ester, methyl n-butanoate	pungent, etherial, fruity	C5H10O2		710
methyl pentanoate	methyl valerate, pentanoic acid, methyl ester, methyl valerianate	sweet, tutti frutti, juicy bubble gum-like	C6H12O2		823
methyl hexanoate	methyl caproate, methyl hexoate, methyl capronate, methyl hexylate	ethereal fruity, pineapple	C7H14O2		924
(E)-3-hexenyl acetate	trans-3-hexenyl acetate, (3E)-3-hexenyl acetate, (E)-3-hexenol acetate	sharp, fruity, green	C8H14O2		1005
(E)-pinocarvyl acetate	trans-pinocarvyl acetate, 2(10)-pinen-3-ol, acetate, trans-(-)-		C12H18O2		1300
carvyl acetate	carveol acetate, p-mentha-6,8-dien-2-ol, acetate, l-carvyl acetate	green, spearmint, herbal	C12H18O2		1334
Monoterpenoid alcohols					

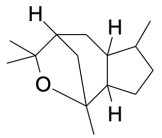
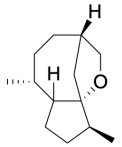
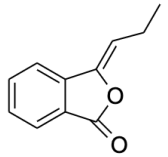
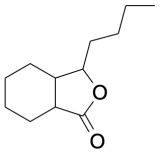
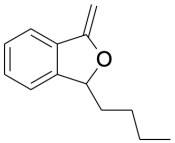
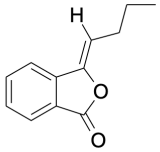
linalool	linalol, linalyl alcohol, allo-ocimenol, 2,6-dimethyl-2,7-octadien-6-ol	citrus, floral	C10H18O		1106
(*E*)-2,8-p-menthadiene-1-ol	p-mentha-2,8-dien-1-ol, cis-p-menth-2,8-dienol, 1-methyl-4-prop-1-en-2-ylcyclohex-2-en-1-ol	fresh, minty	C10H16O		1122
terpinene-4-ol	(+)-terpinen-4-ol, (+)-4-terpineol, (S)-p-menth-1-en-4-ol, S-origanol	menthol, woody	C10H18O		1184
(*E*)-p-mentha-1(7),8-dien-2-ol	trans-1(7),8-p-menthadien-2-ol, trans-p-mentha-1(7),8-dien-2-ol	camphor, menthol, phenol	C10H16O		1186
α-terpineol	terpineol, p-enth-1-en-8-ol, dl-αterpineol	citrus, woody, lemon	C10H18O		1200
dihydrocarveol	8-p-menthen-2-ol, 1,6-dihydrocarveol, neodihydrocarveol	green, minty, sweet	C10H18O		1202
(*E*)-carveol	trans-carveol, p-mentha-6,8-dien-2-ol, trans-, trans-carveole	spicy, caraway, spearmint	C10H16O		1217
(*Z*)-carveol	(-)-cis-carveol, p-mentha-6,8-dien-2-ol, cis-, (4R,6R)-cis-carveol	spicy, caraway	C10H16O		1220

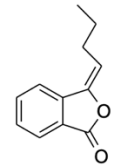
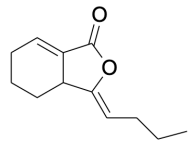
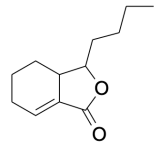
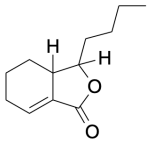
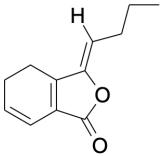
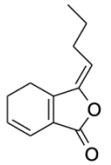
thymol	2-isopropyl-5-methylphenol, 5-methyl-2-isopropylphenol, thymic acid, thyme camphor	herbal, thyme, phenolic	C10H14O		1292
carvacrol	o-thymol, 5-isopropyl-2-methylphenol, isopropyl-o-cresol	spice, woody, camphor	C10H16O		1308
Monoterpenes					
α-thujene	3-thujene, origanene, Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	woody, green,	C10H16		932
α-pinene	2-pinene, acintene A, 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, pin-2(3)-ene	fresh, woody	C10H16		940
camphene	comphene, 2,2-dimethyl-3-methylenenorbornane, 2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane	citrus, cooling	C10H16		955
sabinene	sabinen, 4(10)-thujene, bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	citrus, pine, spicy	C10H16		978
β-pinene	pseudopinene, nopinene, 2(10)-pinene, 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane	green, nutmeg,	C10H16		980

myrcene	β-myrcene, 7-methyl-3-methyleneocta-1,6-diene, β-geraniolene	balsam, fruity,	C10H16		992
p-mentha-2,8-diene	1-methyl-4-prop-1-en-2-ylcyclohex-2-en-1-ol, 2-cyclohexen-1-ol,1-methyl-4-(1-methylethenyl)-	fresh, minty	C10H16O		1001
α-phellandrene	p-mentha-1,5-diene, menthadiene, 1,3-cyclohexadiene, 2-methyl-5-(1-methylethyl)-, dihydro-p-cymene	citrus, herbal, green	C10H16		1005
d-3-carene	3-carene, carene, car-3-ene, 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene	citrus, pine, herbal	C10H16		1018
α-terpinene	p-mentha-1,3-diene, terpilene, 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	terpenic, pine	C10H16		1020
β-phellandrene	p-mentha-1(7),2-diene, 3-isopropyl-6-methylenecyclohex-1-ene, 2-p-menthadiene	minty, terpenic	C10H16		1026
o-cymene	4-isopropyltoluene, dolcymene, o-cymol, 1-isopropyl-4-methylbenzene	cumin, lemon	C10H16		1030
limonene	dipentene, cinene, cajeputene, p-mentha-1,8-diene	citrus, pine, minty	C10H16		1033

β-ocimene	ocimene, 3,7-dimethylocta-1,3,6-triene	warm, floral, herbal	C10H16		1050
terpinolene	isoterpinene, terpinolen, αterpinolene, p-mentha-1,4(8)-diene	fresh, woody, sweet, pine	C10H16		1097
p-mentha-1,3,8-triene	1,3,8-p-menthatriene, p-menthatriene, 1-methyl-4-prop-1-en-2-ylcyclohexa-1,3-diene	terpenic, camphoreous	C10H14		1138
pentyl cyclohexa-1,3-diene	1-pentylcyclohexa-1,3-diene, pentylcyclohexadiene		C11H18		1161
γ-terpinene	p-mentha-1,4-diene, crithmene, moslene, γterpinen,	sweet, citrus	C10H16		1064
dihydrocarvone	p-menth-8-en-2-one, 1,6-dihydrocarvone, (+)-dihydrocarvone	herbal, minty, mentholic	C10H16O		1200
L-carvone	2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enone, 1-carvone, carvol	spearmint, herbal, minty	C10H14O		
D-carvone	(S)-(+)-carvone, (S)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enone, (S)-(+)-p-mentha-6,8-dien-2-one	caraway, herbaceous, spicy	C10H14O		1256
Sesquiterpenes					

α-copaene	copaene, 8-isopropyl-1,3-dimethyltricyclo(4.4.0.02,7)dec-3-ene	woody, spicy, honey	C15H24		1394
β-caryophyllene	(-)-trans-caryophyllene, caryophyllene, (E)-β-caryophyllene	sweet, woody, spice	C15H24		1444
α-humulene	αcaryophyllene, 3,7,10-humulatriene, 1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)-	woody	C15H24		1477
valencene	(+)-valencene, valencen, (3R,4aS,5R)-4a,5-dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	sweet, fresh, citrus	C15H24		1503
α-selinene	eudesma-3,11-diene, 2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	pepper, orange, amber	C15H24		1505
β-selinene	β-eudesmene, (4aR,7R,8aS)-7-isopropenyl-4a-methyl-1-methylenedecahydronaphthalene	herbal	C15H24		1509
cuparene	(+)-cuparene, (R)-cuparene, 1-methyl-4-[(1R)-1,2,2-trimethylcyclopentyl]benzene	woody, cedar, floral	C15H22		1511

kessane	(1S,2R,5R,6R,8R)-1,5,9,9-tetramethyl-10-oxatricyclo[6.2.2.02,6]dodecane		C15H26O		1537
liguloxide		floral	C15H26O		1541
Phthalides					
3-propylidene phthalide	propylidene phthalide, 1(3H)-isobenzofuranone, 3-propylidene-, (Z)-, 3-propylidene-2-benzofuran-1-one	celery, herbal, lovage	C11H10O2		1601
3-butylhexahydrophthalide	3-butyl-hexahydro-isobenzofuran-1-one, Hexahydro-3-butylphthalide, (3R,3aR,7aS)-3-Butylhexahydro-1(3H)-isobenzofuranone	celery	C12H20O2		1646
3-n-butylphthalide	butylphthalide, 3-butylisobenzofuran-1(3H)-one, 3-butyl-1,3-dihydro-2-benzofuran-1-one	celery, herbal, phenolic	C12H14O2		1658
(*Z*)-3-butylidenephthalide	n-butylidenephthalide, 1(3H)-isobenzofuranone, 3-butylidene-, (3Z)-, ligusticum lactone	celery, herbal	C12H12O2		1685

(E)-3-butylidenephthalide	1(3H)-isobenzofuranone, 3-butylidene-, (3E)-	herbal, lovage, celery	C12H12O2		1707
sedanenolide	senkyunolide A, (S)-sedanenolide, 1(3H)-isobenzofuranone, 3-butyl-4,5-dihydro-, (S)-	herbal	C12H16O2		1729
(E)-sedanolide	trans-neocnidilide, sedanolide, 3-butyl-3a,4,5,6-tetrahydro-3H-2-benzofuran-1-one	herbal, celery	C12H18O2		1735
(Z)-sedanolide	cis-neocnidilide,	herbal, celery	C12H18O2		
(*Z*)-ligustilide	(3Z)-3-butylidene-4,5-dihydro-2-benzofuran-1(3H)-one, 3-butylidene-4,5-dihydro-1(3H)-isobenzofuranone, cis-ligustilide	herbal, celery	C12H14O2		1741
(*E*)-ligustilide	(E)-3-butylidene-4,5-dihydroisobenzofuran-1(3H)-one, trans-ligustilide, (3E)-3-butylidene-1,3,4,5-tetrahydro-2-benzofuran-1-one	sweet, spicy	C12H14O2		1797

477 *Linear retention indices taken from DB-5 column using NIST and authentic standards for reference

Project introduction and aims

Celery *(Apium graveolens)* is a globally grown and consumed stalky green vegetable that is utilised in multiple cultures and cuisines including French, Italian and Cajun, combined with onions, carrots, bell peppers or tomatoes to form the base of many soups, stocks, and sauces (Rożek, 2007). This is due to the distinct aroma profile possessed by celery, comprising a variety of monoterpenes, sesquiterpenes, alcohols, aldehydes and most importantly, phthalides (Macleod & Ames, 1989; van Wassenhove, Dirinck, Vulsteke & Schamp, 1990; Uhlig, Chang & Jen, 1987; Kurobaysahi, Kuono, Fujita, Morimitsu & Kubota, 2006)). Phthalides, including 3-butylphthalide, sedanenolide and neocnidilide, are high-boiling compounds abundant in Ligusticum and Angelica species such as celery, lovage and celeriac with odour characteristics including celery, herbal, cooked celery and have been identified as the characteristic compounds in celery (Uhlig et al, 1987; Kurobayashi et al. 2006; Karmakar, Pahari & Mal, 2014). Additionally, celery and celery seed essential oil are commonly used as herbal remedies due to the medicinal properties that they possess, used to treat a range of illnesses from high blood pressure to ischemic strokes. The phthalide compounds mentioned above have been identified to possess many health benefits, playing a role in reducing blood pressure, aiding in cardiac performance and increasing cerebral blood flow (Lin, Chan, Chung & Li, 2005). For this reason, synthesised *dl-3-n*-butylphthalide has been approved as a drug treatment for ischemic strokes (Yan, Feng & Zhang, 1998). Non-volatile compounds present in celery have also been noted to possess potential health benefits including a range of phenolic acids and flavonoids, particularly apigenin, which has been observed to retain excellent bioavailability accompanied by antioxidant, anti-inflammatory, and anticancer properties (Drewnowski & Gomez-Carneros, 2000; Guerra, Carrozzi, Goñi, Roura & Yommi, 2010). Furthermore, the micronutrient content of celery contributes additionally to the health benefits, with vitamins including A, C and K, and minerals such as potassium, folate, and sodium (Malhotra, 2012). For the reasons discussed above, it is clear why celery is such a popular and commonly consumed vegetable.

As a traditional vegetable, celery has been used in recipes dating back to 1623 by the French for flavouring purposes, whereas research into the aroma composition dates to 1963 by Gold and Wilson whereby celery juice was distilled, and the essential oil was extracted and analysed through gas chromatography (GC), identifying 17 compounds. Following on from this, a plethora of investigations have been completed on various celery extracts and using a range of separation techniques. All agree that

monoterpenes, sesquiterpenes and phthalides constituent the basic aroma profile of celery and the most reported compounds in celery fall into these groups. However, there is huge variety in the range of other compound groups identified (alcohols, aldehydes, ketones) as well as the contribution towards the aroma profile from all these compounds. Van Wassenhove, Girinck, Vulsteke & Schamp (1990) identified 28 compounds in the essential oil of four different celery cultivars across two years whereas Phillippe, Suvarnalatha, Sankar & Suresh (2002) identified 29 compounds in celery seed oil that was grown in two regions of India. 40 compounds were identified in the fresh material of Celebrity cultivar using high vacuum-low temperature distillation combined with GC/GC/Flame Ionisation detector (FID), GC/mass spectrometry (GC/MS) and GC/OPA by MacLeod and Ames (1989). Collating all the data together, it is obvious that the chemical composition of celery will change depending on the material tested, cultivar, geographical location, and conditions of growth. Additionally, sensitivity differences in the method of extraction and analysis can cause changes.

Due to the influence of these factors, the importance of authors stating this information is clear, otherwise their data becomes unrepeatable and leads to misinterpretation of the data. Looking at the investigations that have previously been completed, there are few datasets that include all the variables stated above. Furthermore, there has been no experiment whereby the aroma composition of the same genotypes are investigated in a multi-year and multi-site experiment, where the influence of external factors (temperature, humidity, soil and water composition, agronomy) and internal factors (genotype, maturity) upon the aroma composition of celery is studied. Therefore, this project aims to conduct a multi-site and multi-year experiment whereby these factors and their influence can be studied on eight genotypes of celery in the years of 2017-2021 in both the UK and Spain. Using a solid phase microextraction gas chromatography/mass spectrometry, the aroma composition of these celery genotypes can be identified and combining with sensory profiling using a trained panel, any differences in the aroma profile and the impact of the perceived flavour can be investigated.

As previously mentioned, celery is a culinary vegetable that has been commonly used since 15th century however, the preference of celery flavour is a topic that has not been investigated, in fact there has been no research looking to the consumer preference of celery, the drivers of preference and finally, what attributes consumers want in their celery. Answering these questions would help improve the quality of

celery that is produced through (i) directing breeders on new celery hybrids that contain the desirable consumer qualities (ii) educating celery and fresh produce growers on factors that will influence the flavour quality of their crops (iii) recommend cultivars that produce optimal qualities when taking their growing environment into consideration. The project aims and questions that were addressed in this thesis are listed below:

Aims

- To determine and quantify flavour compounds contributing to the organoleptic properties of celery
- Investigate the effect of genotype, maturity, harvest year, geographical location, and agronomic techniques on the volatile composition of celery
- Link the volatile profile with sensory profiling data to allow associations to be drawn between flavour biochemical analysis and human sensory perception
- Identify consumer preferences and drivers of preference within celery

Research Questions

- What are the key aroma compounds and what aroma do they contribute to celery?
- What are the key biochemical drivers of differences in the aroma composition?
- Can changes in the aroma composition lead to noticeable changes in the sensory profile?
- What attributes do consumers find desirable in celery?
- What are the drivers of preference in celery?
- Can we create a new hybrid of celery based on its metabolite profile that displays the potential to meet consumer demand?

The thesis structure is divided into eight chapters, the first chapter investigated the celery aroma literature that has been previously compiled, identifying the "gap" in current knowledge. Following on from this, chapter 2 contains results from a preliminary experiment where the aroma composition profile of 24 parental genotypes identified the most abundant compounds within celery. Chapters 3, ,4 and 5 focused on different environmental factors and their influence on the aroma composition, using the same eight genotypes throughout. Moving on to chapter 6, we investigated the development of aroma across maturity using two genotypes harvested at three time-points where we identified the aroma compounds key

562 to the typical mature celery aroma. Chapter 7 combines three parental genotypes used throughout the
563 project with their hybrids that were presented to a consumer panel to investigate the drivers of preference
564 within celery as well as to identifying the attributes that consumers find desirable in celery. To conclude,
565 the final chapter includes an overall discussion and conclusion and highlights the potential of future work.

566 The celery material used in chapters 2, 3, 4, 5, 6 and 7 was in the form of dried celery. Preliminary
567 analysis was completed whereby fresh and dried celery were compared and although differences in the
568 relative abundance were observed, we identified all compounds that were commonly reported in literature.
569 Freeze-drying was used as a method of preservation to ensure there was no difference in aroma quality
570 across the days in which the SPME GC/MS was completed and while differences in the aroma profile were
571 observed using this preparation method, it was required to ensure all samples were subjected to the same
572 postharvest conditions and preservation prior to analysis. Had we used fresh material, quality loss would
573 have been observed along with aroma differences between analysing the first and last samples, thus
574 introducing an unintended variable to the experiments. By freeze drying the samples as soon as they arrived
575 at the university, the differences observed between genotypes and variables were due to the independent
576 variables being investigated and were not confounded by the degradation of samples over shelf life.
577 Analysis comparing the difference between the aroma profile of fresh and dried material found a decrease
578 in certain volatile compounds (alcohols, aldehydes, esters) but characteristic and other commonly reported
579 compounds were identified in all genotypes.

580 Similar findings were also observed in literature where Nurzyńska-Wierdak, Gruszecki and Kosior
581 (2018) compared freeze-drying with oven-drying in two celery genotypes. Losses in the aroma profile were
582 observed (no significance stated) but also, differences in the aroma quality were seen between the
583 genotypes, concluding that genotypes retain their different aroma profile qualities when freeze-drying.
584 Furthermore, certain compounds were retained at a higher abundance than convection drying.

585 On the other hand, Lisiewska and Kmiecik (1998) found that freeze-drying chives as a method of
586 preservation meant that the typical, distinct odour was retained compared to other preservation methods
587 and Diaz-Maroto, Palomo, Castro, González Viñas and Pérez-Coello (2004) reported that freeze-drying
588 basil led to no significant differences to the typical taste and flavour. Rołson, Osińska, Wajs-Bonikowska
589 (2013) compared freeze-drying with oven-drying, freezing and fresh material with lovage leaves. All
590 preservation methods saw a decline in volatile content when compared to fresh, however, using freeze-

591 dried and oven-dried resulted in a higher celery odour intensity. The highest percentage of phthalides were
592 identified in freeze-dried material. Finally, Hoffman (2007) identified that freeze-dried material represented
593 a more typical and intense aroma than convectionally-dried materials. It was for these reasons that freeze-
594 dried as a method of preservation was used.

595

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CHAPTER 1: Investigating the factors that influence the aroma profile of *Apium graveolens*: A review (As published in Food Chemistry, 2021, 345 (128673), see Appendix I for pdf)

1.4. Abstract

Celery *(Apium graveolens)* is a regularly consumed vegetable, providing strong, distinct flavours to dishes as well as health benefits. Constituents of the aroma profile of celery include a range of volatile compounds (terpenes, phthalides and aldehydes) that contribute to its characteristic odour and flavour. Vast amount of research has been completed on the aroma profile of celery. However, there is limited information stating the cultivar, origin and geographical location, despite that research on a plethora of other crops has indicated that these are key factors driving crop performance and quality attributes. This paper characterises the underlying biochemistry that determines the aroma profile of celery, whilst investigating the genetic and environmental influences leading to its variation. We make recommendations for minimum standards (MIAPAE: Minimum Information About a Plant Aroma Experiment) that should be adopted by the scientific community prior to publication of data relating to flavour and aroma characterisation of crops.

1.2. Introduction

Celery is a member of the Apiaceae or Umbelliferae family, known for the shape of its aromatic flowers called umbels. Crops belonging to this family exhibit distinct flavours including parsley, carrot, fennel, dill, and coriander (Terry, 1989). Celery is most frequently used during cooking as well as consumed in its raw state in salads or with condiments (Rożek, 2007). Celery is thought to be part of the "holy trinity" in many cuisines, combined with bell peppers and onions to form the Cajun holy trinity or combined with carrots and onions to form "Soffritto" in Italian cooking.

There are three main subspecies of *A. graveolens*: leaf celery (*Apium graveolens* L. subsp. *Secalinum*), stalk celery (*Apium graveolens* L. subsp. *Dulce*) and root celery, also known as celeriac (*Apium graveolens* L. subsp. *Rapaceum*). Stalk celery and celeriac are consumed often as vegetables globally, whereas leaf celery or Chinese celery is commonly cultivated and consumed in East Asian countries. Currently on the market, there is an assortment of celery produce available for consumption

659 which is presented in a variety of formats; prepacked whole celery (the celery base, long petioles, and
660 leaves, often cut below any knuckles), prepared celery sticks (chopped petioles with no leaves or
661 knuckles) and celery hearts (chopped, with inner petioles; exposing the heart of the celery).
662 Furthermore, celery can be grown as a white, green, or pink variety. Varieties can also be found in a
663 range of heights and appearances including noticeable ribs along the petioles, low knuckles or bowing
664 petioles.

665 Studies have shown that petioles and leaves share similar volatile compounds, however it is
666 often seen that the leaves are much more aromatic than the petioles and a higher yield of essential oil is
667 gained from the leaves (Li, Hou, Wang, Tan, Xu & Xiong, 2018). Typically, it is the celery petioles
668 that are often consumed in the UK; however, the leaves are consumed in other countries and form part
669 of salads or as a garnish for traditional dishes. Conversely, the aromatic herb coriander, also a member
670 of the Apiaceae family, is used regularly in cooking but the seeds and leaves are utilised.

671 Celery is a versatile plant grown for many functions; the seed, which commonly undergoes
672 extraction to obtain essential oil, can be used as a flavouring agent but also for medicinal uses. The seed
673 has been reported to have excellent anti-inflammatory and antioxidant potential. Kaufman, Cseke,
674 Warber, Duke, and Brielmann (1999) identified over two dozen compounds having the above properties
675 including a range of phthalides, chlorogenic acids, flavonoids (apigenin and luteolin) as well as
676 terpenes. Celery is consumed as a salad vegetable and regularly used as a flavouring agent in stock,
677 soups, and bouillons (Malhotra, 2012); its distinct flavour is made up of a combination of volatile
678 compounds that are responsible for the grassy, herbal aroma. These compounds range from aldehydes
679 and esters to terpenes and phthalides, the latter found to contribute most significantly to the
680 characteristic odour of *A. graveolens L.* (Macleod, MacLeod & Subramanian, 1988). These compounds,
681 along with low molecular weight sugars, organic acids, and flavonoids, are responsible for perceived
682 taste and flavour (Rowan, 2011).

683 While celery has been the focal point in a plethora of literature reviews, the majority of these
684 have been general reviews and not focused on collating data from previous studies to identify
685 differences in the aroma profile and what may influence this. For example, a widespread and thorough
686 review completed by Sowbhagya (2014) looked at the chemical, technological and nutraceutical

functions of celery, however, there was limited focus on the aroma and the impact of variety or different environmental conditions on aroma. Conversely, Li et al. (2018) published a critical review on the advances in celery research providing an in-depth review discussing the current technologies as well as the developments in genetic breeding, genomics research and function genes in celery.

Predominantly, research investigating celery flavour utilises the seed or essential oil, with fewer publications looking at the flavour of fresh samples. The flavour profile will change depending on the chemical composition which in turn will change because of genotype, season, the part of the plant that is consumed, the geographical region it is grown, the stage and the quality of harvest (Malhotra, 2012) as well as soil type, methods of extraction and analysis of the volatile components. This review aims to examine and elucidate current literature investigating the aroma compounds present in leaf and stalk celery (*Apium graveolens* L. subsp. *Secalinum; Apium graveolens* L. subsp. *Dulce*), determine how these compounds contribute to flavour and identify factors that play a role in influencing the aroma, thus showing the need for minimum standards to be adopted by the scientific community, allowing for the creation of a repository with potentially replicable and high-quality data.

1.3. Methodology

To carry out the review, the scientific search engines that were used were Web of Science, ScienceDirect and Google Scholar. Web of Science was mainly used as it offers access to a broader variety of scientific datasets which can be searched singly or simultaneously, including BIOSIS Previews, Data Citation Index and Food Science and Technology Abstracts (FSTA). Articles were sorted in accordance with relevance of the search string used.

The following keywords were identified: celery, aroma, postharvest, environment (Table 1.1). These key words were either used in conjunction or separately. Search operators and search strategies were adopted including key word synonyms, truncation, and wildcard symbols in order to help to refine or widen the search. Search strategies were vital for the refinement of the journals used for this review as a vast quantity of journals have previously investigated celery, with close to 3000 journals available for use (Table 1.2).

Table 1.1: Key words and synonyms used for searching databases.

Main Key word	Synonym
Celery	• *Apium graveolens* • Umbelliferae • Apiaceae • Cultivar • Crop
Aroma profile	• Volatile • Essential oil • Flavour • Odour • Terpenes • Phthalides • Secondary metabolites
Postharvest	• Maturity • Ripening • Shelf-life • Quality
Environment	• Geographical location • Season

Table 1.2: Key words search results in Web of Science

Search string	Full text available online	Relevant
Celery	2,925	3
Celery aroma profile	6	2
Volatile content of celery	11	2
Volatiles of celery essential oil	25	12
Phthalide content of celery	36	13
Celery postharvest	16	2

There were no limitations on dates of papers used, many papers found were published from 1969-present and references were exported to Mendeley reference manager. Furthermore, peer-reviewed journals and journals where full-text access was available were preferred. Originally, papers were considered for evaluation depending on the information they included such as harvest date, cultivar used and cultivar origin, however, this meant many papers were eliminated due to the absence of information of this nature.

1.4. Volatile compounds contributing to aroma and flavour

Within nature, volatiles are comprised of a diverse range of organic compounds that occur naturally, performing multiple functions; from plant and insect signalling through pheromones to food

743 whereby flavour compounds influence organoleptic properties (Pichersky & Gershenzon, 2002). In
744 plants, a range of biosynthetic pathways occur leading to the formation of different products. It has been
745 identified that agents of primary metabolism are the original precursors for the biosynthetic pathways
746 that lead to volatile synthesis such as proteins, carbohydrates, fatty acids, and amino acids (Croteau &
747 Karp, 1991; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008). For example, amino acid degradation
748 will lead to the synthesis of phenylpropanoids and benzenoids. These are the precursors involved in the
749 synthesis of aromatic alcohols, aldehydes, and esters through the shikimate pathway (Vogt, 2010).
750 Whereas in food, flavour compounds can be synthesised through several pathways for example, cooking
751 methods such as grilling or roasting, causing the formation of flavour compounds through the Maillard
752 reaction.

753 Table 1.3 shows a collection of volatile compounds including terpenes, alcohols, aldehydes and
754 phthalides that have been identified in celery from published data. This is accompanied by Table 1.4,
755 which contains the environmental and genotypic data that was included in the studies to build Table
756 1.3. It can be seen in Table 1.3 that there is a variety of compounds present in celery that contribute to
757 its aroma. Although most of the literature focuses on the terpene and phthalide content, the number of
758 other compounds present in celery including alcohols, esters and aldehydes should not be ignored as
759 these are responsible for fresh, grassy and green notes. The reporting levels of these compounds remain
760 relatively low in comparison to terpenes and phthalides, with *(E)*-2-hexen-ol, *(Z)*- 3-hexenal, and
761 hexanol only being reported a handful of times.

762 Completing the review has shown that the aroma compounds present in *A. graveolens* differ
763 considerably depending on cultivar, geographical location, processing, extraction method and the
764 material used. Table 1.3 shows the compounds most reported, and these are: limonene (17 times), 3-*n*-
765 butylphthalide (15 times), β-pinene (14 times), α-pinene and myrcene (13 times), *(Z)*-caryophyllene
766 and β-selinene (12 times). Out of alcohol, ester and aldehyde compounds, the highest reported
767 compound is *(Z)*-3-hexenol (6 times) followed by linalool (4 times). Out of the 21 papers, Wilson (1967)
768 and Gold & Wilson (1963) reported the highest number of aldehydes and alcohols.

769 **Table 1.3:** Summary of volatile compounds identified in celery as reported in studies since 1963.

Compound Name	Aroma descriptor[a]	Reference[b]																					Composition range (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total	
Aldehydes																								
hexanal	green, fatty, leafy		X							X		X					X						4	0.1 - 2.7
3-methylbutanal	fruity, chocolate, fatty		X					X															2	tr - 0.87
2-methylbutanal	musty, cocoa, nutty		X																				1	0.l7 - 0.45
furfural	sweet, almond, baked bread		X																				1	0.35 - 1.1
(*Z*)-3-hexenal	green												X				X						2	n/a
phenylacetaldehyde	honey, floral rose, sweet				X																		1	tr - 0.13
heptanal	green, herbal, fatty											X					X						1	0.1
octanal	citrus, orange peel, green											X					X						2	tr
nonanal	waxy, aldehydic, fresh				X							X											1	tr - 0.26
undecanal	waxy, soapy, floral																X						1	n/a
dodecanal	waxy, soapy, citrus																X						1	n/a
citronellal	waxy, floral, herbal																X						1	n/a
(*E*)-2-nonenal	green cucumber, aldehydic												X										1	n/a
Alkane																								
2-methylpentane												X											1	0.1
3-methypentane												X											1	0.1
hexane												X											1	0.1

octane												X										1	0.1
nonane												X										1	0.3
Alcohols																							
(*Z*)-3-hexenol	green												X			X	X	X			X	X	6	tr - 3.96
1-hexanol	green, fruity, apple															X	X	X					3	tr - 0.36
2-hexanol														X									1	1.2 - 1.3
heptanol	musty, leafy, herbal																X						1	n/a
(*E*)-2-hexenol	green, leafy, fresh, grassy																	X					1	n/a
linalool	citrus, floral			X				X				X			X								3	tr - 0.80
(*E*)-2,8-p-menthadiene-1-ol	fresh, minty							X										X					2	tr - 0.20
(*Z*)-2,8-p-menthadiene-1ol	fresh																	X					1	n/a
borneol	balsam, camphor, herbal			X																			1	1.4
geraniol	floral, fruity, rose			X																			1	0.6
thymol	herbal, thyme, phenolic			X					X														2	0.70 - 6.1
terpinene-4-ol	menthol, woody		X									X				X							3	tr - 1.19
dihydrocarveol	green, minty, sweet																	X					1	n/a
α-terpineol	citrus, woody, lemon											X				X		X					3	tr - 0.1
(*Z*)-carveol	spicy, caraway							X								X		X					3	tr - 3.4
carvacrol	spice, woody, camphor								X														1	1.9 - 3.4
limonene-1,2-diol	cool, minty																	X					1	n/a
(*E*)-carveol	spicy, caraway, spearmint																	X					1	n/a
(*E*)-p-mentha-1(7),8-dien-2-ol	camphor, menthol, phenol																	X					1	n/a
(*E*)-1(7)8-p-menthadiene-2-ol																		X					1	n/a

eugenol	sweet, warm			X				X															2	0.1 - 3.0
citronellol	floral, leather, waxy				X																		1	0.12
globulol	floral, rose				X																		1	3.56
Alkene																								
(E,Z)-undeca-1,3,5-triene	fresh, green, greasy											X	X										2	tr
pentylcyclohexadiene			X					X	X			X											4	0.2 - 4.5
Esters																								
2-octen-1-yl acetate	green, citrus, vegetable				X			X															2	tr - 5.38
(E)-3-hexenyl-1-acetate	sharp, fruity, green																		X				1	0.25
carvyl acetate	green, spearmint, herbal						X		X	X							X		X				4	tr - 25
bornyl acetate	woody, pine, herbal								X														1	tr - 0.2
α-terpinyl acetate	sweet, herbal, bergamot			X													X						2	0.1
phenylethyl propanoate	floral, red rose, fruity				X																		1	0.61
(*Z*)-3-hexenyl pyruvate	green, oily, melon																X						1	n/a
(E)-pinocarvyl acetate								X											X				1	tr - 1.0
Monoterpenes																								
α-thujene	woody, green,		X	X			X					X		X									5	tr - 7.5
α-pinene	fresh, woody		X	X		X	X	X	X		X	X		X	X	X				X	X		13	tr - 9.59
camphene	citrus, cooling		X			X	X		X			X		X	X					X	X		9	tr - 0.29
sabinene	citrus, pine, spicy		X			X	X	X	X		X	X		X									9	tr - 1.72
β-pinene	green, nutmeg,		X			X	X	X	X		X	X		X	X	X				X	X	X	14	tr - 11.51
myrcene	balsam, fruity,		X	X	X	X	X	X	X		X	X	X	X	X	X	X				X		13	tr - 20.97

α-phellandrene	citrus, herbal, green										X			X							X		3	0.1 - 0.28
d-3-carene	citrus, pine, herbal				X										X	X							4	tr
α-terpinene	terpenic, pine			X								X		X									3	0.1 - 0.5
p-cymene	cumin, lemon		X				X	X		X	X	X		X		X							8	tr - 0.31
limonene	citrus, pine, minty		X	X	X	X	X	X	X	X	X	X		X	X	X	X			X	X	X	17	tr - 84
β-phellandrene	minty, terpenic							X				X											2	tr - 0.6
β–(*E*)-ocimene	sweet, herbal		X				X		X		X	X		X	X					X			8	0.1 - 12.50
β–(Z)-ocimene	warm, floral, herbal						X	X	X		X										X		5	tr - 10.1
γ-terpinene	sweet, citrus		X	X		X	X	X			X	X		X		X				X			10	tr - 78.24
dihydrocarvone	herbal, minty, mentholic							X								X			X				3	tr - 50.0
L-carvone	spearmint, herbal, minty					X				X									X				3	0.19 - 10.0
p-mentha-1,3,8-triene	terpenic, camphoreous									X	X	X											3	tr - 2.3
Sesquiterpenes																								
α-copaene	woody, spicy, honey				X									X						X			3	tr - 0.82
(*E*)-caryophyllene	sweet, woody, spice				X		X		X		X												4	0.1 - 8.1
(*Z*)-caryophyllene	clove, pepper, woody		X	X	X	X		X				X		X	X	X				X	X	X	12	tr - 10.5
α-humulene	woody		X				X	X	X					X						X	X	X	8	tr - 8.3
ar-curcumeme							X	X				X											3	tr - 0.4
β-selinene	herbal		X	X	X	X		X	X	X		X		X	X					X		X	12	0.6 - 16.3
α-selinene	pepper, orange, amber		X			X	X	X		X		X		X	X	X				X			10	tr - 2.8
(Z)-β-guaiene	woody, spicy, powdery						X																1	2.6
cuparene	woody, cedar, floral				X																		1	0.64 - 2.11

(*E*)-β-farnesene	woody, citrus, herbal				X						X											2	0.1 - 1.27
kessane					X		X	X	X			X		X								6	0.6 - 5.34
liguloxide							X															1	tr
spathulenol	earthy, herby, fruity			X	X									X								2	tr - 4.43
Phthalides																							
3-butylhexahydrophthalide	celery		X						X			X							X			X	5	tr - 1.2
3-n-butylphthalide	celery, herbal, phenolic	X	X	X	X			X	X	X		X	X	X	X	X	X		X			X	15	tr - 20.0
(*Z*)-3-butylidenephthalide	celery, herbal	X	X	X					X			X				X	X						7	0.1 - 30.5
(E)-3-butylidenephthalide	herbal, lovage, celery	X			X							X											3	1.0 - 20.1
cnidilide	celery, herbal		X									X											2	tr - 41.0
sedanenolide	herbal	X	X	X				X	X	X		X		X		X							9	0.2 - 39.5
(E)-sedanolide	herbal, celery											X											1	5
(Z)-sedanolide	herbal, celery											X											1	1.4
(*Z*)-ligustilide	herbal, celery		X		X		X		X			X				X							6	tr - 47.31
sedanolide	herbal, celery	X	X					X	X	X			X	X		X			X			X	11	0.2 - 45.2
(*E*)-ligustilide	sweet, spicy		X		X					X		X	X	X	X	X							9	0.1 - 6.95
Other compounds																							
2-pentylfuran	green, fruity, earthy				X					X						X							3	tr - 0.35
camphor	camphoreous			X												X							2	tr - 0.6
pentylbenzene					X			X		X						X							4	tr - 1.84
2-undecanone	waxy, fruity, fatty				X																		1	0.42 - 0.54
caryophyllene oxide	sweet, fresh, spicy				X		X	X	X					X									4	tr - 4.11
apiole	parsley, herbal			X			X			X	X												4	0.1 - 23.2
Total Compounds Identified		**5**	**28**	**22**	**24**	**11**	**21**	**29**	**25**	**15**	**14**	**40**	**8**	**24**	**13**	**24**	**17**	**12**	**7**	**11**	**10**	**9**		

[a] Odour descriptors identified using The Good Scents Information System. [b] (1) Uhlig *et al.*, 1987 (2) Van Wassenhove *et al.*, 1990 (3) Sellami *et al.*, 2012 (4) Shojaei *et al.*, 2011 (5) Sorour, 2015 (6) Rożek *et al.*, 2016 (7) Phillippe *et al.*, 2002 (8) Marongiu *et al.*, 2012 (9) MacLeod *et al.*, 1988 (10) Orav *et al.*, 2003 (11) MacLeod & Ames, 1989 (12) Kurobayashi *et al.*, 2006 (13) Wolski

772 *et al.*, 2004 (14) Jian-Qin *et al.*, 1990 (15) Tang *et al.*, 1990 (16) Gold & Wilson, 1963 (17) Wilson, 1967 (18) Wilson, 1970 (19) Ehiabhi *et al*,. 2013 (20) Deng *et al*,. 2003. (21) Lund *et al*, 1973;
773 tr = value was less than 0.1; n/a = data not available
774

775 **Table 1.4:** Summary of Environment x Genotype using the references found in Table 1.3.

Ref[a]	Variety used	Cultivar origin	Geographical location of growth	Year(s) grown	Material tested	Extraction and analysis method
1	Utah 52-70, Giant pascal, Chinese Heug-Kunn, French dinant, Golden self-blanching, Camlyn, Florida 2-14, Clean-cut Harris	N/A	Michigan, USA	1985	Fresh	Solvent extraction and separated by HPLC and identified by GC/MS
2	Blancato, Avon Pearl, Golden Spartan, Loret	N/A	Roeselare-Rumbeke, Belgium	1986 and 1987	Essential oil	Extracted by simultaneous steam distillation-extraction (likens-Nickerson) and identified by high-resolution multi-dimensional gas chromatography with FID
3	N/A	N/A	Soliman, Tunisia	2008	Essential oil and fresh	Extracted with solvent extraction and hydrodistillation and identified using GC/FID
4	Wild Type	N/A	Koohrang, Bazoft and Samsami, Iran	2008	Essential oil	Extracted by hydrodistillation and identified using GC/MS
5	N/A	N/A	Agriculture Research Centre, Egypt	2013	Fresh and dried	Extracted by hydrodistillation and identified using GC/MS
6	Safir	Netherlands	Lublin, Germany	2019	Fresh	Extracted by steam distillation and identified using GC/MS/MS
7	Gaudich	Punjab, India	Kanpur and Punjab, India	N/A	Celery seed oil	Oils sourced for the study and identified using GC/MS
8	N/A	Europe	Italy and Portugal	N/A	Fresh	Extracted by SFE and hydrodistillation and identified using GC/FID and GC/MS

9	N/A	Libya	Libya, brought fresh	N/A	Fresh	Extracted by steam distillation and identified using GC/FID and GC/MS
10	N/A	Estonia	Brought fresh	N/A	Fresh and air-dried essential oil	Extracted by SDE and identified by capillary GC and GC/MS
11	Celebrity	N/A	Brought fresh	N/A	Fresh	Extracted by high vacuum-low temperature distillation and identified using GC/GC/FID, GC/MS and GC/OPA
12	N/A	N/A	Nagano Prefecture, Japan brought fresh	N/A	Fresh	Extracted by hydrodistillation followed by SAFE and identified using GC/FID, GC/MS and
13	N/A	N/A	N/A	N/A	Fresh	Extracted by solvent extraction and identified using GC/ITMS
14	N/A	N/A	N/A	N/A	Celery seed oil	Extracted by steam distillation and identified using GC/MS and GC/FTIR
15	N/A	N/A	Brought fresh	N/A	Fresh	Solvent extraction and identified using GC and GC/MS
16	N/A	N/A	Brought fresh	N/A	Celery juice	Extracted by steam distillation, fractions were collected in portions of the apparatus (column-bottom, chilled water trap, ice trap, salt and ice trap, dry-ice trap and liquid nitrogen trap). Identified using GC, GC/FID and GLC
17	N/A	N/A	N/A	N/A	Essential oil	Extracted by batch and continuous steam distillation followed by solvent extraction, and identified using GC/MS F&M
18	N/A	N/A	N/A	N/A	Essential oil	Extracted by batch and continuous steam distillation, identified using GC/MS
19	N/A	N/A	Nigeria	N/A	Essential oil	Extracted by hydrodistillation and identified using GC/MS

20	N/A	N/A	Research Centre for Plants, Shenghai	N/A	Fresh	HS-SPME-GC/MS was using for extraction and identification
21	Utah 5270 and Flormart		Florida	November 1972, April and July 1973	Essential oil	Extracted by steam distillation, volatile content determined by “Bromate Titration Method” and were separated using GLC.

776 [a] Refer to Table 1.3 for references.

Table 1.4 lists all the various isolation and analytical methods that have been used across the studies to construct Table 1.3. The most popular method of extraction is hydrodistillation (HD) followed by gas chromatography/mass spectrometry (GC/MS). Although HD is a traditional method of extraction that is regularly used throughout industry, the high temperatures used can contribute to the thermal degradation of some volatile components (Oreopoulou, Tsimogiannis & Oreopoulou, 2019). Victório, Riehl & Lage (2009) compared the volatile content using simultaneous distillation–extraction (SDE), HD and static headspace methods on *Aplinia zerumbet* (Pers). Although they found a difference in the composition of the essential oil between these processes, they concluded that all methods were suitable for the analysis of volatiles, however, SDE is more suitable for analysing smaller quantities of plant material (Victório, Riehl, & Lage, 2009).

Using a method where volatiles can be isolated from a matrix at room temperature under a vacuum, will prevent thermal degradation of compounds and improve recovery rates. MacLeod and Ames (1989) used low temperature high vacuum distillation and identified 40 compounds including 13 monoterpenes, 12 phthalides and five sesquiterpenes as well as several alcohols, alkenes, and alkanes. Utilising high vacuum distillation allows for the separation of higher boiling compounds such as phthalides, which have been shown to be difficult to isolate and characterise in previous studies shown by Orav, Kailas and Jegorova (2003). Here six phthalides isomers were identified but the correct characterisation of these isomers could not be completed.

In terms of analysis, most of the studies (Table 1.4) used 1D GC in order to analyse celery volatiles. However, with this method, correct characterisation of phthalides was shown to be limited and even in some studies, no phthalides were identified. The utilisation of 2D GC has shown to aid in the correct separation of phthalides as well as the characterisation of phthalide isomers (Bartschat, Beck, & Mosandl, 1997; MacLeod & Ames, 1989; van Wassenhove et al., 1990a).

Only one study by Deng, Song, Zheng, Hu & Zhang (2003) analysed fresh celery samples by extracting the volatiles present in the headspace using solid phase micro-extraction (SPME) followed by GC/MS. However, investigating celery as an essential oil has shown to yield results with more identifiable compounds than SPME as shown by MacLeod & Ames (1989); van Wassenhovet et al. (1990a); Philippe et al. (2002) and Shojaei et al. (2011) (Table 1.3, reference 11, 2, 4 and 7).

Orav et al. (2003) and Sorour, Hassanen and Ahmed (2015) compared the differences in volatile content between fresh and dried celery material and concluded that processing the celery through methods such as freeze drying, or air drying should not alter the presence of aroma compounds but only the abundance of certain compounds. This was confirmed by Orav et al., (2003) who investigated the difference of aroma profiles in fresh celery and air dried, oven dried and freeze-dried celery, showing that there was little difference between the processing methods in terms of the presence or absence of compounds; but differences were observed in terms of the concentrations of certain compounds (e.g., a decrease in limonene and a slight increase in phthalide concentration). Table 1.3 also shows the variation in percentage composition between compounds. Although variation is expected when so many variables are involved, certain compounds show an extreme variation; the biggest occurring within the monoterpenes, particularly for limonene and γ-terpinene. Both compounds have been identified to be very common monoterpenes in celery as shown by van Wassenhove et al. (1990a), identifying limonene and γ-terpinene as the most abundant compounds across four varieties. Variation caused by abiotic and biotic factors, such as maturity and environment, influence these compounds. Thus, showing the importance of examining the same cultivar across different seasons in different geographical locations. Although not as vast, variation between the reported composition of phthalides can be seen, particularly with cnidilide, (*Z*)-ligustilide and sedanolide. Characterising phthalides and their enantiomers correctly has been shown to be difficult using 1D GC and hydrodistillation techniques. This would explain the variation between extraction processes.

Furthermore, out of the 21 papers that were used to build Table 1.3, 13 papers mentioned the geographical region inm which the cultivar under investigation was grown, seven provided the celery cultivar name, seven provided growing and harvesting dates, five mentioned the cultivar origin, three completed a multisite experiment, three used more than one cultivar and only one repeated the experiment the following year (Table 1.4). Not one paper used one single cultivar in a multisite experiment that was repeated the following season. The vast quantity of research that has been completed on celery and its aroma profile can only be described as partial and inconclusive. Clearly, there is variation in the aroma profile and simply studying one cultivar, grown in one location, in one

year is not a sufficient sample size or experiment to conclude the following compounds are the only compounds to be present in celery. There was no compound that was detected in every study on celery.

It is clear from Table 1.4 that many authors do not record basic information regarding the provenance of their samples, this would enable some consideration of the genetic and environmental influences on aroma compounds. Other communities have developed standards for minimum information required for characterising raw materials used in experimental datasets and it is recommended that the flavour science community also adopts a similar approach.

Plant phenotyping experiments (and it can be argued that flavour and aroma are a subset of phenotype) are already required to adhere to standards. The proposed guidelines for the correct handling of data from plant phenotyping experiments to allow for data reuse and combining are known as the "Minimum Information About a Plant Phenotyping Experiment" (MIAPPE). These guidelines contain a checklist of attributes that would aid in the understanding of the plant phenotypic data and how it was obtained. The checklist of attributes can be categorised into the following sections: general metadata, timings and locations, environments, treatments, experimental design, sample collection and processing and observed variables (Cwiek-Kupczyńska et al., 2016). Similarly, MIAME: Minimum Information About a Microarray Experiment present six fundamentals that enable the correct interpretation of results and experimental repetition including: the raw data for each hybridisation as well as the final processed data for the set of hybridisations, essential sample annotation (experimental factors), experimental design, annotation of the array and essential protocols (laboratory and data processing) (Brazma et al., 2001).

Following a similar attribute checklist to MIAME and MIAPPE, Table 1.5 presents MIAPAE: 'Minimum Information About a Plant Aroma Experiment', describing the minimal information that would allow for accurate interpretation and correct repetition of the experiment. Including the attributes presented in Table 1.5 allows for sufficient information to be provided, ensuring experiments whereby the aroma of plants is profiled can be interpreted, verified, and repeated correctly, with the goal of facilitating the formation of superior datasets.

858 **Table 1.5:** Recommended attribute checklist for plant aroma experiments.
859

Checklist section	Attribute	Recommended information to include
Experimental design	Field	Replication, block design, harvest protocol
	Laboratory	Replication, analytical method protocol including extraction procedure, use of standards (internal and external), temperature programs, QCs, statistical analysis and quantification methods
Presenting Results		Chemical classes, triplicate range of relative abundance for chemical compounds, semi-quantification of data, P-value, LRIs (experimental and expected), method of LRI identification, units
Sample information	Seed	Preparation, source, pre-treatments
	Plant	Taxon, common name, origin, cultivar, age and life stage at harvest
	Plant extract	Type of extract used e.g., essential oil, fresh or dried material
Timing and location	Timing	Start and duration of experiment, timings between the stages of harvest and processing
	Location	Growth, post-harvest, processing and storage location
Environment	Met data	Average day and night temperature (°C), rainfall (mm), day and night length (hours)
	Agronomic practices	Treatments, watering and irrigation
	Nutrients	Fertiliser composition and amount added, soil salinity
	Postharvest	Temperature of storage (°C), transport between facilities, processing and storage conditions
Raw material collection, processing and storage	Collection	Plant organ of interest, method of collection
	Processing	Method of processing, duration, location and temperature
	Storage	Method of storage, duration, location and temperature

860
861 Although in some cases it is not possible to follow experiments exact and reproduce identical
862 results, it is possible to follow the same experimental design, particularly when it comes to replicating
863 laboratory conditions. Whilst there is no doubt there will be differences in compound abundances, by
864 following the same extraction protocol, using the same analytical instrument along with the stated
865 temperature program, similar compound groups may be identified. Addressing all information that is
866 required of MIAPAE (Table 1.5), we hope to build a repository whereby experiments completed
867 following MIAPAE can be used to provide guidance for future experiments on plant aroma including

what outcomes to expect when following specific conditions and optimum extraction processes and temperature programs to follow to identifyy desired chemical compounds. Furthermore, through standardisation we aim to improve the quality of data that is presented to authors.

The variation in compounds identified in celery between experiments investigating the aroma profile can be seen clearly (Table 1.3) and with different cultivars, experimental designs, processing methods and instrumental analysis, however, it is difficult to compare these results. Using the proposed MIAPAE standards, whereby information on the experimental design, sample collection, processing and testing is included, experiments can either be replicated or variables changed/introduced to allow for further comparison, collation of datasets and eventually leading a public repository with the purpose of providing high-quality plant aroma data.

1.4.1. Terpenes

The aroma of raw celery is often described as fresh, herbal, woody and citrusy, and the main contributors to these descriptors are terpenoids, sesquiterpenes and monoterpenes. These are all major components that constitute the aroma profile in celery, as well as ubiquitous across many other flowers, herbs, spices, and food stuffs.

Terpenes play a diverse range of roles in nature and in industry, from insect and plant signalling to fragrances and flavourings. Terpenes are mostly hydrocarbons and are constituents of essential oils. Isoprene, a unit made up of five carbons, is the building block for terpene synthesis and when biosynthesis occurs, isoprene forms either acyclic, cyclic, or polycyclic compounds (Parker, 2015). Celery contains a range of monoterpenes, two isoprene units ($C_{10}H_{16}$), and sesquiterpenes, made up of three isoprene units ($C_{15}H_{24}$) and these can be cyclic or bicyclic in structure, including: limonene, β-pinene, β-selinene and β-caryophyllene. The structure of β-caryophyllene includes a nine-membered ring that is fused to a cyclobutene ring (Figure 1.1).

Within *A. graveolens*, there has been a wide range of terpenes reported in literature including a variety of monoterpenes and sesquiterpenes. Monoterpenes such as d-limonene (62.4–70.3%) and *(I)*-β-ocimene (10.1–10.5%) contributed the largest proportion of volatiles present in fresh celery grown in Estonia (Orav et al., 2003) (Table 1.3, reference 10), whereas, Jian-Qin et al. (1990) (Table 1.3,

reference 14) identified in celery seed oil d-limonene (72.16%), β-selinene (12.17%) and α-selinene (2.05%) as the most abundant terpenes.

Limonene (18,000–37,000 μg/kg), λ-terpinene (6,000–16,500 μg/ kg) and β-pinene (436–1,205 μg/kg) were most abundant across the four varieties used in an investigation carried out by van Wassenhove et al. (1990a) using blanching varieties grown in Belgium (Table 1.3, reference 2). The variation across the four cultivars used in this study provides evidence that there is a genetic basis for flavour deviation between cultivars. Throughout literature, limonene is the most abundant terpene, with an odour often described as citrus, fresh and lemon. However, limonene is not a key characteristic aroma compound, with a reported odour threshold range of 0.50–0.59 ppb orthonasal and 0.46–0.62 ppb retronasal (Plotto, Margaría, Goodner, Goodrich & Baldwin, 2004).

A study carried out by Deng et al., (2003) utilised SPME GC/MS to analyse the volatile constituents making up celery, identifying many compounds including monoterpenes and terpenoids. Obtaining a cultivar grown in Shanghai, Deng et al. (2003) confirmed the high proportion of limonene present (32.22 % relative contents), followed by α-pinene (16.56 % relative contents), and β-ocimene (9.5 9% relative contents). These values differ considerably when comparing literature (Table 1.3) suggesting that multiple factors play a role in celery flavour including geographical location and cultivar (Deng et al., 2003*)*.

1.4.1.1. Biosynthesis of terpenes

Biosynthesis of terpenes occurs from isopentane either through the mevalonic acid pathway (appendix II) (MVA-pathway) from acetyl-CoA or the non-mevalonate pathway (appendix III). During the MVA-pathway, the pyrophosphorylation of mevalonic acid leads to the production of mevalonic acid pyrophosphate (MVA-PP), decarboxylation and dehydration of MVA-PP will result in the formation of isopentenyl diphosphate (IPP). IPP can be isomerized to produce dimethylallyl diphosphate (DMAPP). The bonding of IPP with DPP leads to the synthesis of geranyl pyrophosphate (GPP), which is the precursor of monoterpenes, and then the bonding of a further IPP molecule forms farnesyl pyrophosphate, the precursor of sesquiterpenes (Schwab et al., 2008). Alternatively, isoprene can also be synthesised through the non-mevalonate pathway or the MEP/DOXP, which similarly to the MVA-pathway, leads to the production of IPP and DPP. However, the MEP/DOXP-pathway occurs

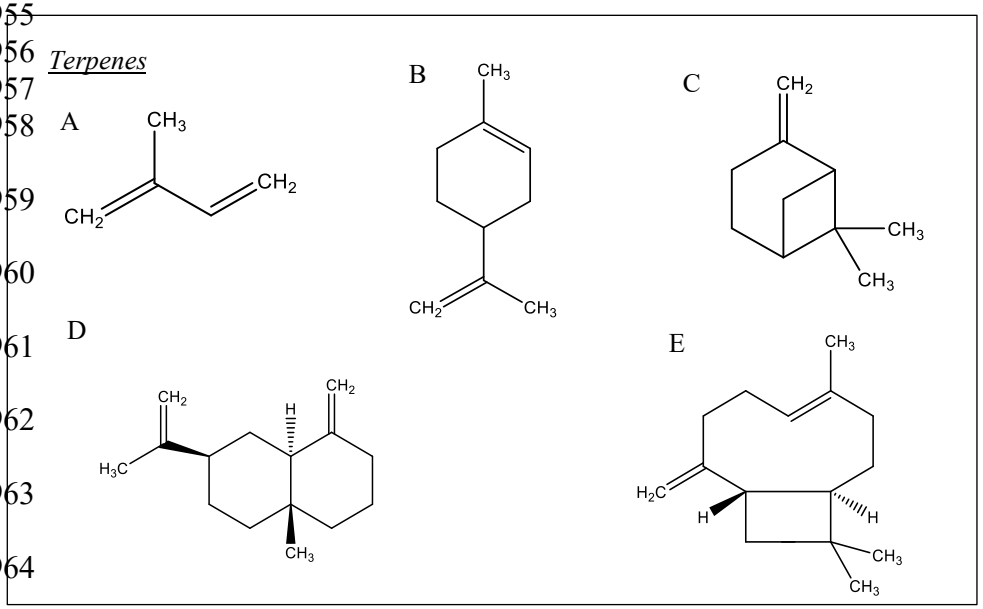
more predominantly in green plants, operating in the plastids, utilising D-glyceraldehyde 3-phosphate bonding with pyruvate to form 1-deoxy-D-erythritol (DXP). This eventually leads to the production of DMAPP, IPP and GPP to synthesise predominantly monoterpenes and some sesquiterpenes. In contrast, the MVA-pathway operates in the cytosol and synthesises mostly sesquiterpenes, sterols and triterpenes (Kuzuyama & Seto, 2012).

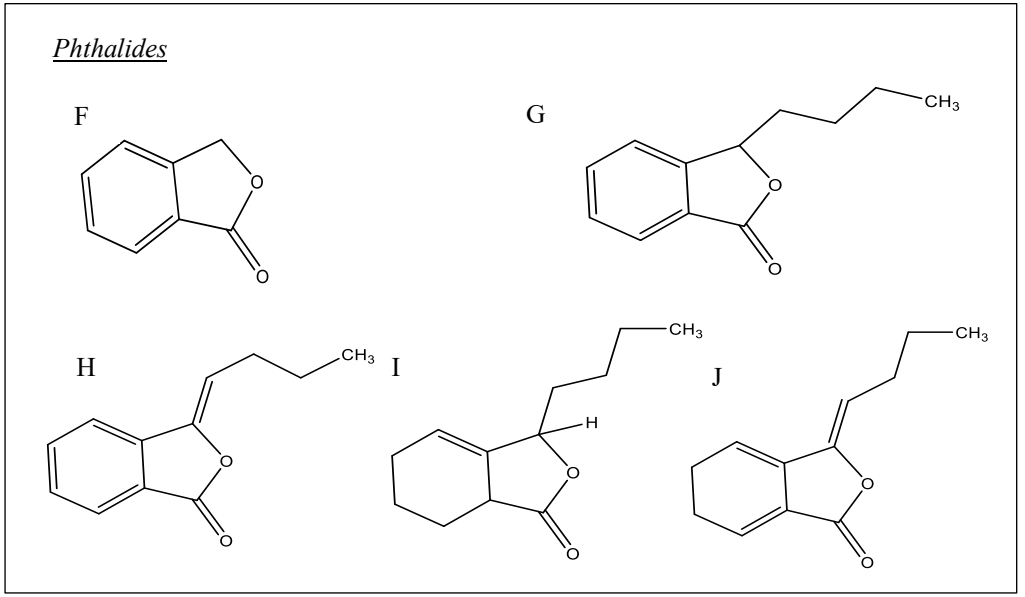
Due to the abundance of terpenes present within celery and their simple structure, mono- and sesquiterpenes are ideal starting materials for many other compounds however, the presence of terpenes can potentially be detrimental to the plant, due to their high oxidative potential. For example, limonene, the most abundant compound in celery can transform into a range of derivatives including cis- and trans- carveol, L-carvone and α-terpineol, all of which have been found in celery (Bicas, Dionísio & Pastore, 2009; Turner, Lignou, Gawthrop & Wagstaff, 2021a, b & c). Furthermore, through the oxidation of limonene, α -, and - β-pinenes, pinocarvone and various ketones were obtained.

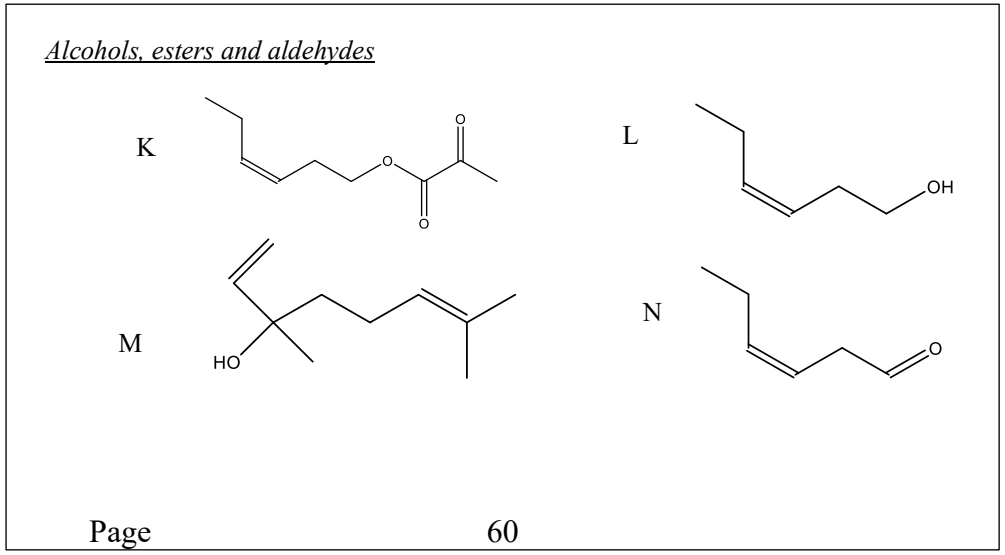
These reactions occur within celery due to cytochrome P450 oxygenases, abundant in many plant cells, it is known for its ability to oxidise monoterpenoids along with catalysing the production of many other important secondary metabolites (Figueiredo, Almendra, Barroso & Scheffer, 1996). Furthermore, the simple structure of monoterpenes also allows for the formation of further compounds depending on the attachment of various functional groups. In this insistence, NADP+ oxidoreductase becomes a key enzyme in catalysing the biosynthesis of monoterpenoid alcohols in celery including linalool, thymol, borneol and terpineol (Ikeda et al., 1991).

The diversity of the terpenes that have been identified in celery is vast (Table 1.3) along with the aroma characteristics that they contribute. An explanation for the diverse range of terpenes identified is due to how susceptible the terpene structure is to modification with the aid of various oxidative enzymes that display unspecific substrate and regiospecificity and therefore, broaden the range of compounds that are synthesised (Pichersky & Raguso, 2016). Ehrlich and Raven (1964) hypothesised that a munitsunitore diverse terpene content is a response of plant defence mechanisms and that these newly synthesised compounds are adaptive in response to a change in the environment. Compounds that are synthesised in response to stress are either new, more complex compounds or modification of the skeleton of a current compound with the addition of new functional groups.

Figure 1.1: A range of volatile compunds that occur and contribute to the typical aroma of celery; isoprene (A), limonene (B), β-pinene (C), β-selinene (D), β-caryophyllene (E), 1(3H)-isobenzofuranone (F), butylphthalide (G), 3-butylidenephthalide (H), (Z)-ligustilide (I), sedanenolide (J), (Z)-3-hexenyl pyruvate (K), (Z)-3-hexen-1-ol (L), linalool (M) and (Z)-3-hexenal (N).







This can be observed in compounds such as limonene, phellandrene, α- and β-pinene and o-cymene whereby close similarlities can be seen in the compound structure.

1.4.2. Phthalides

Phthalides are naturally sourced in plants, being particularly abundant in *Ligusticum* and *Angelica* from the Apiaceae family (Karmakar, Pahari & Mal, 2014). Celery, celeriac and lovage are rich sources of phthalides and these compounds hold many health benefits; they are biologically active compounds playing roles on the central nervous system and cardiac performance, aiding in anti-thrombotic modulation, and providing protection against cerebral ischaemia and high blood pressure (Lin, Chan, Chung, & Li, 2005). An example of one of the health benefits from consuming phthalides can be observed Yang Feng and Zhang (1998) whereby a significant increase of cerebral blood flow in cerebral ischemia rats when *dl*-3-*n*-butylphthalide was used as treatment. More recently, a 90-day administration of *dl*-3-*n*-butylphthalide was completed, whereby the administration of *dl*-3-*n*-butylphthalide had significantly more favourable outcomes than Ozagrel, a drug commonly used to treat strokes (Cui et al., 2013). From the evidence provided above, along with a plethora of other supporting investigations, *dl*-3-*n*-butylphthalide a phthalide synthesised from 3-*n*-butylphthalide, was approved by the China Food and Drug Administration as a new drug for the treatment of strokes in 2002.

1.4.2.1. Biosynthesis of phthalides

Structures and biosynthetic pathways of phthalides have been suggested previously but they remain ambiguous, and little is known about these compounds. One pathway way has been suggested by Karmakar et al. (2014) (appendix IV). They hypothesised that phthalide is originally synthesised from tetraketide **(2)** which in turn, is formed from the condensation of four acetic acid **(1)** bonded by the action of polyketide synthase. According to Karmakar et al. (2014), dialdehyde **(8)** is synthesised through the condensation of the tetraketide unit to orsellinic acid **(3)** though various enzymes (ketoreductase, cyclases and aromatases). Then, orsenllic acid is subject to methylation, regiospecific oxidation and decarboxylation **(4**–**7)**. An intramolecular Cannizzaro reaction **(9)** occurs producing phthalide **(10)** from dialdehyde. Phthalides are classified according to their substitution at C-3 and the oxidation occurring within the benzene ring (Karmakar et al., 2014). This can be seen in Figure 1, where

994 the double bonds within the benzene ring change along with the arrangement present at C-3 to produce
995 a different compound.

996 To date, all naturally occurring phthalides are derived from 1(*3H*)- isobenzofuranone consisting
997 of one benzene ring bonded with a γ-lactone between carbon atoms. 1(*3H*)-Isobenzofuranone has the
998 simplest phthalide structure, C8H6O2 (Lin et al., 2005). Multiple phthalides have been identified in
999 celery including: phthalide, 3-butylphthalide, 3-butylidenephthalide, (*Z*)-ligustilide and sedanenolide
1000 (Figure 1.1).

1001 Using enantioselective multidimensional gas chromatography, Bartschat et al. (1997) analysed
1002 3-butylphthalide enantiomers and eight 3-butylhexahydrophthalide stereoisomers in celery, celeriac,
1003 celery seed and fennel extracts. From this, 3-butylphthalide enantiomers (3*S* and 3*R*) were identified
1004 with 3*S* enantiomer showing to be the preferred configuration in all extracts. Furthermore, 3-
1005 butylhexahydroxyphthalides (3*R*,3a*R*,7a*S* and 3*S*,3a*R*,7a*S*) were detected and shown to be generated in
1006 high enantiomeric purity in celery and celeriac extracts. Bartschat et al. (1997) stated that the high
1007 enantiomeric purities of these compounds suggest that they may be synthesised with high
1008 stereoselectivity; originating from partially hydrogenated phthalides such as sedanolide and
1009 sedanenolide, known key contributors to *A. graveolens* odour.

1010 Often in literature, the stereochemical aspects of these phthalide compounds have been
1011 neglected including the impact these have upon sensory characteristics. MacLeod and Ames (1989)
1012 analysed the volatile components present in supermarket purchased celery and celeriac using GC,
1013 GC/MS and GC odour port assessment (GC/OPA) and positively identified 12 phthalides in both
1014 extracts including two 3-butylhexahydrophthalide isomers. Although the stereochemistry was not taken
1015 into consideration, these two isomers were shown to possess different odours according to GC/OPA.
1016 The first isomer identified exhibited a “sweet, sickly, cooked celery” and “braised celery, peppery,
1017 smoky” in celery and celeriac respectively. The second isomer was not identified in celery but was
1018 described as “celery, fruity, fragrant” in celeriac. MacLeod and Ames (1989) discussed how having a
1019 substitution of an alkyl group at C3 would lead to a less celery odour compared to an alkylidene
1020 substitution whereby a more intense celery odour due to the alkylidene group increased from C1 to C4.
1021 This agrees with findings by Gold & Wilson (1963) who identified four alkylidene phthalides in celery

juice distillate fractions that possessed a strong characteristic celery odour and were identified as the principal odour components of celery.

There has been conflicting evidence on whether phthalides are truly present as earlier studies were unable to separate and characterise phthalide compounds including 3-butylhexahydroxyphthalides enantiomers and the sedanolides. Uhlig et al. (1987) investigated the effect of phthalides on the flavour of celery using eight different cultivars of varying origins but grown in Grand Rapids, Michigan (Table 1.3, reference 1). Dichloromethane extracts of celery stem tissue were separated by HPLC and identified using GC/MS. The peak area per gram of total solids of butylphthalides (butylphthalide, *trans*- and *cis*-butylidene phthalide), sedanenolide and sedanolide were identified. Sedanolide was absent in six out of eight cultivars tested and they suggested that this result could be due to technical error, as the HPLC was unable to resolve minute quantities of sedanolide from sedanenolide. Within the cultivars, there was over six-fold variation in the abundance of different compounds, with butylphthalide abundance ranging from 250 to 1540 peak area per g total solids (Uhlig et al., 1987). In Uhlig's study, five phthalides were identified, almost half of the phthalides identified by MacLeod and Ames (1989).

For sensory evaluation, Uhlig presented the plant tissue from the samples diluted in water to six trained panellists, whereby the intensity of celery flavour was evaluated on a nine-point hedonic scale (1 = no celery flavour and 9 = extremely strong celery flavour). These flavour scores were correlated with the phthalide content, leading Uhlig to conclude that the variation of phthalide content across cultivars resulted in significant differences in the perception of celery flavour (Uhlig et al., 1987).

Phthalides, although lower in abundance than terpenes, are much more odour-active, exhibiting flavour dilution factors of around 15,000 before the limit of detection is reached and can be seen to be characteristic compounds of celery aroma (Kurobayashi et al., 2006). Sedanenolide has an odour threshold value of 0.14 – 0.60 mg/L depending on the enantiomer (Oguro & Watanabe, 2011) and 3-*n*-butylphthalide has a value of 0.00001 mg/L (Bartschat et al., 1997). Furthermore, Lund, Wagner, and Bryan (1973) identified the odour threshold of phthalide compounds that expressed a celery-like odour. These included sedanolide (1 mg/L), 3-*n*-butylphthalide (10 mg/L) and hexahydro-3-*n*-butylphthalide (2 mg/L) as well as β-selinene (1 mg/L), although the latter were identified to not exhibit a characteristic celery odour when compared with sedanolide and 3-*n*-butylphthalide, they were still considered to be

contributors to the fresh celery aroma. Out of these compounds, sedanolide was identified as the most characteristic compound to the celery odour.

1.4.3. Alcohols, aldehydes, and esters

Few published papers focus on the presence of other volatiles such as alcohols, esters, and aldehydes. These compounds are vital to the aroma, with odours described as green, fresh, citrus, and floral. Shojaei et al. (2011) studied the chemical composition of three ecotypes of wild celery (Bazoft, Koohrang and Samsami) grown in three different regions of Iran in 2008 and identified a range of aromatic compounds using GC–MS analysis (Table 1.3, reference 4). Within the three ecotypes, at least 22 compounds were identified and phthalides made up much of the chemical composition. Compounds such as 2-octen-1-ol acetate, pentylbenzene and 2-undecanone were reported at much lower abundances, yet at similar concentrations to sesquiterpenes. Gold and Wilson (1963) investigated the volatile flavour substances present in celery juice, identifying 38 compounds comprising of aldehydes, esters, alcohols, terpenes and phthalides (Table 1.3, reference 16). Gold and Wilson identified the ester (*Z*)-3-hexenyl pyruvate as a principal odour constituent using a dry ice trap, with odour descriptors such as green, vegetative, and floral green tea (Gold and Wilson, 1963).

Wilson (1967) identified and quantified the alcohol composition of celery essential oil using column chromatography on two celery essential oils. Using this method of separation allowed him to identify that the two essential oils were comprised of 10 to 15% alcohol, including hexan- 1-ol, *(Z)*-3-hexene-1-ol and *(E)*-2-hexene-1-ol as well as terpene alcohols; *(E)*- and *(Z)*-2,8-p-menthadiene-1-ol (Table 3, reference 17). He concluded that although these alcohol compounds did not possess aromas that were typical of celery, they were still important contributors to the overall aroma and flavour (Wilson, 1967).

1.4.3.1. Biosynthesis of alcohols, aldehydes, and esters

In plants, alcohols, aldehydes, and esters originate from saturated and unsaturated fatty acids such as linolenic acid and are formed predominately by three processes: α-oxidation, β-oxidation, and the lipoxygenase pathway. Initially, saturated, and unsaturated fatty acids are bound to acyglycerols as triacylglycerides and are released as free fatty acids via enzymatic oxidative (acyl hydrolase)

degradation of lipids. The lipoxygenase pathway, which leads to the synthesis of short-chain aldehydes and alcohols (C6 and C9), involves multiple enzymes including lipoxygenase (LOX), hyperoxide lyase (HPL) and alcohol dehydrogenase (ADH). LOX catalyses the conversation of linolenic acid to 9-hydroperoxide or 13-hydroperoxide.

With the use of enzymes or *β*-oxidation, aroma compounds are formed such as 3-*(Z)*-hexenol, *(E)*-jasmone and 3-*(Z)*-hexenyl acetate. For example, hexanal is a linolenic acid-derived aldehyde with a fatty, green odour, it is synthesised through a series of enzymatic reactions using LOX, HPL, 3Z,2E-enal isomerase and alkenal oxidoreductase (Schwab & Schreier, 2002; Stumpe & Feussner, 2006). Figure 1 shows the compound structure for: (*Z*)-3-hexenyl pyruvate, (*Z*)-3-hexen-1-ol, linalool and (*Z*)-3-hexenal, these are just a selection of alcohols, aldehydes and esters that have been identified in celery. Compounds known as green leaf volatiles (GLVs) are synthesised in the plant when subject to biotic and abiotic stresses. These include compounds such as 3-(*Z*)-hexanol, 3-(*Z*)-hexenyl acetate and hexanal, these compounds often have green, fatty odours, important to celery aroma.

The biosynthesis of aldehydes, alcohols and ketones have been shown to increase over time in food and due to the similarities in structure between aldehydes and ketones, their stability and reactivity also remains similar. Although ubiquitous in nature, the synthesis of these compounds can occur through lipid and protein degradation and lipid oxidation. In plants, the degradation of fatty acids through oxidation occurs in the peroxisomes and plays a role in plant response to abiotic and biotic stresses. Not studied in celery, compounds such as 1-octen-3-one, (E,Z)-2,6-nonadienal, Z-3-hexenal, (Z,Z)-3,6-nonadienal and 3-methylbutanal have been associated with ‘off-odours’ (Sucan, 2004), many of which have been identified in celery, have been observed to be products of β-oxidation (Turner et al., 2021 a,b,c). Lipid oxidation can also be induced through light exposure; linoleic acid is particularly susceptible to photooxidation, which in turn influences the beginning of the LOX pathway, synthesising compounds such as hexanal and (*Z*)-3-hexenol.

1.5. Genetics and the aroma of celery

Over the years, there has been a focus on improving yield to increase product availability as well as to decrease cost paid by the consumer. However, this means that there has been a lack of focus

on the quality of crops and therefore, important traits such as flavour have been ignored. Key aspects of quality include nutritional content, post-harvest quality, being free of disease and eating quality. There has been a lot of focus on developing disease-resistant celery lines, particularly to Fusarium yellows (*Fusarium oxysporum* f. sp. *apii*) which is one of the biggest diseases to threaten celery production worldwide. It was Orton, Hulbert, Durgan, and Quiros (1984) who developed the first Fusarium-resistant celery line using a celeriac accession (Orton et al., 1984). Furthermore, breeding of late bolting or slow bolting variety has also been emphasised to improve yield, particularly during the winter-spring season to extend the season (Li et al., 2018).

There are multiple reasons as to why emphasis on breeding for flavour has been low. Breeders carry out taste tests during the development phase whereby taste attributes such as bitterness and sweetness are scored, and lines are rejected if unpalatable. Nevertheless, breeders do not have the tools available to select for flavour, in addition to the need to select for the maintenance and consistency of flavour (Klee, 2010). Determining the flavour would require sensory profiling analysis to be completed on a whole breeding population using a trained panel, as well as laboratory work to identify and quantify the aroma compounds present. This can be a lengthy and expensive process. Using transcriptome sequencing would help identify genes that are being expressed in the same cultivar that has been taken into different environments and grown, providing information on the differences in gene expression. However, genetics only show the potential flavour of the crop, factors such as the environment, handling and damage and cooking will alter the flavour profile and taste (Klee, 2010).

Conversely, work completed by Thappa et al. (2003) investigating the variation of aroma compounds in celery seed and leaf oil, particularly focused on reducing the limonene and increasing the phthalide content to improve the flavour quality for consumption. Although this study concentrated on seed varieties, the success in producing a genetically improved celery expressing a reduced limonene content shows that *A. graveolens* can be modified to exhibit desired properties (Thappa et al., 2003).

Although there have been advances in biotechnology, the celery genome remained unconstructed only until recently, whereby previously, the genome of the carrot was the only member of the Apiaceae family with the genome constructed. Li et al. (2020) reported the genome sequence of *A. graveolens L.* with a total sequence length of 2.21 Gb and 34,277 predicted genes which is larger

than the carrot sequence. The completion of this work allowed Li et al. (2020) to identify significant genes involved in disease resistance and secondary metabolite synthesis and metabolism. Focusing on terpenoid synthase family genes, three developmental stages were monitored using previous transcriptome data to analyse the expression of these terpenoid synthase proteins. During the first two stages of development, these proteins were seen to be expressed at a higher abundance than stage 3, signifying that terpenoid metabolism is involved in the growth and development of celery (Li et al., 2020).

1.6. Abiotic factors and the aroma of celery

It is difficult to predict the flavour profile of a crop at the point of consumption as multiple factors and interactions between the environment and genotype will contribute to any variations that may occur. Although the genotype will determine the capacity of the crop to synthesise the chemical components of the flavour profile, environmental factors play an important role in determining the phenotype (or chemotype). This in turn influences flavour, causing crops of the same variety to develop different secondary metabolite profiles such as polyphenols and volatiles, in different growing environments (Raffo, Sinesio, Moneta, Nardo, Peparaio & Paoletti, 2006). A response to abiotic stress is to synthesise aromatic compounds that protect the crop, which ultimately affects postharvest quality (Yan, Yu, Xu, Gu & Zhu, 2014). This means that edge effects in the field can impact on volatile content. Crop plants grown on the borders of the field may exhibit a different volatile content to individuals of the same cultivar grown in the middle of the field, where there is more protection from pests and unfavourable weather conditions. Short chain aldehydes and alcohols (C6 and C9) are known to be produced by plants in response to wounding occurring during harvest and storage. These compounds are GLVs and are important contributors to the characteristic aroma of celery but also play an important role in the plant defence strategies though intra and interplant volatile signalling. The evidence suggests that once damage has occurred, GLVs form, released and detected by other plants, evoking a defence system in response (Matsui, 2006; Scala, Allmann, Mirabella, Haring, & Schuurink, 2013).

A study carried out by Yan et al. (2014) showed that celery grown in soil in a drier climate, or ‘more stressful’ environment expressed a higher bitterness through increased polyphenols to protect the

crop against abiotic and biotic stresses. Yan et al. (2014) utilised a deep sequencing method to identify how miRNAs interact under heat stress, recognising that, although different varieties of celery have similar morphology, the miRNA population being expressed to withstand biotic and abiotic factors of their surroundings (Yan et al., 2014). Furthermore, the colour of the petiole can be manipulated through placement of planting and white celery can be produced by planting seeds in a shaded area. Here, the crop is away from direct sunlight and thus the production of chlorophyll is inhibited, and the crop remains white in colour (Sowbhagya, 2014).

Exposure to alternative environmental conditions and sequencing the genes expressed will help identify which parts of the genome respond to different environmental stimuli such as soil composition, season, and climate (Stoop & Pharr, 1994). From this, it can be identified which genes expressed are also connected to flavour compounds.

D'Antuono, Neri and Moretti (2002) found that changing the nitrogen levels in the soil can lead to a change in the flavour profile of celery. Using the cultivar Darklet and varying nitrogen concentrations, they found that higher doses of nitrogen led to a higher sedanenolide and lower monoterpene (limonene) content (D'Antuono et al., 2002). Thappa et al. (2003) reported that a high limonene content may lead to an unpalatable celery and a celery exhibiting higher phthalide content can be more desirable. Conversely, the application of nitrogen fertiliser on celery crop was shown to have a negative influence over the volatile composition of the crop, as identified by van Wassenhove, Dirinck, Schamp, and Vulsteke (1990b). Applying organic and mineral nitrogen fertiliser to two different varieties of celery saw a large decrease in the volatile content, particularly in the phthalide compounds.

Furthermore, the influence of irrigation on the chemical composition of the essential oil of *A. graveolens* was investigated by Rożek, Nurzyńska-Wierdak, Sałata, & Gumiela (2016), whereby an increase in a range of monoterpenes (α-pinene, cymene, limonene) can be seen in the petioles. However, a decrease can be seen in compounds such as myrcene, caryophyllene and *(Z)*-β-ocimene. In terms of phthalides, only (*Z)*-ligustilide was identified in the petioles of celery at 0.05% when no irrigation was used but was not identified when irrigation was applied (Rożek et al., 2016).

On the other hand, Khalid & Hussein (2012) investigated the effect of cattle and liquid manures on the essential oil content of celery grown at the Experimental Farm of National Research Centre, Egypt across two seasons. The essential oil was extracted using hydrodistillation and analysed using GC/MS. Overall, statistical differences were observed when using a liquid manure and it was concluded that the use of a combination of liquid and cow manure gave the "best essential oil production". Although an increase in the phthalide content was witnessed, a closer look shows that there was no statistically significant change and in fact there was a decrease in the monoterpene content. An increase in acetate esters including *trans*-pinocarvyl acetate and *cis*-carvyl acetate can be seen, as well as in sesquiterpenes such as β-selinene, β-humulene and β-caryophyllene (Khalid & Hussein, 2012). While there was a positive influence on the essential oil content (%) and yield when using liquid and cow manures, there was minimal influence on the essential oil constituents and the impact these manures had on the flavour profile could be questioned (Kokotkiewicz and Luczkiewicz, 2016).

Finally, the time of harvest would have an influence on the aroma of celery, although it has been shown that this is only minimal. Lund et al. (1973) were able to show seasonal and varietal differences from the oils recovered from celery waste from a packinghouse in Florida, using two varieties and taking waste trimmings and stalks in different seasons (November, April, and July). A slight difference was observed in the composition of the waste trimmings from all cuts; sedanolide and β-selinene, identified as important compounds to the celery odour in this study and exhibited a decrease from 3.09 % and 4.00 % in November to 2.68 % and 3.67 % in April respectively. Limonene was not detected at all in the April harvest. They attributed this difference to the higher proportion of stalks in the waste in April rather than leaf trimmings and concluded that using an oil with a higher leaf content leads to a better quality of oil for flavouring. Varietal differences are more obviously observed, whereby compounds marked as celery-like odour compounds are shown to either be lower or not detected in the second variety used in this study, it can be expected that this variety will have a less "typical" celery odour (Lund et al., 1973).

1.7. Post-harvest environment and the aroma of celery

The flavour of the crop can be influenced post-harvest due to poor harvesting techniques, incorrect handling, or storage conditions. The optimum storage conditions for celery include a temperature of 0 °C with a high relative humidity of 95 % (Malhotra, 2012). This maintains the desired organoleptic properties and appearance qualities over storage, however when the temperature is increased to 10 °C, these desired properties start to change. Viña and Chaves (2003) studied the textural differences and changes in fresh cut celery stored at 0 °C and 10 °C for 27 days. Sampling occurred at day 0, 7, 14, 21 and 27. Firstly, after seven days, strong yellow discolouration of the petioles was witnessed, and texture changes described as a "loss of crispiness" occurred. They further acknowledged the development of "off-odours" when samples were stored at 10 °C for 21 days, accompanied by rot and micro-organism decay. Twenty-one days is not a typical duration for the supply chain and these senescence characteristics would not be experienced by the consumer. Furthermore, this assessment was only completed through visual inspection (Viña & Chaves, 2003). It is likely that these off-odours were produced earlier on in the experiment, but not at a noticeable level to be detected by the human nose until day 21. Without the use of a fully trained nose, this becomes a very subjective method of monitoring organoleptic property changes. Using a GC/MS method would confirm the presence and identification of the off odours that were produced.

Preservation methods such as drying (freeze-drying and convection drying) and their influence on the aroma profile on the essential oil of two cultivars of celery were investigated by Nurzyńska-Wierdak, Gruszeck & Kosior (2018). Using convection drying, a larger number of compounds were retained including limonene and β-selinene, whereas freeze-drying allowed a higher retention of myrcene. The effect of drying on the phthalide content is unclear as they were not identified in either cultivar. Although harvest time and cultivar used had an impact on the essential oil content, they concluded that convection drying allows for a higher yield of essential oil than freeze-drying (Nurzyńska-Wierdak et al., 2018). Overall, freezing has been shown as the optimum preservation method in terms of retaining the volatile constituents of celery essential oil when comparing to fresh celery (Kokotkiewicz & Luczkiewicz, 2016; Rosłon, Osińska, & Gajc-Wolska, 2010; Rosłon, Osińska, & Wajs-Bonikowska, 2013).

It is known that vegetables belonging to the Apiaceae family are capable of synthesising furanocoumarins. These compounds are synthesised from coumarin through the shikimate pathway and become key compounds involved in the synthesis of many polyphenols as well as responsible for phototoxic skin reactions in humans (Christensen, 2018). Growing plants in harsh environments such as extreme UV radiation, dramatic temperature changes and pest attacks (Chaudhary, Ceska, Warrington & Ashwood-Smith, 1985). Furanocoumarins are secondary metabolites present in a limited number of plant families including: Moraceae, Apiaceae and Rutaceae and are involved in plant defence and environmental adaptation (Dugrand-Judek et al., 2015). Chaudhary et al. (1985) identified levels of furocoumarins was at its highest in celery that showed signs of fungal infections after 22 to 29 days of storage. There was a statistically significant increase in the levels of 5-methoxypsoralen, 8-methoxypsoralen and psoralen compared with fresh celery. These furocoumarins are defence compounds with antimicrobial properties, synthesised in response to the biotic stress (Chaudhary et al., 1985).

A review completed by Forney (2008) identified processes during postharvest handling on fresh-cut produce that caused significant flavour loss. Forney identified two kinds of mechanisms that cause flavour loss, the first being metabolic changes due to the synthesis of flavour compounds, including compounds that produce off-odours. Metabolic changes are subject to the crop physiology, which in turn is influenced mainly by environmental factors. The second mechanism is diffusional changes in product flavour, whereby the volatile compounds transfer out of the crop. Where metabolic changes are dependent on the plant physiology, diffusional changes are reliant on the chemical and physical properties of the flavour compound itself. The determination of the flavour of celery post-harvest is dependent on these two mechanisms which in turn, are dependent on the environment in which the crop is kept (Forney, 2008).

1.8. Conclusion

Using the data that has been collated in Table 1.3, showing the aroma compounds in various celery varieties, the aroma profile of celery is complex, consisting of an assortment of compounds ranging from terpenes and phthalides to alcohols and aldehydes. Terpenes and phthalides are most

consistently reported throughout literature, with less emphasis placed upon other compounds such as alcohols, esters, and aldehydes. However, this does not mean the latter are any less significant contributors to the aroma of celery.

Given the vast amount of work that has been already completed, there is rarely a dataset that states the variety of celery used, the season and location in which it was sampled and whether repetitions were completed over multiple time points in multiple sites. Therefore, very few papers provide insight into the aromatic variance that may be attributed to environmental factors, as distinguished to those due to the genetic influence of variety. When the cultivar variety is specified, there is an impact of genetics on aroma, since all sources express different aroma compounds. Providing minimal standardised information such as geographical location of growth and cultivar would help build a bigger and better library to help understand the impact these factors have upon the aroma profile of celery, and we recommend the adoption of MIAPAE standards for flavour and aroma publications on all crops.

Preference of celery flavour by consumers is an area that needs further investigation to help improve the quality of celery that is produced, alongside an understanding of how the postharvest environment further changes the organoleptic profile of the crop as it moves through the supply chain. Furthermore, linking sensory profiling and consumer liking with flavour chemistry is an untouched topic and making this connection will provide information for producers and retailers on how celery quality is perceived and how important sensory attributes, such as flavour and aroma, are to influencing consumer preference. The availability of the celery genome sequence now makes targeted breeding for these biochemically driven traits a realistic possibility for vegetable plant breeders to pursue so that lines can be developed that have distinct flavour profiles.

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CHAPTER 2: Determining the most abundant volatile compounds present in celery using 24 genotypes

2.1. Introduction to chapter

This chapter describes preliminary work that was carried out at the very beginning of the project to identify genotypes that would be used in subsequent experiments. Celery, a culinary vegetable that is regularly used in cuisines, has been analysed numerous times and found to possess a variety of monoterpenes, sesquiterpenes and phthalides. Monoterpenes including limonene, α- and β- pinene and γ-terpinene, sesquiterpenes including β-caryophyllene, α- and β-selinene and finally, phthalides such as 3-n-butylphthalide and sedanenolide are the most reported compounds identified in celery as well as some of the most abundant. Before we investigated the influences of the aroma profile within celery, it was important that we determined the aroma composition of the parental genotypes that would be used throughout the project. Tozer Seeds Ltd selected 30 different genotypes of celery from a range of origins that all displayed different characteristics including resistance to fusarium, self-blanching and strong flavoured (Appendix V). These were blind coded as lines 1-30. It was decided by the sponsors that once analysis was complete, using statistical analysis, ten extremes, five genotypes expressing a significantly high volatile content and five genotypes expressing a significantly low volatile content, would be taken forward for sensory analysis. These ten genotypes will also be subject to genetic crossing in which each genotype will be crossed with another genotype where we can eventually study the maternal and paternal inheritance that occurs within celery. Thirty celery genotypes were originally sown but only 24 of these germinated and successfully grew to commercial maturity. These 24 still possessed a range of qualities and origins and provided a suitable diversity set from which to draw the genotypes that were used for the rest of the project.

The goal of this chapter was to confirm the aroma composition of the 24 genotypes of celery, ensuring that the most reported compounds were identified in these and to identify the genotypes that expressed the statistically highest and lowest relative abundance. We hypothesised that there would be significant differences caused by genotype, thus leading to significant differences in the odour profiling.

2.2. Introduction

Apium graveolens, or celery, is a green leafy vegetable with long fibrous petioles that are used regularly in cooking for soups, stocks and sauces as well as consumed raw in salads. The aroma composition has been studied by a plethora of authors as shown in Chapter 1, whereby Table 3 collates compounds identified in previous studies and compares the variation in percentage composition within celery. The most reported compounds that comprise the aroma profile are monoterpenes, that contribute fresh, citrus and earthy odours, sesquiterpenes contributing woody, floral and pine odours (Turner, Lignou, Gawthrop & Wagstaff, 2021a) and phthalides which have been identified as the characteristic aroma compounds of celery (Kurobayashi, Kouno, Fujita, Morimitsu & Kubota, 2006). These compounds, predominately sedanenolide, 3-butylphthalide and neocnidilide possess strong odours with odour characteristics including "celery" and "herbal" (Macleod & Ames, 1989). All these volatile compounds contribute towards the distinct flavour profile constitute celery.

Popular in its consumption and grown globally, the depth of research into celery and its volatile contents is surprisingly low. Although many studies have identified the most abundant constituents of celery, there has been very few that investigate the factors that influence these and the impact that compositional changes will have upon the sensory characteristics. D'Antuono, Neri and Moretti (2002) investigated the application of various nitrogen levels on the essential oil of celery waste trimmings whereby an increase in nitrogen led to a decrease in limonene and other low boiling monoterpenes yet an increase of phthalides was observed, leading to improved flavour quality. Conversely, van Wassenhove, Dirinck, Schamp and Vulsteke (1990) observed a decrease in both terpene and phthalide content when organic and/or inorganic fertilizer was applied to two celery cultivars. Although both papers discussed losses and/or gains in flavour quality; without the completion of sensory analysis or consumer acceptance trials, the decline or improvement of flavour quality and whether this is acceptable to the consumer cannot be stated nor examined. Alternatively, Raffo, Sinesio, Moneta, Nardo, Peparaio and Paoletti (2006) completed sensory profiling using a trained panel and descriptive analysis to assess the internal quality of fresh and cold stored celery petioles, identifying significant differences in appearance, mouthfeel, flavour, and taste attributes in four different celery cultivars which corresponded to the significant differences observed in the chemical profile.

As identified in chapter 1, the data already available presenting the aroma composition of celery expresses a clear variation due to cultivar, season, and geographical location. Differences in geographical location would also present further variation due to differences in agronomy, water availability and water and soil composition. This study aims to identify the compounds that are of highest abundance in celery and examine the variation between the 24 genotypes from a single trial. From here, ten genotypes that expressed the highest and lowest relative abundance of commonly reported compounds in celery were taken forward and presented to the trained sensory panel where the odour profile of the freeze-dried material of these extreme genotypes will be assessed. From this information, the ten genotypes were further reduced to eight genotypes which were used throughout the wider project. These eight genotypes, representing the "extremes' of the original 24 were grown in both Spanish and UK locations between the years 2017 and 2021 to investigate the aforementioned influences.

2.3. Materials and Methods

2.3.1. Celery material and MIAPAE standard

2.3.1.1. Sample information

The 24 parental genotypes used in this experiment were chosen by the sponsors of the project, Tozer Seeds Ltd, due to differences in internal and external characteristics, genetic origin as well as some being parents of commercial hybrids. The genotypes, their origins and their main attributes are listed in Appendix V. Prior to GC/MS analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis.

2.3.1.2. Timing, Location and Environment

Celery seed (*Apium graveolens*) of 24 parental genotypes supplied by Tozer Seeds Ltd (Cobham, United Kingdom) was grown in commercial conditions and harvested in Cambridgeshire (United Kingdom) by G's Fresh Ltd (Ely, United Kingdom 52°21'12.9"N 0°17'15.6"E) during September 2017.

The UK site was on sandy loam soils with naturally high groundwater and a peaty surface. Trials were grown alongside commercial celery products and subject to commercial conditions including application of agronomic techniques, fertilizer, and irrigation as commercial celery. 20 – 25 mm of overhead irrigation was used, and standard commercial fertiliser, pest and disease control regimes were applied. Seeds were sown in late April and transplanted in early June for harvest early September. The average daily air temperature was 16.6 °C with an average daily rainfall of 0.1 mm and relative humidity 86.7 %. Prior to harvest, the celery was subject to regular in-field assessment to ensure standards for commercial quality were met, including visual and taste tests. These celeries were harvested within a close timeframe of the commercial produce also being grown in the field, acting as an indicator for commercial maturity.

2.3.1.3. Raw material collection, processing, and storage

The celery was grown in three randomised blocks in the centre of the field to reduce any influence from edge effects at a density of 10 plants m^{-2} and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles and sealed in labelled bags for transportation to the University of Reading (United Kingdom). Samples were immediately frozen at -80 °C for one week and subsequently freeze-dried for five days. Samples were then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ) and stored in an airtight container for a maximum of two weeks before analysis with gas chromatography/mass spectrometry (GC/MS).

2.3.2. Chemicals Reagents

For GC/MS analysis, calcium chloride, propyl propanoate and the alkane standard C6-C25 (100 µg/mL) in diethyl ether were obtained from Merck (Poole, UK).

2.3.3. Solid Phase Microextraction (SPME) Followed by GC/MS

The celery sample (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and 50 µl of 100 mg/L propyl propanoate (internal standard) then filled to 5 mL using HPLC-grade water in a 15 mL SPME vial fitted with a screw cap. Samples were analysed by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m 250 µm 0.25 µm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of the injector, interface and detector was 250 °C and the sample injection mode was splitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions.

2.3.4. Odour profiling of dried samples

Sensory evaluation was carried out using quantitative descriptive analysis (QDA^{TM}) to determine the odour characteristics of 11 dried celery samples, and the characteristics were estimated quantitatively. The trained sensory panel at the Sensory Science Centre (University of Reading, n=12; 11 female and 1 male) was used to develop a consensus vocabulary to describe the odour characteristics of 11 celery genotypes. During the development of the sensory profile, the panellists were asked to

describe the appearance and odour of the samples to produce as many descriptive terms as seemed appropriate. References were used to help confirm the characteristics of certain attributes including dried fruit (dried apricots and raisins) and cooked celery (boiled). The terms were discussed by the panellists as a group, with the help of the panel leader and this led to a consensus of nine attributes for assessment. Celery powder (5 g) was presented to the panel in glass vials for assessment according to Turner et al. (2021b).

2.3.5. Statistical analysis

The approximate abundance relative to the internal standard was calculated using the peak area data collected by SPME GC/MS analysis and semi-quantitative data for each compound identified in the SPME GC/MS analysis were analysed by both one-way analysis of variance (ANOVA) and principal component analysis (PCA) using Spearman's Correlation on XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($P<0.05$) between the celery genotypes. This data is shown in Table 1. SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out ANOVA of sensory panel data. The means from sensory data were taken over two sessions for all assessors and correlated with the relative abundance means from the instrumental data via PCA using XLSTAT.

2.4. Results and discussion

2.4.1. Using SPME GCMS, significant differences in the relative abundance between 24 genotypes were identified

In total, 37 volatile compounds were detected in the headspace of the 24 celery genotypes including 19 monoterpenes, ten sesquiterpenes, five phthalides, two monoterpenoid alcohols and one aldehyde (Table 2.1). Quantitative differences were observed between the 24 genotypes and one-way ANOVA revealed significant differences between genotypes. However, 20 compounds expressed no significant difference between genotypes including α-thujene, camphene, γ terpinene, (E)-3-butylphthalidene phthalide and sedanenolide.

Monoterpenes were identified with the highest relative abundance within the aroma profile of all celery genotypes, with limonene expressed as the most abundant compound within celery (Table 2.1). Similar findings were identified in literature, with Orav, Kailas and Jegorova (2003) identifying limonene to comprise up to 62.4 % of the aroma profile of Estonian grown dried celery leaves. The monoterpene compounds identified by Orav, Kailas and Jegorova were also identified in the current study including β-pinene, myrcene and γ-terpinene. Genotypes 19, 12 and 6 were determined to contain the highest relative abundance of limonene of 143, 128 and 123 mg/L respectively, whereas genotypes 18, 22 and 25 expressed the lowest relative abundance of limonene of 21, 37 and 39 mg/L, respectively. Similarly to limonene, γ-terpinene displayed high relative abundance in genotypes 2, 6 and 15 (87, 56 and 43 mg/L, respectively) with odour characteristics including woody, lemon/lime-like and herbal (Turner et al, 2021a).

A similar pattern was observed within sesquiterpenes, whereby genotype 12 expressed high abundance of β-caryophyllene and β-selinene, both compounds commonly identified in celery within literature and expressed the highest relative abundance across all genotypes for sesquiterpene compounds (Table 2.1). The latter compound was identified to possess a celery-like odour. Lund, Wagner, and Bryan (1973) examined the essential oil of celery waste recovered from a packing house and through odour evaluation of components, they determined β-selinene to possess a celery-like odour and to be a contributor to celery-like quality. Lund, Wagner, and Bryan also identified caryophyllene and humulene, sesquiterpenes that were observed in this study. Macleod and Ames (1989) determined the volatile components of celery and celeriac using GC, GCMS and GC-odour port assessment and they identified sesquiterpenes to comprise 3 % of the total volatile aroma of celery with caryophyllene and β-selinene accounting the highest proportion, 1.2 and 1 % of the aroma profile, respectively. The odour characteristics of β-caryophyllene, α-humulene and α-selinene were not reported, however, β-selinene was determined to express a fragrant odour in celery.

1632 **Table 2.1**: The relative abundance of volatile compounds identified in the headspace of 24 parental genotypes of celery

				Relative abundance (mg/L)[c]																							
Code	Compound	LRI[a]	ID[b]	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	22	23	25	26	31	p-Value[d]
	Aldehydes																											
AL1	*m*-tolualdehyde	1083	B[1]	0.76± 0.05	0.16± 0.23	0.58± 0.78	0.29± 0.11	0.52± 0.73	0.55± 0.78	0.06± 0.09	nd	0.64± 0.09	nd	1.7± 2.0	0.47± 0.16	nd	nd	nd	0.10± 0.14	nd	nd	0.45± 0.45	0.48± 1.6	0.77± 1.1	0.66± 0.91	nd	nd	ns
	Monoterpenes																											
M1	α-thujene	933	B[2]	0.44± 0.39	0.37± 0.24	0.44± 0.12	0.25± 0.14	0.63± 0.20	0.92± 0.40	0.39± 0.03	0.24± 0.12	0.31± 0.17	0.48± 0.10	3.6± 0.78	0.24± 0.09	0.42± 0.16	0.40± 0.21	0.26± 0.03	0.45± 0.28	0.16± 0.04	0.76± 0.30	0.25± 0.05	0.18± 0.05	0.62± 0.18	0.39± 0.17	0.37± 0.19	0.53± 0.20	ns
M2	α-pinene	942	A	1.9± 1.4 abcd	1.8± 0.83 abcd	1.8± 0.38 abcd	1.2± 0.39 ab	3.7± 0.75 cd	4.0± 1.3 d	1.5± 0.12 abc	1.1± 0.44 a	1.1± 0.41 ab	2.0± 0.28 abcd	1.9± 0.19 abcd	1.1± 0.20 a	1.7± 0.09 abc	1.7± 0.61 abc	1.3± 0.36 ab	1.6± 0.36 abc	0.46± 0.10 a	3.3± 0.72 bcd	1.3± 0.17 ab	0.53± 0.14 a	1.7± 0.31 abc	0.79± 0.24 a	1.1± 0.42 a	2.4± 0.72 abcd	***
M3	camphene	957	A	0.93± 0.62	0.76± 0.23	1.0± 0.14	0.60± 0.15	1.3± 0.21	1.3± 0.32	0.79± 0.06	0.60± 0.15	0.69± 0.25	4.1± 4.4	0.96± 0.10	0.73± 0.07	0.91± 0.18	0.59± 0.42	0.67± 0.15	0.95± 0.21	0.55± 0.04	1.4± 0.13	0.76± 0.15	0.51± 0.04	1.0± 0.17	0.67± 0.23	0.60± 0.19	1.1± 0.23	ns
M4	sabinene	980	A	0.44± 0.42 a	0.19± 0.20 a	0.33± 0.10 a	0.18± 0.15 a	0.76± 0.36 a	0.94± 0.47 a	0.27± 0.12 a	0.19± 0.08 a	0.25± 0.19 a	0.49± 0.18 a	0.82± 0.27 a	0.17± 0.12 a	0.27± 0.20 a	0.36± 0.18 a	0.19± 0.03 a	0.34± 0.09 a	0.13± 0.10 a	0.74± 0.37 a	0.21± 0.17 a	0.23± 0.21 a	0.41± 0.20 a	0.21± 0.10 a	0.29± 0.20 a	0.47± 0.20 a	*
M5	β-pinene	988	A	5.2± 3.9 a	10± 3.1 ab	1.3± 0.22 a	7.2± 3.4 ab	16± 1.2 ab	18± 7.7 ab	9.5± 1.8 ab	6.9± 2.7 ab	5.8± 2.7 a	17± 4.7 ab	14± 3.9 ab	3.1± 0.71 a	8.3± 2.1 ab	7.5± 3.5 ab	8.0± 0.95 ab	10± 3.5 ab	1.8± 0.56 a	24± 9.2 b	5.8± 3.5 a	3.6± 1.4 a	4.4± 1.1 a	6.8± 2.9 ab	7.3± 4.0 ab	13± 5.5 ab	**
M6	myrcene	990	A	2.7± 1.7 a	2.3± 0.91 a	2.7± 0.52 a	1.9± 0.73 a	7.4± 2.5 ab	7.1± 2.7 ab	2.3± 0.23 a	2.5± 0.61 a	1.6± 0.59 a	4.1± 0.89 a	15± 3.3 b	1.9± 0.62 a	1.9± 0.39 a	1.6± 1.3 a	2.3± 0.53 a	2.7± 0.75 a	1.1± 0.28 a	8.1± 3.7 ab	1.8± 0.25 a	0.93± 0.27 a	25± 8.0 c	1.3± 0.38 a	2.6± 1.2 a	3.4± 1.6 a	***
M7	δ-2-carene	1004	B[2]	0.06± 0.09	nd	0.04± 0.06	0.03± 0.04	0.10± 0.07	0.13± 0.09	nd	0.02± 0.02	0.02± 0.03	0.05± 0.06	0.08± 0.11	nd	0.02± 0.03	0.06± 0.04	0.02± 0.03	0.04± 0.06	nd	0.06± 0.08	nd	nd	0.04± 0.05	0.03± 0.04	0.04± 0.05	0.07± 0.06	ns
M8	δ-3-carene	1020	A	0.86± 0.90 a	0.45± 0.23 a	1.1± 0.20	0.52± 0.18 a	1.1± 0.25 a	1.5± 0.51 a	0.47± 0.13 a	0.56± 0.10 a	0.42± 0.06 a	1.0± 0.24 a	1.3± 0.41 a	0.59± 0.25 a	0.74± 0.28 a	0.58± 0.41 a	0.58± 0.17 a	0.50± 0.33 a	0.45± 0.04 a	1.5± 0.49 a	0.62± 0.05 a	0.41± 0.04 a	0.91± 0.05 a	0.63± 0.16 a	0.90± 0.23 a	1.0± 0.26 a	**
M9	o-cymene	1030	A	9.6± 4.8 abc	9.4± 4.4 abc	14± 3.7 abcd	6.8± 1.8 ab	16± 3.4 abcd	23± 3.6 d	7.4± 1.1 abc	7.9± 3.1 abc	7.7± 1.9 abc	8.2± 0.95 abc	19± 1.7 cd	7.2± 1.4 abc	10± 0.62 abcd	7.9± 2.8 abc	6.1± 1.3 ab	9.1± 0.46 abc	3.4± 0.10 a	18± 8.6 bcd	6.1± 0.71 ab	3.4± 0.52 a	9.1± 2.0 abc	4.7± 0.64 a	10± 5.2 abcd	12± 5.4 abcd	***
M10	limonene	1035	A	67± 11 abc	55± 15 abc	86± 9.1 abc	61± 17 abc	113± 26 bc	124± 32 bc	67± 7.7 abc	90± 11 abc	47± 17 ab	103± 27 abc	128± 17 bc	52± 16 ab	57± 13 abcd	80± 15 abc	69± 4.7 abc	85± 21 abc	21± 5.0 a	143± 35 c	58± 15 abc	38± 13 ab	96± 23 abc	40± 14 ab	71± 23 abc	79± 28 abc	***
M11	(E)-β-ocimene	1049	A	1.2± 0.39 ab	0.42± 0.41 ab	1.3± 0.61 ab	0.87± 0.49 ab	1.8± 0.87 ab	2.7± 1.7 b	0.53± 0.13 ab	0.75± 0.23 ab	0.30± 0.21 ab	0.32± 0.23 ab	2.2± 0.81 ab	0.63± 0.31 ab	0.62± 0.19 ab	0.48± 0.25 ab	0.47± 0.05 ab	1.0± 0.50 ab	0.20± 0.07 a	1.9± 1.0 ab	1.3± 0.52 ab	0.78± 0.36 ab	0.66± 0.22 ab	0.29± 0.08 ab	0.47± 0.37 ab	0.62± 0.26 ab	*
M12	γ-terpinene	1065	A	19± 1.2	88± 11	21± 3.9	12± 5.6	12± 8.6	57± 24	13± 2.1	11± 2.9	13± 5.1	25± 8.4	41± 10	12± 5.0	17± 5.3	44± 2.4	12± 2.6	17± 7.1	5.2± 1.3	40± 20	9.6± 2.3	5.8± 2.5	20± 6.3	11± 3.7	17± 7.6	25± 11	ns
M13	terpinolene	1096	A	0.76± 0.72	0.88± 0.23	1.1± 0.26	0.69± 0.41	1.4 ±0.33	1.5± 1.0	0.70± 0.05	0.96± 0.24	0.52± 0.19	0.95± 0.35	1.2± 0.32	0.68± 0.52	0.76± 0.14	0.82± 0.40	0.66± 0.11	0.84± 0.15	0.31± 0.11	1.6± 0.82	0.77± 0.20	0.36± 0.13	0.90± 0.23	0.41± 0.91	0.71± 0.14	0.46± 0.26	ns
M14	allo-Ocimene	1132	B[3]	1.8± 1.4 abcd	0.72± 0.32 ab	2.0± 0.43 abcd	1.2± 0.48 abcd	3.9± 1.5 bcde	5.5± 2.2 e	0.91± 0.11 abcd	1.1± 0.32 abcd	0.43± 0.15 ab	0.11± 0.08 a	4.3± 1.0 cde	0.85± 0.31 abc	0.84± 0.18 abc	0.77± 0.37 ab	0.85± 0.25 abcd	1.9± 0.81 abcd	0.26± 0.04 a	4.4± 2.5 de	2.5± 0.58 abcde	1.2± 0.80 abcd	0.65± 0.16 ab	0.36± 0.14 a	0.99± 0.46 abcd	0.92± 0.41 abcd	***
M15	neo-allo ocimene	1134	B[4]	0.20± 0.22	0.07± 0.06	0.18± 0.14	0.12± 0.09	0.37± 0.28	0.48± 0.34	0.11± 0.10	0.10± 0.08	0.04± 0.04	nd	0.35± 0.26	0.08± 0.06	0.08± 0.06	0.08± 0.06	0.05± 0.03	0.16± 0.13	nd	0.39± 0.28	0.26± 0.18	0.14± 0.10	0.07± 0.05	0.04± 0.03	0.09± 0.07	0.09± 0.07	ns
M16	p-menthatriene 1,3,8	1136	B[5]	0.82± 0.15	0.57± 0.06	0.79± 0.77	0.50± 0.51	0.76± 0.82	1.4± 1.5	0.33± 0.36	0.30± 0.27	0.05± 0.04	0.01± 0.02	1.2± 1.1	0.43± 0.42	0.37± 0.36	0.40± 0.49	0.35± 0.34	0.68± 0.77	0.02± 0.02	0.92± 1.2	0.62± 0.65	0.46± 0.50	0.34± 0.42	0.22± 0.29	0.26± 0.26	0.15± 0.13	ns
M17	pentylcyclohexa1, 3, diene	1166	B[6]	1.8± 0.94	1.3± 0.74	1.5± 0.72	1.5± 0.55	2.0± 0.29	2.8± 0.80	1.1± 0.10	1.0± 0.16	1.4± 0.46	2.6± 1.7	2.5± 0.13	1.4± 0.74	1.5± 0.44	2.0± 1.3	1.3± 0.63	1.8± 0.64	0.14± 0.05	1.6± 1.1	1.7± 0.21	1.2±0 .39	0.98± 0.32	0.74± 0.25	1.1± 0.28	1.9± 0.92	ns
M18	pinocarvone	1166	B[6]	0.43± 0.15	0.35± 0.21	0.44± 0.28	0.42± 0.41	1.6± 1.2	1.2± 1.1	0.55± 0.48	0.50± 0.12	0.83± 0.98	1.7± 2.1	0.90± 0.56	0.40± 0.44	0.48± 0.42	1.3± 0.61	0.42± 0.38	0.63± 0.56	0.19± 0.10	2.8± 0.52	0.69± 0.76	0.13± 0.07	0.61± 0.36	0.38± 0.35	0.72± 0.75	2.0± 2.6	ns
M19	L-carvone	1050	A	7.3± 5.0	6.0± 1.9	6.7± 1.8	6.0± 2.5	8.7± 3.8	7.4± 2.1	5.0± 0.33	4.6± 1.2	6.8± 1.7	10± 3.9	9.4± 2.1	5.5± 1.6	5.9± 1.5	9.6± 4.1	6.5± 0.95	6.3± 2.3	3.9± 2.7	12± 4.6	4.6± 0.69	4.9±1 .6	6.5± 1.4	4.3± 0.98	7.3± 2.6	7.7± 2.6	ns
	Monoterpenoid alcohols																											

MA1	terpinen-4-ol	1185	A	0.22±0.31	0.03±0.05	0.11±0.10	nd	0.26±0.31	0.25±0.35	0.25±0.35	0.25±0.18	0.07±0.10	0.01±0.02	0.11±0.16	0.06±0.08	0.09±0.12	0.09±0.13	0.08±0.11	0.07±0.10	0.13±0.12	0.20±0.28	0.13±0.18	0.06±0.08	0.15±0.15	0.09±0.13	0.14±0.20	0.13±0.18	ns
MA2	carvacrol	1317	A	0.11±0.08	0.23±0.08	0.13±0.02	0.23±0.14	0.16±0.15	0.17±0.15	0.16±0.05	0.56±0.31	0.09±0.02	0.03±0.05	0.32±0.14	0.12±0.09	0.17±0.05	0.09±0.03	0.05±0.04	0.09±0.08	0.03±0.02	0.12±0.11	0.26±0.16	0.06±0.04	0.05±0.03	0.04±0.03	0.14±0.05	0.11±0.01	ns
	Sesquiterpenes																									
S1	cyclosativene	1356	B[7]	0.02±0.03	nd	0.05±0.04	0.02±0.03	0.24±0.22	0.06±0.04	0.06±0.05	0.10±0.08	0.04±0.03	0.13±0.09	0.14±0.15	0.08±0.09	0.08±0.06	0.10±0.07	0.16±0.12	0.18±0.12	0.07±0.05	0.36±0.30	0.07±0.08	0.11±0.08	0.03±0.04	0.15±0.11	0.07±0.05	0.02±0.02	ns
S2	α-ylangene	1385	B[5]	nd	0.17±0.24	0.09±0.13	0.15±0.21	0.16±0.22	0.13±0.18	0.07±0.10	0.15±0.21	0.05±0.07	0.06±0.09	0.19±0.27	0.06±0.08	0.01±0.01	0.04±0.05	0.12±0.17	0.08±0.12	0.02±0.03	0.11±0.16	0.11±0.15	0.06±0.08	0.02±0.03	0.06±0.08	0.10±0.14	0.07±0.10	ns
S3	α-copaene	1392	A	nd[a]	0.04±0.06[a]	0.03±0.03[a]	0.03±0.03[c]	0.76±0.12	0.05±0.03[a]	0.04±0.02[a]	0.36±0.12 abcd	0.08±0.03 ab	0.53±0.04 cde	0.09±0.03 ab	0.03±0.03[a]	0.04±0.02[a]	0.07±0.05[a]	0.52±0.24 cde	0.57±0.06 de	0.11±0.02 ab	1.2±0.31[f]	0.17±0.12 abc	0.32±0.06 abcd	0.02±0.01[a]	0.46±0.07 bcde	0.12±0.05 ab	0.04±0.01[a]	***
S4	β-caryophyllene	1444	A	0.51±0.27 ab	0.76±0.53 abc	1.3±0.45 abc	0.35±0.13[a]	1.3±0.32 abc	1.2±0.21 abc	0.55±0.02 ab	1.7±0.63 bc	0.29±0.01[a]	1.3±0.19 abc	1.6±0.76 bc	0.62±0.18 ab	0.37±0.09[a]	0.86±0.01 abc	0.71±0.27 abc	1.3±0.33 abc	0.33±0.06[a]	1.9±0.41[c]	0.26±0.07[a]	0.26±0.08[a]	1.0±0.26 abc	0.32±0.06[a]	1.2±0.39 abc	0.68±0.14 abc	***
S5	aromadendrene	1462	A	0.02±0.03	0.06±0.08	0.05±0.07	0.03±0.04	0.09±0.12	0.07±0.10	0.04±0.05	0.10±0.14	0.03±0.02	0.07±0.10	0.07±0.10	0.03±0.03	0.03±0.05	0.05±0.07	0.07±0.09	0.05±0.07	0.02±0.02	0.06±0.08	0.02±0.03	0.03±0.04	0.02±0.03	0.02±0.03	0.05±0.06	0.02±0.03	ns
S6	α-humulene	1475	A	0.08±0.07[a]	0.31±0.29 ab	0.14±0.08 ab	0.11±0.06 ab	0.38±0.16 ab	0.33±0.98 ab	0.20±0.05 ab	0.38±0.18 ab	0.10±0.03[a]	0.47±0.23 ab	0.62±0.40[b]	0.11±0.04 ab	0.15±0.04 ab	0.23±0.04 ab	0.19±0.16 ab	0.27±0.04 ab	0.08±0.01[a]	0.31±0.02 ab	0.26±0.08 ab	0.08±0.02[a]	0.13±0.02 ab	0.06±0.01[a]	0.19±0.05 ab	0.20±0.03 ab	*
S7	β-selinene	1507	B[8]	0.52±0.48	4.9±0.66	1.0±0.48	0.49±0.21	1.0±0.30	2.0±0.31	0.55±0.20	1.3±0.70	0.71±0.56	0.52±0.27	2.6±1.5	1.5±1.1	1.0±0.88	0.51±0.16	0.81±0.45	1.5±0.22	0.16±0.03	1.8±0.17	1.5±1.4	0.44±0.14	0.60±0.16	0.42±0.12	0.92±0.35	0.55±0.13	ns
S8	valencene	1515	A	1.1±0.28[a]	1.8±1.3[a]	1.6±1.3[a]	0.51±0.36[a]	0.32±0.25[a]	0.98±0.29[a]	1.1±0.06[a]	0.17±0.12[a]	1.4±1.1[a]	0.49±0.16[a]	5.6±1.8[a]	2.5±2.0[ab]	1.8±1.4[a]	0.45±0.13[a]	0.55±0.11[a]	0.35±0.12[a]	0.38±0.17[a]	1.1±0.12[a]	1.8±1.3[a]	0.37±0.12[a]	1.2±0.13[a]	0.40±0.13[a]	0.39±0.02[a]	2.3±0.54 ab	***
S9	α-selinene	1518	B[9]	0.36±0.30[a]	0.20±0.41[a]	0.13±0.05[a]	0.06±0.05[a]	0.27±0.08[a]	0.19±0.07[a]	0.08±0.07[a]	0.28±0.18[a]	0.07±0.02[a]	0.25±0.08[a]	0.45±0.25[a]	0.15±0.06[a]	0.08±0.03[a]	0.18±0.05[a]	0.25±0.13[a]	0.35±0.05[a]	0.08±0.06[a]	0.44±0.17[a]	0.11±0.04[a]	0.14±0.05[a]	0.12±0.03[a]	0.15±0.04[a]	0.18±0.07[a]	0.12±0.03[a]	*
S10	kessane	1555	B[6]	0.66±0.26[a]	3.8±0.24[b]	1.2±0.59 ab	0.32±0.09[a]	0.12±0.09[a]	1.3±0.35 ab	0.64±0.10[a]	0.17±0.02[a]	1.1±0.48 ab	0.16±0.08[a]	3.7±1.1[b]	2.2±0.72 ab	1.6±0.44 ab	0.13±0.09[a]	0.06±0.09[a]	0.14±0.03[a]	0.07±0.06[a]	0.76±0.04[a]	1.7±0.29 ab	0.10±0.07[a]	0.62±0.10[a]	0.05±0.03[a]	0.05±0.08[a]	1.5±0.45 ab	***
	Phthalides																											
P1	3-n-butylphthalide	1676	B[6]	3.1±1.6[a]	5.0±3.7[ab]	3.0±0.83[a]	2.2±0.46[a]	3.2±1.1[a]	5.1±0.55 ab	2.8±0.44[a]	5.1±1.4[ab]	2.5±0.23[a]	3.3±0.49[a]	7.3±0.52[b]	4.8±1.3[ab]	2.6±0.28[a]	3.1±0.84[a]	2.4±0.53[a]	4.5±0.19 ab	2.2±0.64[a]	4.3±0.76 ab	3.3±0.23[a]	2.5±0.60[a]	2.6±0.32[a]	1.3±0.22[a]	2.3±0.24[a]	2.4±0.34[a]	***
P2	(E)-3-butylidenephthalide	1684	B[6]	0.22±0.32	0.43±0.41	0.36±0.09	0.21±0.04	0.30±0.05	0.38±0.03	0.23±0.01	0.41±0.02	0.17±0.05	0.27±0.02	0.39±0.04	0.26±0.09	0.18±0.03	0.18±0.02	0.16±0.04	0.24±0.02	0.09±0.01	0.24±0.04	0.16±0.03	0.12±0.01	0.15±0.03	0.08±0.01	0.17±0.06	0.14±0.05	ns
P3	sedanenolide	1746	B[10]	5.7±3.0	8.7±5.9	4.2±1.1	3.5±1.3	5.3±1.9	8.2±0.66	4.5±1.1	7.9±3.1	3.0±0.77	5.7±0.84	15±1.9	6.3±2.2	12±3.8	4.5±1.5	3.4±0.97	6.6±1.4	6.3±4.8	6.3±0.85	3.5±2.3	3.4±1.3	4.3±0.58	2.0±0.43	3.7±0.95	3.8±0.92	ns
P4	neocnidilide	1754	B[6]	0.14±0.09[a]	0.32±0.20 ab	0.46±0.29 abc	0.20±0.06 ab	0.40±0.12 ab	0.43±0.02 ab	0.58±0.11 abc	0.63±0.19 bc	0.15±0.03[a]	0.19±0.04 ab	0.18±0.04 ab	0.33±0.10 ab	0.17±0.02[a]	0.15±0.03[a]	0.17±0.03[a]	0.40±0.05 ab	0.46±0.11 abc	0.51±0.10 abc	0.47±0.01 abc	0.91±0.30[c]	0.31±0.06 ab	0.20±0.04 ab	0.22±0.03 ab	0.18±0.03[a]	***
P5	(E)-ligustilide	1762	B[10]	0.17±0.15	0.42±0.43	0.51±0.56	0.17±0.12	0.13±0.05	0.26±0.08	0.21±0.02	0.22±0.06	0.17±0.06	0.27±0.09	0.30±0.07	0.32±0.16	0.14±0.02	0.16±0.03	0.12±0.03	0.17±0.06	0.08±0.01	0.25±0.04	0.18±0.05	0.11±0.03	0.14±0.03	0.08±0.01	0.16±0.09	0.19±0.11	ns

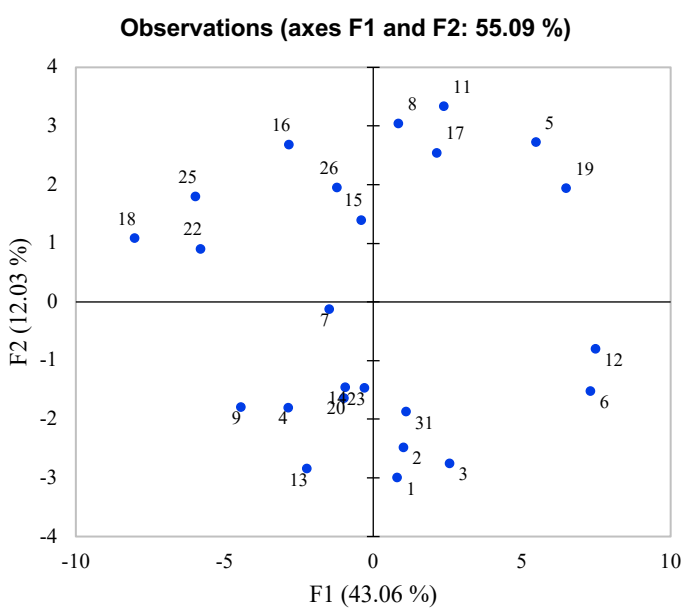
[a] Linear retention index on a HP-5MS column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited [1]Radulovic et al. (2010); [2]Adams et al. (2005); [3]Halik et al. (2006); [4]Su et al. (2006); [5]Bylaite & Meyer (2006); [6]Andriamaharavo (2014); [7]Custer (2009); [8]Yu et al. (2007); [9]Zeng et al. (2007); [10]Turner et al. (2021b). [c] approximate abundance relative to the internal standard to the internal standard, propyl propanoate; means labelled with letters are significantly different ($p < 0.05$) according to the one-way ANOVA; nd, not detected. [d] Probability, obtained by ANOVA; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

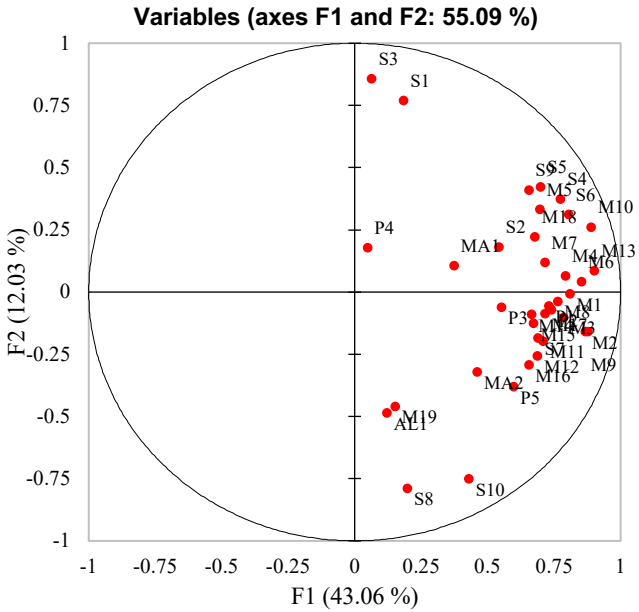
Additionally, phthalides have been determined to possess strong celery-like odours and are named as the characteristic aroma compounds of celery, particularly sedanenolide and butylphthalide; both compounds were identified in the present experiment (Table 2.1). Kurobayashi et al. (2006) characterised the aroma compounds extracted by SAFE and analysed through GCMS and AEDA using GC/O in the leaves and stalks of celery. The leaves of celery expressed a much higher concentration of sedanenolide and butylphthalide than the stalks, however when both the leaves and stalks were boiled, the stalks retained their phthalide content, but a significant loss was observed in the boiled celery leaves. Using AEDA, sedanenolide and butylphthalide displayed the highest flavour dilution factor in both leaves and stalks. Genotype 12 and 8 both expressed high relative abundance of sedanenolide (14 and 7.8 mg/L) and butylphthalide (7.2 and 5.0 mg/L) and will most likely be perceived with a strong celery-like odour. Conversely, genotype 25 was identified with the lowest relative abundance of both sedanenolide and butylphthalide, expressing 1.9 and 1.2 mg/L, respectively. Although genotype 18 was identified with a high abundance of sedanenolide (6.2 mg/L), a much lower abundance of butylphthalide was identified, 2.2 mg/L (Table 2.1). Macleod and Ames (1989) determined sedanenolide as the phthalide to comprise the highest proportion of the aroma composition in celery (28 %) followed by cis-sedanolide (5 %) and (z)-ligustilide (3.5 %) and all these compounds were identified to exhibit odour descriptors such as “celery” and “pungent”. Genotype 12 appears to be significantly different to genotypes 18, 22 and 25 throughout volatile analysis, expressing significantly higher abundances across most compounds, particularly when compared to genotype 25. Completing principal component analysis allowed us to visualise these differences and draw associations between genotypes and their metabolic profiles (Figure 2.1).

Principal components one (F1) and two (F2) explained 55.09 % of the total variation within the presented dataset and it can be observed that the first axis separates genotypes 1, 2, 3, 4, 6, 7, 9, 12, 13, 14, 20, 23 and 31 from the others whereas the second axis separates genotypes 1, 2, 3, 5, 6, 8, 11, 12, 17, 19 and 31. The genotypes observed on the right hand side of the plot display a high association with the volatile compounds identified in GCMS (Table 2.1), whereas those displayed on the opposite side of the plot display a much lower association (Figure 2.1). Those genotypes presented on the left side of the biplot would be perceived as less aromatic when compared to those presented on the right side of

the biplot due to their lower relative abundance in aromatic compounds. This confirms genotypes 18, 22 and 25 to be the most different to genotype 12 in terms of the volatile profile and we hypothesise that this will lead to a significant difference in the sensory characteristics of these genotypes. In addition to genotype 12, genotype 6 also expresses a high association with volatile compounds. This is due to the high relative abundance of monoterpenes including α- and β-pinene, limonene, γ-terpinene and β-caryophyllene and butylphthalide. Genotypes 5, 8 and 11 displayed a high abundance with many commonly reported compounds in celery including camphene, limonene, L-carvone and β-caryophyllene. These genotypes also expressed a high abundance in both butylphthalide and sedanenolide. This is reflected within the PCA (Figure 2.1) whereby these genotypes express a close association with these volatile compounds.

Genotypes chosen for odour analysis by the trained panel were selected according to their aroma profile displayed in Table 2.1 and Figure 2.1. Genotypes 12 and 6 expressed a high abundance in all volatile compounds including all phthalide compounds and although not as high as these genotypes, genotypes 5, 8 and 11 were also chosen to be taken forward, representing “high extremes”. On the other hand, genotypes 9, 18, 22 and 25 represented the “low extremes” due to their low abundance and association with volatile compounds. We expected these genotypes to display contrasting sensory profiles, particularly genotype 12 and 25. Genotype 15 was also taken forward for sensory profiling as it fell in between the high and low extremes, displaying neither the highest nor lowest in abundance for volatile content.





AL1	m-tolualdehyde	M19	L-carvone
M1	α-thujene	MA1	terpinen-4-ol
M2	α-pinene	MA2	carvacrol
M3	camphene	S1	cyclosativene
M4	sabinene	S2	α-ylangene
M5	β-pinene	S3	α-copaene
M6	myrcene	S4	β-caryophyllene
M7	δ-3-carene	S5	aromadendrene
M8	δ-2-carene	S6	α-humulene
M9	o-cymene	S7	β-selinene
M10	limonene	S8	valencene
M11	β-(E)-ocimene	S9	α-selinene
M12	γ-terpinene	S10	kessane
M13	terpinolene	P1	3-butylphthalide
M14	allo-ocimene	P2	(E)-3-butylidene phthalide
M15	neo allo-ocimene	P3	sedanenolide
M16	p-1,3,8-menthatriene	P4	neocnidilide
M17	pentylcyclohexa1, 3, diene	P5	(E)-ligustilide
M18	pinocarvone		

(A) **(B)** **(C)**

Figure 2.1. Principal component analysis of 24 celery samples harvested in 2017 UK showing correlations with volatile compounds. (A) Projection of the samples; (B) Distribution of variables; (C) Compound codes as appear in plot (B).

2.4.2. Odour analysis of dried celery material

The odour profile of the ten celery samples was generated by a trained panel who came to the consensus of nine terms for the quantitative assessment of freeze-dried powdered celery, eight of these assessed the odour (Table 2.2). Out of the eight odour attributes that were profiled by the panel, one attribute, brown fruits, was identified to be significantly different to the other odour attributes between samples. The assessment of odour using dried powder is not a powerful method for profiling the aroma of celery, however few significant assessor x sample interactions were identified suggesting that the panellists scored the samples in a consistent manner.

Table 2.2: Quantitative appearance and odour assessment of ten celery powders

	Score [A]										
Attribute	5	6	8	9	11	12	15	18	22	25	P-value [B]
Colour (white to green)	33 [d]	42 [bcd]	38 [cd]	48 [b]	45 [bc]	66 [a]	49 [b]	45 [bc]	60 [a]	6.2 [a]	***
Fresh fennel (low to high)	28	28	29	30	27	27	27	30	27	24	ns
Dusty (low to high)	27	29	28	30	31	32	32	29	29	26	ns
Green (low to high)	23	23	22	20	22	18	24	20	21	15	ns
Cooked celery (low to high)	20	26	25	25	24	28	23	27	27	22	ns
Musty (low to high)	9.8	4.7	12	9.9	8.3	11	10	9.9	10	5.5	ns
Brown fruits (low to high)	3.3 [ab]	3.0 [b]	3.5 [ab]	3.3 [ab]	3.8 [ab]	3.8 [ab]	3.0 [b]	3.8 [ab]	4.1 [ab]	5.1 [a]	*
Sweet (low to high)	22	21	19	23	18	19	18	24	22	24	ns
Paint (low to high)	1.7	0.2	1.7	3.6	1.2	1.0	0.9	1.1	1.8	0.2	ns

[A] Mean score of two replicate samples taken from the trained panel (n=12). Means labelled with letters (a,b,c,d,e) are significantly different ($p < 0.05$) according to the Assessor x Sample interaction; Means not labelled with the same letters

are significantly different ($p < 0.05$). [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. Anchors used in sensory test are mentioned below the attribteu name

Monoterpenes, sesquiterpenes and phthalides were only reported in this study as they are the most frequently reported compounds in the celery literature. By expanding the search for volatile compounds to include compound groups such as aldehydes, alcohols, ketones and esters, a more representative aroma profile of celery was constructed as these undoubtedly contribute to the distinct aroma of celery, especially the green, fruity odour characteristics. Gold and Wilson (1963) identified 38 compounds in the essential oil of celery juice including a range of aldehydes, esters and alcohols and highlighted (Z)-3-hexenyl pyruvate as a characteristic odour compound in celery with odour descriptors including “green” and “vegetative”.

Subsequently, Wilson (1967) focussed on quantitating the alcoholic composition of celery essential oil, identifying the aroma composition to comprise of 10 to 15 % alcohols including hexanol and (Z)-3-hexenol as well as a range of monoterpenoid alcohols such as (E)- and (Z)-2,8-p-menthadiene-1-ol. Although these compounds are not formally considered characteristic compounds of celery, they provide odour characteristics such as green, fresh, and vegetative making them important compounds to the overall aroma and flavour of celery. Furthermore, the use of freeze-dried material for sensory analysis has been shown to be an inappropriate method due to distortions occurring from the state of the material, influencing attributes such as musty and stalky to be used by the panel (Table 2.2). We hypothesise that the main sensory characteristics of celery occur upon consumption where the taste, flavour and mouthfeel attributes can be assessed; these attributes having a higher influence over the perception. Therefore, using fresh material and presenting it to a panel will also allow for a better representative sensory profile of celery to be produced as we can ask the panel to assess the attributes of the petiole of each genotype during scoring.

2.5. Conclusion

From the results presented in this chapter, genotype caused a significant difference in the relative abundance of aroma compounds identified in the headspace of 24 parental genotypes of celery.

Limonene, β-caryophyllene, sedanenolide, myrcene and o-cymene were the most abundant compounds within the 24 genotypes in varying abundances.

Genotypes 5, 6, 8, 11 and 12 also displayed high relative abundance in commonly reported compounds including those that have been labelled as characteristic compounds of celery, phthalides. Genotype 12 expressed the highest relative abundance of most compounds. These five genotypes were identified as “high extremes” from the initial population. Conversely, genotype 25 displayed the lowest relative abundance of the majority of compounds identified and, therefore, we expected to be most different from genotype 12 when presented to a trained sensory panel. Furthermore, genotypes 9, 18 and 22 expressed a low relative abundance across many compounds. Together with genotype 25 these were identified as “low extremes” from the initial population. These two genotype groups represented the high and low extremes according to their volatile content and were taken forward for odour assessment using the trained panel, along with genotype 15 which displayed a volatile content that was neither higher nor lower than the genotypes mentioned above.

Unlike the volatile content, minimal significant differences were observed in the odour profile of the freeze-dried samples with only brown fruits expressing a significant difference between the ten genotypes. We concluded that the use of dried material for odour assessment was not an appropriate method and that once fresh material was used, we expected to identify significant differences in the sensory profile that were explained by the differences in the aroma profile of these celery genotypes.

There is currently limited research to support the influence of genotype over the aroma composition in celery and whether this has a significant influence over the sensory characteristics of celery. Within this preliminary study, we identified that genotype clearly has an impact of the aroma content of celery, however, to achieve accurate sensory data, we must present fresh celery samples to the trained panel. Linking sensory perception with the aroma profile will provide fresh produce growers with a better understanding of the sensory properties of celery and the impact of genotype. Equally, growing the same eight genotypes across different years and geographical locations will expose the crops to varying environments; examining the importance of selecting genotype according to the desired sensory profile based off the environment in which the celery will be grown in.

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CHAPTER 3: Investigating the relationship of genotype and climate conditions on the volatile composition and sensory profile of celery *(Apium graveolens)*

3.1. Introduction to the paper (as published in Foods 2021 volume 10, issue 6, 1335)

Upon completion of the literature review, it was clear that the work already published investigating the aroma profile of celery had gaps in the data provided by authors in the form of growing and experimental information. This meant that any interpretation of data or experiment repetition would be missing information key to accurate interpretation of the results. Therefore, we put forward the Minimum Information for A Plant Aroma Experiment (MIAPAE) that, when followed, will ensure the data that is produced is reproducible and that as many variables as possible are described and/or controlled. With the aim of addressing the issues highlighted in the literature review, we presented the experimental data in this thesis following the MIAPAE standards that were set out in the previous chapter and, although we cannot include the exact cultivar name of the celery genotypes used in this project, we kept to these standards which are outlined in the materials and methods.

The aroma profile of celery has been investigated in multiple experiments previously, however very few research papers have utilised multiple genotypes and grown them in different environments to examine the influence of factors such as temperature, rainfall, and humidity on the aroma composition of celery. Furthermore, none have used a trained sensory panel to determine whether these changes cause a significant change in the sensory characteristics of celery. Using two different harvest years (2018 and 2020), the paper aimed to (1) develop and use a method that identifies the compounds in celery, matching those previously identified in the literature (2) to identify any changes in the aroma composition when eight genotypes were subject to the same agronomic practice, grown in the same field but exposed to different climate conditions each year and (3) to use a trained sensory panel to link any compositional biochemical changes to changes in the sensory profile. We aimed to identify compound groups that respond differently to the changes in growing conditions, potentially as a stress response, and then to determine how this affected the sensory characteristics of celery. The information

collected in this chapter will educate fresh produce growers on the influencers of the celery sensory profile and whether environmental conditions have a positive or negative effect on the crop.

Sections 3.2 – 3.7 were published in Foods 2021 volume 10, issue 6, 1335. (See appendix VI for the pdf version of the manuscript).

3.2 Abstract

Apium graveolens is a biennial crop grown across the globe for its stalks, leaves and seed and is known for its distinct flavour and strong taste. Various extraction methods on fresh and dried celery and its essential oil are reported in the literature examining the aroma profile of this crop and demonstrating that its volatile composition is determined by variables including cultivar, season, geographical location, and agronomic practices. This study investigated the volatile and sensory profile of eight celery genotypes grown over two years (2018 and 2020) in the same location in the UK. Solid-phase-micro-extraction followed by gas chromatography-mass spectrometry were used to determine the volatile compounds present in these genotypes and sensory evaluation using a trained panel to assess the sensory profile of fresh celery. Significant differences ($p < 0.05$) in the volatile composition and sensory profile were observed and influenced by both genotype and harvest year. Two genotypes exhibited similar aroma composition and sensory profile between the years. Celery samples harvested in 2018, which possessed air temperatures that were considerably warmer than in 2020, exhibited higher proportions of sesquiterpenes and phthalides and we hypothesise that the higher proportions were generated as a response to heat stress. Studying the relationship between the genotype and the environment will provide clear information to guide growers in how to consistently produce a higher quality crop.

3.3. Introduction

Celery is a vegetable that belongs to the Apiaceae family which is grown across the globe, consumed regularly and forms part of the “holy trinity” or “Soffritto” in cooking, used raw in salads or with condiments (Rozėk, 2007). The investigation of the aroma and flavour of celery has been studied

using a range of extraction techniques, such as solvent assisted flavour extraction (SAFE) and solid phase microextraction (SPME), combined with instrumental analysis, such as gas chromatography/mass spectrometry (GC/MS) on celery leaf, petiole, and seed. The consensus is that terpenes (monoterpenes and sesquiterpenes) and phthalides make up most compounds present in the flavour profile. Phthalides have been shown to be key contributors to typical celery aroma (3-n butylphthalide, sedanenolide and (E)- ligustilide and (Z)-ligustilide) and possess odour descriptors such as “celery”, “herbal” and “green” (Macleod & Ames, 1989; Kurobayshi, Kouno, Fujita, Morimitsu & Kubota, 2006). The composition of alcohol, aldehyde and ester compounds have been poorly represented in literature. Although they are not characteristic compounds to celery odour, their importance should not be neglected as these compounds contribute to green, fresh and woody notes that are important to the overall celery aroma. Wilson (1967) identified and quantified 13 alcohols in celery essential oil using gas chromatography including n-hexanol, cis-3-hexene-1-ol and dihydrocarveol. Wilson commented on the pleasant aroma of these compounds and concluded that although they are not characteristic compounds of celery, they complete the typical flavour and aroma of celery.

In a recent review by the authors (Turner, Lignou, Gawthrop & Wagstaff, 2021), the complexity of the aroma profile is discussed and the variation within reported datasets caused by differences in cultivar, geographical location of growth, agricultural techniques as well as extraction and analysis techniques are highlighted. To overcome these variances, Turner et al. (2021) recommended the use of Minimum Standards About a Plant Aroma Experiment (MIAPAE), ultimately leading to a repository of data whereby accurate interpretation of results and correct experimental repetition can occur. Importantly, it was demonstrated that the genotype alone does not determine the final flavour outcome, but other factors during preharvest (cultivar, climate, and agronomy) and postharvest (harvest techniques and storage conditions) simultaneously influence the final composition (Turner et al. 2021; Malhorta, 2021). The application of alternative agronomic practices, including varying nitrogen levels in soil, the use of irrigation systems and inorganic/organic fertilisers, as well as growing celery in different geographical regions have all been shown to influence the aroma composition of celery (D’Antuono, Neri & Moretti, 2002; Rozėk, Nurzyńska-Wierdak, Sałata & Gumiela, 2016; Khalid & Hussein, 2012; Shojaei, Ebrahimi & Salimi, 2011; Philippe, Suvarnalatha, Sankar & Suresh, 2002).

Rożek, Nurzynska-Wierda and Kosior (2013) explained the consequences of agricultural techniques on the volatile composition of leaf celery essentials, while van Wassenhove, Dirinck, Schamp and Vulsteke (1990) concluded that the use of fertiliser (organic and/or inorganic) resulted in a decrease in terpene and phthalide content.

Limited research has been conducted on the impact of the environment on the volatile composition of celery, with few studies using the same cultivar over multiple sites and seasons that are compliant to MIAPAE (Turner et al, 2021). Van Wassenhove, Dirinck, Vulsteke and Schamp (1990) investigated the volatile composition of four celery cultivars grown in two seasons (1986 and 1987) on sandy loam fields in Belgium. Although differences in the composition were observed, their focus was not on the variation of composition but more on the validity of their method to identify and separate terpenes and phthalides in celery. Genotypic and seasonal differences were observed in the total terpene and phthalide content of all four cultivars (van Wassenhove et al, 1990). Lund, Wagner, and Bryan (1973) also reported differences in the oil composition of celery (Utah 5270) waste trimmings between November 1972 and July 1973, yet no seasonal significant differences were shown. Conversely to van Wassenhove et al. (1990), a much smaller group of compounds were investigated by Lund et al. (1973) that numbered around 12 compared to the 33 compounds identified by van Wassenhove et al. (1990). This suggests that the harvest year has minimal impact over the volatile composition. Alternatively, Shojaei, Ebrahimi and Salini (2011) showed the impact of the environment on the volatile composition by testing one species of wild celery (*Kelussia odoratissima*) sampled across three different regions of Iran. They identified trans-ligustilide as the main compound from the three locations contributing various percentages 47.31, 37.55 and 33.73. There were also variations in the presence of compounds throughout three ecotypes; the Bazoft ecotype was found to contain fewer compounds than the ecotypes grown in Koohrang and Samsani (Shojaei, Ebrahimi & Salini, 2011).

The aim of this study was to investigate the relationship between genotype and the environment on the volatile composition of eight celery genotypes grown in the UK across two different years (2018 and 2020). In addition, sensory evaluation using a trained panel was used in order to understand how chemical and physiological changes lead to differences in organoleptic perception and used to identify interactions between compound groups and climate. Ultimately, this information would assist breeders

and growers to develop and select cultivars that are optimal for specific growing climates and to produce a consistent quality product.

3.4. Materials and Methods

3.4.1. Celery Material and MIAPAE Standards

3.4.1.1. Sample Information

The eight genotypes used in this study were chosen based on their differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each genotype used in this study, the origins of these parental breeding lines and their images postharvest can be found in the appendix VII.

3.4.1.2. Timing, Location and Environment

The celery seeds (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd. (Cobham, UK) were grown in commercial conditions and harvested in Cambridgeshire (UK) by G's Fresh Ltd. (Ely, UK, 52°21°12.9°N 0°17°15.6° E) during the spring/summer of 2018 and 2020. The celery was grown in a field with commercial celery products and treated by the same agronomic techniques and conditions as commercial celery, including identical fertiliser application and exposure to water. For both years, 20–25 mm of overhead irrigation was used, and standard commercial fertiliser, pest and disease control regimes were applied. In 2018, plugs were transplanted mid-June after growing in the nursery for 22 days and then harvested 91 days later. The average daily air temperature was 18.2 °C with an average soil temperature of 23.8 °C, 0.2 mm of rainfall daily and an average relative humidity of 88.1 %. In 2020, the plugs were transplanted late April after growing in the nursery for 24 days and were harvested 76 days later. The average daily air temperature was 14.3 °C with an average soil temperature of 15.4 °C, 0.05 mm daily rainfall and an average relative humidity of 74.8 %. Prior to the harvest, the celery is tested regularly in-field to ensure standards for commercial quality are met, including visual and taste tests. The celeries were harvested within a close timeframe compared to the commercial produce also being grown in the field.

3.4.1.3. Raw Material Collection, Processing and Storage

The celery was grown in three randomised blocks in the centre of the field to reduce any influence from edge effects at a density of 10 plants m^{-2} and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles and then sealed in labelled bags for transportation to the University of Reading (United Kingdom). Celery samples used for sensory evaluation were refrigerated for one day, while samples for aroma analysis were immediately frozen at 80 °C for one week and subsequently freeze-dried for five days. Samples were then milled into a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ, USA) and then stored in an airtight container for a maximum of two weeks before analysis with gas chromatography-mass spectrometry (GC/MS).

3.4.2. Chemical Reagents

For GC/MS analysis, calcium chloride, propyl propanoate and the alkane standard C6–C25 (100 µg/mL) in diethyl ether were obtained from Merck (Poole, UK).

3.4.3. Solid Phase Microextraction (SPME) Followed by GC/MS

Celery (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and filled to ~5 mL using HPLC-grade water in a 15 mL SPME vial fitted with a screw cap. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA). The SPME fibre stationary phase was composed of 75 µm divinylbenzene/Carboxen™ on polydimethylsiloxane, Supelco (Bellefonte, PA, USA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m 250 µm 0.25 µm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min.

The temperature of the injector, interface and detector was 250 °C and the sample injection mode was splitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions.

3.4.4. Sensory Evaluation of Fresh Celery Samples

Sensory evaluation was carried out using quantitative descriptive analysis (QDA^{TM}) to determine the sensory characteristics of the eight celery samples and the characteristics were estimated quantitatively. The trained sensory panel at the Sensory Science Centre (University of Reading, n = 12; 11 female and 1 male) was used to develop a consensus vocabulary to describe the sensory characteristics of the eight celery genotypes. During the development of the sensory profile, the panelists were asked to describe the appearance, odour, taste, flavour, mouthfeel, and aftereffects of the samples in order to produce as many descriptive terms as seemed appropriate. References were used to help confirm the characteristics of certain attributes including fresh and dried fennel, salad rocket, flat leaf parsley and fresh coriander. The terms were discussed by the panelists as a group, with the help of the panel leader, and this led to a consensus of 22 and 24 attributes for the 2018 and 2020 harvest, respectively. The sensory assessment of the samples was carried out in a temperature-controlled room (22 °C) under artificial daylight and in isolated booths, each equipped with an iPad. Celery petioles were chosen to be as uniform as possible. The first outer petioles were removed and discarded. The next ring of petioles was used, and these were washed with filtered water and cut to 15 cm petiole length prior to serving to the panellists at room temperature. The panellists scored in duplicate for each sample in separate sessions. Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Guelph,

ON, Canada) was used to acquire the data. Samples, coded with three-digit random numbers, were provided in a monadic balanced order, with sample sets randomly allocated to panelists. The panellists were asked to assess the appearance first; to break the petiole in half to assess the odour; to bite from the middle for taste, flavour, and mouthfeel; and then after 30 s delay to assess the aftereffects. The intensity of each attribute for each sample was recorded on a 100-point unstructured line scale. Between samples, the panellists cleansed their palate with water and crackers.

For the 2020 harvest, due to the COVID-19 pandemic restrictions, the trained panel assessed the samples from home in July 2020. Vocabulary refreshment and training sessions occurred prior to scoring virtually on the Teams platform. Samples were prepared similarly to 2018 but were sent out to panellists using chilled transport couriers. The panellists completed their scoring simultaneously using Compusense Cloud software whilst on video on Teams.

3.4.5. Statistical Analysis

The percentage composition was calculated from the data collected by SPME GCMS analysis. Quantitative data for each compound identified in the SPME GC/MS analysis were analysed by both one-way and two-way analysis of variance (ANOVA) and principal component analysis (PCA) using Spearman's correlation on XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine the sample means that differed significantly ($P < 0.05$) between harvest maturities and the celery genotypes. These data are shown in Table 3.1. Only those compounds exhibiting significant differences between harvest year, genotype, and their interaction (harvest year x genotype) were included in the principal component analysis. To compose the PCA plots that combine both sensory and instrumental data, the volatile data was added as supplementary data on top of the flavour and aroma attributes.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out ANOVA of sensory panel data. The means from sensory data were taken over assessors and correlated with the percentage composition means from the instrumental data via PCA using XLSTAT.

3.5. Results and Discussion

3.5.1. Volatile Composition

In total, 86 compounds were identified in the headspace of the eight celery genotypes in both harvest years (2018 and 2020) and listed in Table 3.1. Sixty-five compounds were identified in 2018 across eight genotypes, including: 22 monoterpenes, ten sesquiterpenes, eight aldehydes, five alcohols (three of which are classified as monoterpenoid alcohols) and five phthalides. Nine additional compounds were identified in the headspace of the same genotypes from the 2020 harvest including: 22 monoterpenes, 13 sesquiterpenes, five phthalides and five alcohols (including three monoterpenoid alcohols).

Quantitative differences were observed between the two harvest years (E) as well as the eight genotypes (G) used in this study. Two-way ANOVA revealed more significant differences between aroma composition caused by the harvest year compared to the genotype, although differences caused by the genotype were still observed. Most alkanes and compounds including nonanal, α-thujene, camphene, sabinene, p-mentha-2,8-dien-1-ol, α-ylangene, (E)-β-caryophyllene and trans-neocnidilide expressed no significant difference in the relative amount between 2018 harvest and 2020 harvest.

Previous research has shown that monoterpenes comprise most of the aroma profile of celery. In this study and for both years, monoterpenes comprised most of the aroma composition of the eight celery genotypes, making up an average of 55% of the aroma composition in 2018 and 88% in 2020, which is a significantly higher proportion of the total profile and confirms previous research. Orav, Kailas and Jegorova (2013) reported similar results in Estonian grown celery, where monoterpenes content comprised 85.3% of total flavour profile. Limonene was one of the most abundant compounds with an average percentage composition of 31% in 2018 and 58% in 2020. Limonene odour has been described as citrusy, pine and minty (Turner et al, 2021a; Turner, Dawda, Wagstaff, Gawthrop & Lignou, 2021). These are not typical descriptors used to describe celery odour and although its prominence is dominant in celery, its contribution to the aroma profile is minimal. Other terpenoid compounds including camphene, α-pinene and β-pinene, γ-terpinene, β-caryophyllene, α-humulene and kessane identified in this study were also detected in many other studies in varying proportions (Rozėk et al., 2016; Khalid & Hussein, 2012; Shojaei, Ebrahimi & Salimi, 2011; Rozėk et al., 2013; van Wassenhove et al., 1990; Orav, Kailas & Jegorova, 2013; Sorour, Hassanen & Ahmed, 2015).

Table 3.1. Percentage composition of volatile compounds identified in the headspace of eight celery genotypes using SPME GC/MS and harvested in 2018 and 2020.

Code	Compound	LRI_{expt} [a]	ID [b]	Percentage Composition (%)[c]														*p* [d]		
				2018								2020								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	E[e]	G[f]	GxE[g]
	Alcohols																					
A1	3-methyl-3-butenol	730	A	0.42± 0.08 [b]	0.31± 0.04 [ab]	0.94± 0.27 [c]	0.35± 0.14 [ab]	0.22± 0.07 [ab]	0.23± 0.06 [ab]	0.30± 0.12 [ab]	0.39± 0.06 [b]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
A2	(E)-2-pentanol	758	A	0.73± 0.28 [ab]	0.42± 0.16 [ab]	0.64± 0.04 [ab]	0.23± 0.08 [a]	0.32± 0.09 [a]	0.65± 0.23 [ab]	1.2± 0.54 [b]	0.50± 0.22 [ab]	tr± 0.01 [a]	tr± 0.01 [a]	0.12± 0.05 [a]	tr± 0.01 [a]	0.15± 0.03 [a]	tr± 0.05 [a]	tr± 0.03 [a]	tr± 0.01 [a]	***	***	***
A3	pentanol	763	A	0.21± 0.06 [ab]	0.11± 0.04 [a]	0.31± 0.20 [ab]	0.13± 0.10 [a]	0.23± 0.15 [ab]	0.39± 0.14 [ab]	0.63± 0.25 [b]	0.28± 0.08 [ab]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.03 [a]	tr± 0.01 [a]	0.10± 0.03 [a]	0.14± 0.02 [a]	0.12± 0.03 [a]	0.10± 0.02 [a]	**	**	**
	Total			1.4	0.84	1.9	0.71	0.77	1.3	2.1	1.2	0.07	0.06	0.18	0.03	0.25	0.21	0.3	0.13			
	Aldehydes																					
AL1	hexanal	800	A	9.7± 0.8	1.3± 0.46	2.6± 0.32	0.65± 0.29	2.0± 0.39	8.9± 2.7	13± 5.5	6.3± 1.2	0.16± 0.05	0.11± 0.02	0.22± 0.1	0.14± 0.03	0.24± 0.03	0.35± 0.25	0.22± 0.05	0.26± 0.15	*	ns	*
AL2	(E)-2-hexenal	849	A	0.18± 0.11	tr± 0.02	tr± 0.02	tr± 0.01	tr± 0.03	0.15± 0.11	0.20± 0.08	0.11 ± 0.05	nd	nd	nd	nd	nd	nd	nd	nd	**	ns	**
AL3	heptanal	901	A	tr± 0.03	nd	0.28± 0.15	0.16± 0.13	0.25± 0.16	0.23± 0.14	0.29± 0.08	0.25± 0.15	nd	nd	nd	nd	nd	nd	nd	nd	**	ns	**
AL4	(E)-2-heptenal	954	A	0.10± 0.22 [a]	1.6± 0.55 [abc]	1.6± 0.23 [abc]	0.5± 0.04 [ab]	1.5± 0.10 [abc]	3.2± 1.5 [bc]	4.2± 1.3 [c]	1.8± 0.97 [abc]	0.18± 0.05 [a]	0.2± 0.07 [a]	0.28± 0.10 [a]	0.36± 0.04 [ab]	0.54± 0.06 [ab]	0.53± 0.16 [bc]	0.46± 0.11 [a]	0.03± 0.04 [a]	***	***	***
AL5	octanal	1003	A	0.10± 0.07	nd	0.49± 0.06	0.27± 0.06	0.39± 0.19	0.51± 0.26	0.51± 0.17	0.51± 0.23	0.18± 0.02	0.16± 0.05	0.22± 0.04	0.25± 0.02	0.19± 0.03	0.24± 0.03	0.25± 0.14	0.15± 0.03	*	*	*
AL7	m-tolualdehyde	1086	B [1]	0.33± 0.07 [ab]	0.24± 0.02 [a]	4.0± 0.28 [d]	1.1± 0.28 [c]	0.95± 0.02 [bc]	0.19± 0.02 [a]	0.26± 0.05 [a]	1.6± 0.29 [c]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.01 [a]	nd [a]	***	***	***
AL8	nonanal	1105	A	0.33± 0.14	0.12± 0.02	0.20± 0.03	tr± 0.01	0.17± 0.03	0.16± 0.1	0.22± 0.17	0.19± 0.09	0.10 ± 0.02	tr± 0.05	0.21± 0.01	tr± <0.01	tr± 0.01	0.11± 0.02	0.14± 0.01	tr± 0.01	ns	ns	ns
AL9	(E,E)-2,6-nonadienal	1156	A	0.21± 0.04 [c]	0.30± 0.03 [c]	0.18± 0.02 [bc]	0.18± 0.04 [bc]	0.17± 0.03 [bc]	0.16± 0.08 [a]	tr± 0.03 [ab]	0.22± 0.08 [c]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
	Total			11	3.6	9.4	3	5.5	14	19	11	0.65	0.57	0.94	0.82	1.1	1.3	1.1	0.52			
	Esters																					
E1	methyl butanoate	717	A	tr ± 0.03	tr± 0.01	tr± 0.02	tr± <0.01	tr± 0.02	tr± 0.04	tr± 0.05	tr± 0.01	nd	tr± <0.01	nd	tr± <0.01	tr± <0.01	tr± <0.01	tr± <0.01	tr± <0.01	ns	ns	ns
E2		1108		nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	tr±	tr±	0.11 ±	tr±	tr±	tr±	nd [a]	tr±	***	***	***

	1-octen-3-yl-acetate		B [2]									0.02 a	0.01 a	0.03 c	0.01 ab	0.01 a	0.01 ab		0.02 b	
E3	(E)-pinocarvyl acetate	1310	B [3]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.36± 0.18 ab	0.38± 0.19 ab	0.43± 0.12 ab	0.14± 0.01 ab	0.43± 0.18 ab	0.55± 0.28 b	0.21± 0.07 ab	0.24± 0.05 ab	***
E4	carveol acetate	1343	B [4]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	tr± 0.02 cd	0.12± 0.05 bcd	0.20± 0.04 d	0.10 ± 0.01 ab	0.18± 0.05 cd	0.10 ± 0.02 bc	tr± 0.01 ab	0.10± 0.02 abc	***
E5	hexy isobutanoate	1378	B [5]	0.10± 0.03 a	0.10± 0.04 a	0.14± 0.02 ab	tr± 0.03 a	tr± 0.05 a	0.16± 0.04 ab	0.32± 0.06 b	0.12± 0.03 ab	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***
	Total			0.14	0.1	0.2	0.07	0.11	0.19	0.36	0.14	0.44	0.52	0.74	0.27	0.65	0.72	0.26	0.4	
	Alkanes																			
ALK1	nonane	900	A	0.41± 0.15 ab	0.32± 0.11 ab	0.43± 0.19 ab	0.14± 0.18 ab	0.13± 0.10 ab	0.28± 0.11 ab	nd a	0.17± 0.02 ab	0.20± 0.11 ab	0.38± 0.14 ab	0.71± 0.29 b	0.36± 0.11 ab	0.51± 0.07 ab	0.39± 0.22 ab	0.29± 0.05 ab	0.27± 0.04 ab	*
ALK2	decane	1000	A	0.80± 0.24 bcd	0.49± 0.13 abcd	nd a	0.37± 0.11 abc	0.60± 0.26 abcd	1.1± 0.21 de	1.7± 0.29 e	0.83± 0.33 cd	0.14± 0.02 ab	0.13± 0.02 ab	0.10 ± 0.11 a	tr± 0.08 a	0.18± 0.02 a	0.31± 0.01 abc	0.19± 0.02 abc	0.14± 0.01 ab	***
ALK3	undecane	1100	A	0.26± 0.15	0.14± 0.09	0.19± 0.11	tr± 0.05	0.24± 0.06	0.14± 0.1	tr± 0.08	0.11 ± 0.06	nd	nd	nd	nd	nd	nd	nd	nd	**
ALK4	dodecane	1199	A	0.48± 0.08	0.37± 0.03	0.46± 0.05	0.31± 0.1	0.33± 0.1	0.44± 0.13	0.46± 0.1	0.44± 0.12	0.39± 0.36	0.38± 0.34	0.18± 0.11	0.10± 0.08	0.11± 0.1	0.11± 0.04	0.10± 0.09	0.08± 0.04	ns
ALK5	tridecane	1299	A	nd	nd	nd	nd	nd	nd	nd	nd	0.61± 0.67	0.58± 0.68	0.23± 0.17	0.14± 0.11	0.13± 0.08	0.11 ± 0.06	0.10 ± 0.06	tr± 0.04	ns
ALK6	tetradecane	1399	A	0.11 ± 0.02	tr± 0.03	tr± 0.02	tr± 0.03	0.10± 0.06	0.10± 0.03	tr± 0.03	0.10 ± 0.02	0.50± 0.48	0.49± 0.21	0.28± 0.23	0.22± 0.1	tr± 0.03	0.14± 0.05	0.14± 0.07	0.11± 0.06	ns
ALK7	pentadecane	1499	A	nd	nd	nd	nd	nd	nd	nd	nd	0.25± 0.19	0.27± 0.19	0.18± 0.08	0.15± 0.08	0.17± 0.04	0.12± 0..02	0.14± 0.04	0.12± 0.03	**
ALK8	hexadecane	1600	A	nd	nd	nd	nd	nd	nd	nd	nd	0.10± 0.06	0.10 ± 0.06	0.10 ± 0.03	tr± 0..03	tr± 0.02	tr± 0.01	tr± 0.01	tr± 0.01	**
ALK9	heptadecane	1700	A	nd	nd	nd	nd	nd	nd	nd	nd	tr± 0.01	tr± 0.02	tr± 0.02	tr± <0.01	0.72± 0.12	0.69± 0.39	tr± 0.01	tr± 0.01	ns
ALK10	octadecane			nd	nd	nd	nd	nd	nd	nd	nd	tr± 0.01	tr± 0.01	tr± 0.01	nd	nd	nd	nd	nd	ns
	Total			2.1	1.4	1.1	0.94	1.4	2.1	2.3	1.6	2.2	2.5	1.8	1.1	1.9	1.9	0.95	0.86	
	Monoterpenes																			
M1	α-thujene	933	B [6]	0.27± 0.09	0.24± 0.08	0.29± 0.13	0.30± 0.11	0.22± 0.1	0.41± 0.19	0.32± 0.14	0.22± 0.13	0.11± 0.02	0.10 ± 0.02	0.10± 0.02	0.14± 0.02	0.11± 0.01	0.24± 0.02	0.15± 0.02	0.14± 0.02	ns
M2	α-pinene	943	A	0.62± 0.05 abcd	0.85± 0.22 bcd	0.52± 0.19 abcd	0.62± 0.18 abcd	1.0± 0.42 d	0.89± 0.20 cd	0.43± 0.20 ab	0.62± 0.31 abcd	0.26± 0.04 abcd	0.14± 0.11 abc	0.20± 0.09 abc	tr± 0.01 a	0.10± 0.01 ab	0.15± 0.01 abc	0.12± 0.01 a	0.40± 0.09 abcd	***

M3	camphene	960	A	2.5± 0.5	0.33± 0.07	0.29± 0.12	0.21± 0.08	0.35± 0.1	0.48± 0.05	0.66± 0.26	0.22± 0.08	0.11± 0.01	0.13± 0.04	0.17± 0.02	0.16± 0.06	0.22± 0.07	0.45± 0.03	0.28± 0.09	0.10 ± 0.03	ns	ns	ns
M4	sabinene	981	A	0.44± 0.13	0.33± 0.04	0.66± 0.39	0.27± 0.04	0.28± 0.05	0.45± 0.03	0.53± 0.13	0.36± 0.06	0.27± 0.02	0.25± 0.01	0.32± 0.04	0.39± 0.03	0.22± 0.08	0.49± 0.05	0.29± 0.05	0.23± 0.04	ns	ns	ns
M5	β-pinene	989	A	3.0± 0.64 ^{ab}	5.2± 1.6 ^{b}	0.96± 0.36 ^{ab}	5.4± 1.6 ^{b}	3.8± 1.6 ^{ab}	2.7± 0.99 ^{ab}	0.79± 0.24 ^{ab}	4.5± 1.1 ^{ab}	2.8± 0.8 ^{ab}	3.9± 1.1 ^{ab}	1.7± 0.39 ^{ab}	5.5± 0.69 ^{b}	3.8± 0.84 ^{ab}	0.13± 0.02 ^{a}	3.1± 0.17 ^{ab}	4.8± 1.1 ^{ab}	**	**	**
M6	myrcene	992	A	1.1± 0.26 ^{a}	1.9± 0.64 ^{a}	2.1± 0.74 ^{a}	2.6± 0.22 ^{a}	1.6± 0.37 ^{a}	2.1± 0.61 ^{a}	0.84± 0.34 ^{a}	1.1± 0.45 ^{a}	1.9± 0.11 ^{a}	2.6± 0.48 ^{a}	7.3± 0.65 ^{b}	7.9± 0.53 ^{b}	2.0± 0.76 ^{a}	1.9± 0.08 ^{a}	1.7± 0.27 ^{a}	2.1± 0.26 ^{a}	***	***	***
M7	α-phellandrene	1013	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.33± 0.02 ^{bc}	0.31± 0.03 ^{b}	0.39± 0.03 ^{cd}	0.30± 0.01 ^{b}	0.40± 0.03 ^{cd}	0.53± 0.03 ^{e}	0.53± 0.02 ^{e}	0.43± 0.03 ^{d}	***	***	***
M8	δ -3-carene	1019	A	0.24± 0.10 ^{ab}	0.23± 0.18 ^{ab}	0.25± 0.04 ^{ab}	0.25± 0.12 ^{ab}	0.22± 0.11 ^{ab}	0.21± 0.10 ^{ab}	0.32± 0.09 ^{b}	0.23± 0.05 ^{ab}	tr± 0.01 ^{ab}	tr± 0.01 ^{ab}	tr± 0.01 ^{a}	tr± 0.01 ^{ab}	nd ^{a}	0.13± 0.10 ^{ab}	nd ^{a}	tr± 0.02 ^{ab}	**	ns	**
M9	α -terpinene	1025	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.46± 0.08 ^{b}	0.42± 0.11 ^{b}	0.37± 0.06 ^{b}	0.35± 0.02 ^{b}	0.32± 0.03 ^{b}	0.37± 0.15 ^{b}	0.30± 0.02 ^{b}	0.48± 0.07 ^{b}	***	ns	***
M10	m-cymene	1032	A	4.3± 0.61 ^{abcd}	3.6± 0.41 ^{abc}	3.5± 0.69 ^{ab}	3.8± 0.43 ^{abc}	3.4± 0.78 ^{ab}	5.0± 0.71 ^{abcde}	2.8± 0.61 ^{a}	3.7± 0.55 ^{abc}	8.9± 1.4 ^{f}	6.6± 2.0 ^{cdef}	5.4± 0.28 ^{abcde}	7.9± 0.27 ^{ef}	4.2± 0.24 ^{abcd}	7.3± 0.20 ^{def}	5.8± 0.68 ^{abcdef}	6.0± 0.47 ^{bcdef}	***	***	***
M11	limonene	1034	A	39± 8.2 ^{ab}	43± 0.56 ^{abc}	33± 5.1 ^{a}	32± 2.3 ^{a}	39± 3.1 ^{ab}	32± 4.5 ^{a}	29± 3.9 ^{cd}	33± 3.1 ^{a}	54± 2.9 ^{bcd}	58± 4.5 ^{bcd}	59± 2.1 ^{cd}	46± 0.27 ^{abc}	65± 2.7 ^{d}	59± 2.1 ^{cd}	61± 1.6 ^{cd}	59± 1.9 ^{cd}	***	***	***
M12	β-(E)-ocimene	1049	B [7]	0.19± 0.03 ^{a}	0.18± 0.07 ^{a}	0.17± 0.05 ^{a}	0.24± 0.03 ^{a}	0.17± 0.02 ^{a}	0.16± 0.02 ^{a}	0.42± 0.08 ^{a}	0.18± 0.02 ^{a}	0.39± 0.04 ^{a}	0.25± 0.06 ^{a}	0.32± 0.11 ^{a}	0.46± 0.05 ^{a}	0.34± 0.08 ^{a}	0.28± 0.04 ^{a}	1.2± 0.22 ^{b}	0.42± 0.09 ^{a}	***	***	***
M13	γ-terpinene	1066	A	4.2± 1.2 ^{ab}	4.3± 1.2 ^{ab}	3.6± 0.60 ^{a}	5.9± 0.28 ^{abcd}	5.6± 0.27 ^{abc}	5.5± 1.4 ^{abc}	2.1± 0.90 ^{a}	5.6± 1.4 ^{abc}	17± 0.86 ^{f}	16± 1.6 ^{f}	10± 1.5 ^{de}	15± 0.67 ^{f}	8.0± 0.36 ^{bcd}	13± 1.3 ^{ef}	9.3± 0.60 ^{ef}	14± 0.27 ^{f}	***	***	***
M14	terpinolene	1097	A	0.62± 0.19	0.89± 0.07	0.53± 0.09	0.43± <0.01	0.36± 0.22	0.73± 0.2	0.57± 0.14	0.9± 0.31	0.75± 0.08	0.73± 0.11	0.76± 0.05	0.69± 0.06	0.79± 0.11	0.82± 0.04	0.84± 0.16	0.86± 0.12	*	ns	ns
M15	*allo*-ocimene	1132	B [8]	0.11± 0.06 ^{a}	0.10 ± 0.01 ^{a}	0.10 ± 0.05 ^{a}	0.31± 0.03 ^{ab}	0.24± 0.01 ^{ab}	0.13± 0.04 ^{ab}	0.31± 0.27 ^{ab}	0.13± 0.08 ^{ab}	0.33± 0.12 ^{ab}	0.14± 0.07 ^{ab}	0.23± 0.03 ^{ab}	0.57± 0.03 ^{b}	0.29± 0.01 ^{ab}	0.27± 0.05 ^{ab}	1.7± 0.36 ^{c}	0.41± 0.04 ^{ab}	***	***	***
M16	*p*-mentha-1,5,8-triene	1135	B [6]	0.26± 0.05 ^{abc}	0.10 ± 0.01 ^{ab}	0.22± 0.02 ^{abc}	0.56± 0.09 ^{d}	0.26± 0.07 ^{abc}	0.13± 0.09 ^{ab}	0.49± 0.17 ^{cd}	0.19± 0.08 ^{ab}	0.10± 0.02 ^{ab}	tr± 0.02 ^{a}	tr± 0.01 ^{ab}	0.12± 0.01 ^{ab}	0.10 ± <0.01 ^{ab}	0.10 ± <0.01 ^{ab}	0.34± 0.11 ^{bcd}	0.10 ± <0.01 ^{ab}	***	***	***
M17	pentylcyclohexa-1,3-diene	1166	B [3]	0.21± 0.05 ^{ab}	0.23± 0.08 ^{ab}	0.25± 0.03 ^{ab}	0.46± 0.11 ^{b}	0.31± 0.03 ^{ab}	0.06 ± 0.04 ^{a}	0.26± 0.16 ^{ab}	0.20± 0.01 ^{ab}	0.36± 0.09 ^{b}	0.34± 0.12 ^{ab}	0.23± 0.01 ^{ab}	0.34± 0.10 ^{ab}	0.27± 0.02 ^{ab}	0.18± 0.02 ^{ab}	0.22± 0.02 ^{ab}	0.25± 0.02 ^{ab}	*	*	*
M18	cis-dihydrocarvone	1208	A	0.39± 0.09 ^{e}	0.36± 0.05 ^{de}	0.35± 0.08 ^{de}	0.19± 0.06 ^{abcde}	0.27± 0.05 ^{cde}	0.18± 0.04 ^{abcd}	0.20± 0.08 ^{abcde}	0.26± 0.02 ^{bcde}	tr± 0.02 ^{ab}	0.10± 0.01 ^{abc}	0.10 ± 0.02 ^{abc}	tr± 0.01 ^{a}	0.10 ± 0.03 ^{abc}	tr± 0.01 ^{a}	0.10 ± 0.02 ^{abc}	tr± 0.01 ^{a}	***	*	***
M19	trans-carveol	1217	B [3]	0.23±	nd	0.10 ±	nd	0.10 ±	0.10 ±	0.16±	0.13±	0.10±	0.13±	0.19±	0.10 ±	0.15±	0.10±	0.10 ±	0.10 ±	*	ns	ns

				0.05		0.06		0.05	0.06	0.06	0.08	0.01	0.03	0.06	0.01	0.01	0.02	0.01	<0.01			
M20	trans-dihydrocarvone	1240	B [9]	0.79± 0.12 d	0.79± 0.14 d	0.67± 0.10 cd	0.41± 0.08 bc	0.57± 0.09 bcd	0.43± 0.05 bc	0.38± 0.06 b	0.59± 0.03 bcd	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
M21	L-carvone	1248	A	0.43± 0.19	0.36± 0.1	0.24± 0.02	0.18± 0.03	0.23± 0.08	0.34± 0.15	0.44± 0.07	0.29± 0.06	0.22± 0.03	0.14± 0.04	0.10 ± 0.01	tr± 0.02	tr± 0.01	nd	tr± 0.03	nd	**	ns	ns
M22	D-carvone	1262	A	0.96± 0.19 cd	0.57± 0.11 abc	1.5± 0.05 d	0.71± 0.06 abc	0.81± 0.13 bcd	0.61± 0.14 abc	0.75± 0.17 abc	1.1± 0.12 cd	0.20± 0.01 ab	0.12± 0.02 ab	tr± 0.02 a	0.10 ± 0.01 abc	0.10 ± 0.01 a	0.21± 0.01 ab	0.15± 0.02 ab	0.10 ± 0.01 abc	***	***	***
M23	thymol	1290	A	0.17± 0.05 c	0.11± 0.14 bc	0.12± 0.04 bc	0.15± 0.09 c	0.10± 0.08 ab	0.10± 0.03 bc	nd a	0.14± 0.11 bc	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
M24	carvacrol	1317	A	0.54± 0.08 e	0.42± 0.09 cde	0.45± 0.03 de	0.60 ± 0.02 e	0.29± 0.03 bcd	0.39 ± 0.03 cde	0.18± 0.04 abc	0.52± 0.04 de	nd a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 ab	tr± 0.01 a	***	***	***
	Total			61	64	50	56	59	53	42	54	89	90	87	86	87	86	87	90			
	Monoterpenoid Alcohols																					
MA1	p-mentha-2,8-dien-1-ol	1122	A	0.10± 0.03	0.15± 0.01	tr± 0.03	0.28 ± 0.03	0.10± 0.02	0.10± 0.04	tr± 0.03	0.14 ± 0.01	tr± 0.01	tr± 0.01	tr± 0.02	tr± 0.01	tr± 0.01	nd	tr± 0.01	tr± 0.01	ns	ns	ns
MA2	dihydrolinalool	1142	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	tr± 0.01 a	tr± 0.01 b	nd a	nd a	tr± 0.01 a	nd a	***	***	***
MA3	trans-pinocarveol	1147	B [10]	0.59± 0.13 c	0.63± 0.17 c	0.30± 0.08 abc	0.20± 0.08 ab	0.28± 0.02 abc	0.35± 0.21 abc	tr± 0.03 a	0.45± 0.10 bc	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
MA4	terpinen-4-ol	1184	A	0.10± 0.01 bc	nd a	tr± 0.03 ab	tr± 0.03 abc	tr± 0.03 ab	tr± 0.07 abc	nd a	0.13± 0.03 c	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
MA5	(E)-8-hydroxylinalool	1349	B [3]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	tr± 0.01 ab	0.10± 0.03 bc	0.10± 0.01 c	tr± 0.01 ab	0.10± 0.01 c	tr± 0.01 ab	tr± 0.01 a	tr± 0.01 ab	***	***	***
	Total			0.79	0.78	0.38	0.53	0.39	0.48	0.06	0.72	0.05	0.13	0.16	0.09	0.09	0.03	0.05	0.05			
	Sesquiterpenes																					
S1	α-ylangene	1384	B [5]	0.26± 0.11	0.24± 0.07	0.17± 0.11	tr± 0.01	0.16± 0.05	0.19± 0.1	0.20± 0.26	0.20± 0.14	0.10± 0.03	0.32± 0.25	0.27± 0.07	0.26± 0.1	0.16± 0.07	0.23± 0.06	0.16± 0.06	0.27± 0.08	ns	ns	ns
S2	α-copaene	1390	A	1.1 ± 0.02 e	0.86 ± 0.01 de	0.62 ± 0.03 bcde	0.10 ± 0.02 a	0.15 ± 0.05 ab	0.49 ± 0.03 abcd	0.78 ± 0.04 cde	0.77 ± 0.05 cde	tr± <0.01 a	0.39± 0.31 abcd	0.30± 0.05 abc	tr± 0.01 a	tr± 0.01 ab	0.17± 0.03 ab	0.30± 0.10 abc	0.42± 0.09 abcd	***	***	***
S3	(E)-β-caryophyllene	1430	B [11]	tr± 0.03	tr± 0.02	nd	nd	tr± 0.04	nd	nd	nd	tr± 0.01	tr± 0.01	tr± 0.01	tr± 0.01	nd	nd	nd	nd	ns	ns	ns
S4	β-caryophyllene	1445	A	4.4± 0.61 cd	5.5± 0.32 d	4.1± 0.43 bcd	2.5± 0.39 abc	4.3± 1.3 cd	4.1± 1.2 bcd	2.4± 0.29 abc	2.2± 0.50 abc	2.3± 0.37 abc	2.9± 0.66 abc	2.4± 0.22 abc	1.3± 0.52 a	1.7± 0.29 ab	2.0± 0.45 abc	0.89± 0.06 a	0.97± 0.19 a	***	***	***
S5	(+)-aromadendrene	1452	A	0.17±	0.21±	0.15±	tr±	0.13±	0.15±	tr±	0.10±	0.10 ±	0.10 ±	0.10±	tr±	tr±	tr±	tr±	tr±	***	***	***

				0.04 de	0.01 e	0.04 cde	0.07 abc	0.03 abcde	0.08 bcde	0.06 abc	0.01 abcd	0.02 abc	0.02 abcd	0.02 abcd	0.01 a	0.01 a	0.01 abc	<0.01 a	0.01 ab			
S6	curcumene	1472	B [12]	0.18± 0.09 cde	0.23± 0.11 e	0.19± 0.06 de	tr± 0.05 abcde	0.15± 0.22 bcde	0.22± 0.19 e	tr± 0.03 abcde	0.12± 0.05 abcde	tr± 0.01 abc	0.10 ± 0.01 abcd	tr± 0.01 abc	tr± 0.01 ab	nd a	nd a	nd a	nd a	***	**	***
S7	α-humulene	1479	A	0.42± 0.16 abc	0.70± 0.58 c	0.38± 0.29 abc	0.49± 1.1 abc	0.51± 0.76 bc	0.40± 0.65 abc	0.18± 1.2 ab	0.26± 0.9 ab	0.30± 0.14 abc	0.51± 0.04 abc	0.24± 0.06 ab	0.30± 0.09 ab	0.40± 0.06 abc	0.14± 0.03 ab	0.12± 0.01 a	0.14± 0.01 ab	***	***	***
S8	α-gurjunene	1495	B [13]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.10 ± 0.02 bc	0.10 ± 0.01 bc	0.10± <0.01 bc	0.10 ± 0.01 ab	0.10 ± 0.01 bc	0.10± 0.02 bc	0.10± 0.03 c	0.10± 0.01 bc	***	ns	***
S9	β-selinene	1508	B [14]	3.0± 0.05 ab	2.7± 0.06 ab	1.5± 0.02 a	4.6± 0.15 b	2.2± 0.19 ab	1.9± 0.12 a	3.3± 0.26 ab	3.0± 0.14 ab	2.5± 0.62 ab	1.6± 0.12 a	0.96± 0.16 a	1.4± 0.28 a	1.2± 0.32 a	0.85± 0.16 a	1.1± 0.23 a	1.7± 0.33 a	***	***	***
S10	valencene	1514	A	nd a	nd a	nd a	2.9± 0.44 b	nd a	nd a	nd a	0.20± 0.07 a	0.15± 0.21 a	0.15± 0.19 a	0.10± 0.01 a	2.6± 0.40 b	0.10± 0.05 a	0.10 ± 0.07 a	0.12± 0.04 a	0.18± 0.08 a	***	***	***
S11	α-selinene	1515	B [15]	0.61 ± 0.02 bc	0.60 ± 0.06 bc	0.43 ± 0.05 abc	0.63± 0.44 bc	0.54 ± 0.04 abc	0.44± 0.03 abc	0.71 ± 0.02 c	0.59± 0.01 abc	0.28± 0.06 abc	0.31± 0.09 abc	0.29± 0.04 abc	0.23± 0.05 ab	0.22± 0.05 ab	0.13± 0.08 a	0.23± 0.06 ab	0.33± 0.03 abc	***	ns	***
S12	kessane	1557	B [3]	nd a	0.12± 0.02 a	nd a	2.8± 0.05 c	nd a	nd a	nd a	nd a	0.26± 0.03 a	0.12± 0.09 ab	tr± 0.01 a	1.7± 0.21 b	0.10 ± 0.01 a	tr± 0.01 ab	tr± 0.01 b	tr± 0.01 a	***	***	***
S13	β-gurjuene$	1560	B [13]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	tr± 0.01 b	tr± 0.01 ab	nd a	tr± 0.03 c	tr± 0.01 ab	tr± 0.01 ab	nd a	nd a	***	***	***
	Total			10	11	7.5	14	8.2	7.9	7.7	7.4	6.1	6.6	4.8	8	3.9	3.8	3	4.2			
	Phthalides																					
P1	3-butylhexahydro phthalide	1662	B [3]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	tr± 0.01 abc	tr± 0.01 ab	tr± 0.01 abc	tr± 0.01 ab	tr± 0.01 ab	tr± 0.01 bc	tr± 0.01 bc	tr± 0.01 ab	***	ns	***
P2	3-n-butylphthalide	1676	A	5.0± 0.01 b	5.2± 0.03 b	9.4± 0.05 c	6.6± 0.01 bc	7.1± 0.03 bc	6.7± 0.01 bc	9.8± 0.06 c	7.0± 0.03 bc	0.73± 0.39 a	0.52± 0.28 a	0.93± 0.30 a	0.88± 0.28 a	0.67± 0.43 a	0.93± 0.60 a	1.6± 0.40 a	1.0± 0.30 a	***	*	***
P3	cis-3-butylidenephthalide	1685	B [3]	0.15± 0.06 b	0.18± 0.05 b	0.36± 0.09 c	0.15± 0.02 bc	0.23± 0.02 b	0.17± 0.07 b	0.25± 0.34 bc	0.18± 0.25 b	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
P4	sedanenolide	1748	A	4.8± 0.30 abcde	9.7± 2.3 cdef	15± 1.9 f	16± 1.6 f	14± 3.0 f	9.5± 2.9 bcdef	11± 3.0 def	13± 2.2 ef	1.3± 0.49 ab	0.78± 0.18 a	2.3± 0.47 abc	1.9± 0.32 abc	1.4± 0.83 ab	3.1± 0.72 abcd	2.6± 0.28 abcd	1.4± 0.36 ab	***	***	***
P5	trans-neocnidilide	1755	B [3]	0.26± 0.03	0.24± 0.03	1.8± 0.02	0.16± 0.04	0.30± 0.06	0.78± 0.06	0.99± 0.04	0.94± 0.04	0.34± 0.1	0.13± 0.05	0.19± 0.22	0.08± 0.02	1.7± 0.88	0.59± 0.22	0.50± 0.06	0.24± 0.06	ns	ns	ns
P6	trans-ligustilide	1764		0.12±	0.14±	0.24±	0.23±	0.25±	0.14±	0.18±	0.18±	tr±	tr±	tr±	tr±	0.10±	tr±	tr±	tr±	***	ns	***

			B [16]	0.02 abc	0.10 abc	0.01 c	0.03 c	0.05 c	0.01 abc	0.09 ab	0.05 ab	0.01 b	0.01 b	0.02 b	0.01 b	0.01 ab	0.01 b	0.01 b	0.01 b			
	Total			10	16	27	23	22	17	22	21	2.4	1.5	3.5	2.9	3.9	4.7	4.7	2.7			
	Oxides																					
O1	(Z)-limonene oxide	1147	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.49± 0.37 ab	0.87± 0.11 bc	0.66± 0.04 bc	1.1± 0.15 c	0.66± 0.05 bc	1.7± 0.26 d	0.73± 0.07 bc	***	***	***
O2	caryophyllene oxide	1610	A	tr± 0.01 ab	0.13± 0.04 b	0.25± 0.05 c	0.10± 0.02 ab	010± 0.07 ab	0.10± 0.02 ab	tr± 0.01 ab	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
	Total			0.04	0.13	0.25	0.05	0.08	0.09	0.02	0	0	0.49	0.87	0.66	1.1	0.66	1.7	0.73			
	Unknowns																					
U1	unknown 1	n/a		0.57± 0.09 abc	0.31± 0.03 ab	0.43± 0.06 ab	0.19± 0.02 ab	0.27± 0.01 ab	0.71± 0.20 bc	1.2± 0.47 c	0.51± 0.29 abc	0.10 ± 0.02 ab	tr± 0.02 a	tr± 0.04 a	tr± 0.01 a	0.11± 0.02 ab	0.18± 0.02 ab	0.13± 0.01 ab	0.10± 0.01 ab	***	**	***
U2	unknown 2	n/a		2.3± 0.63 abc	1.7± 0.03 abc	2.1± 0.06 abc	0.84± 0.02 ab	1.0± 0.01 ab	2.7± 0.20 bc	3.4± 0.47 c	1.5± 0.29 abc	0.28± 0.01 a	0.22± 0.05 a	0.47± 0.10 a	0.14± 0.04 a	0.63± 0.14 ab	0.65± 0.27 ab	0.44± 0.08 a	0.24± 0.05 a	***	*	***
U3	unknown 3	753		nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.14± 0.04 ab	tr± 0.01 ab	tr± 0.01 ab	nd a	tr± 0.01 b	tr± 0.01 ab	tr± 0.01 a	tr± 0.01 a	***	ns	***
U4	unknown 4	1081		nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.07 ± 0.02 b	tr± 0.02 b	0.10 ± 0.01 b	0.10 ± 0.02 b	0.10 ± 0.02 bc	0.11± 0.02 cd	0.15± 0.01 d	0.10 ± 0.01 bc	***	***	***
U5	unknown 5	1279		0.16± 0.06 ab	0.10± 0.01 ab	0.10± 0.01 ab	0.13± 0.03 ab	0.24 ± 0.01 b	0.11 ± 0.01 ab	0.17 ± 0.03 ab	0.10 ± 0.04 ab	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	**	ns	**
U6	unknown 6	1362		0.10± 0.02 ab	0.10± 0.04 ab	nd a	0.16± 0.01 b	tr± 0.04 a	0.10± 0.01 ab	0.10± 0.01 ab	0.10± 0.04 ab	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	*	***
U7	unknown 7	1539		0.25± 0.05 cd	0.33± 0.01 d	0.19± 0.02 bcd	0.10 ± 0.01 ab	0.15 ± 0.06 abc	0.10± 0.08 abc	0.18± 0.15 bcd	0.15± 0.06 abc	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	*	***
U8	unknown 8	1542		tr± 0.01 a	nd a	0.10± 0.03 ab	nd a	0.10 ± 0.04 ab	0.10± 0.04 ab	0.10 ± 0.01 ab	0.10 ± 0.03 ab	nd a	0.10± 0.05 b	0.10± 0.02 b	nd a	0.10± 0.02 b	0.10± 0.02 ab	tr± 0.01 ab	0.11± 0.01 b	***	**	***
U9	unknown 9	1653		nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.10± 0.05 ab	tr± 0.02 a	tr± 0.02 a	tr± 0.01 ab	tr± 0.01 ab	tr± 0.03 a	tr± 0.01 ab	0.16± 0.08 b	**	**	**
U10	unknown 10	1776		nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.04 ± 0.02 ab	tr± 0.01 ab	tr± 0.01 ab	nd a	tr± 0.02 ab	tr± 0.03 ab	tr± 0.01 ab	tr± 0.01 ab	***	ns	**
	Total			3.4	2.5	2.9	1.4	1.8	3.8	5.1	2.4	0.7	0.44	0.67	0.29	1	1.1	0.81	0.72			

[a] Linear retention index on a HP-5MS column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; [1] Radulovic et al. (2010); [2] Adams, (2000); [3]Andriamaharavo, (2014); [4] Stashenko et al. (2003); [5] Lucero et al. (2006); [6] Adams et al. (2005); [6] Sabulal et al. (2007); [7] Havlik et al. (2006); [8] Bylaite & Meyer, (2006); [9] Block et al. (2006); [10] Boulanger et al.

(1999); [11] Cao et al. (2011); [12] Aligiannis et al. (2001); [13] Yu et al. (2007); [14] Zeng et al. (2007); [15] Högnadóttir & Rouseff, (2003); [16]Baccouri et al. (2007) [s] tentatively identified, spectral quality value of 70 was used for this compound. [c] Percentage composition of total peak area divided by compound peak area; means labelled with letters are significantly different ($p < 0.05$) according to the GxE interaction; means of three replicate samples; tr, trace amounts <0.10%; nd, not detected. [d] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [e] Harvest year. [f] Genotype. [g] Harvest year x genotype interaction. Cells have been colour coded; red expresses the genotype with the higher value compared to harvest year; green expresses the genotype with the lower value compared to harvest year; no colour expresses no difference in percentage composition for both years.

Phthalide compounds are known as odour active compounds and main contributors to the characteristic odour of celery (Macleod & Ames, 1989; Lund, Wagner & Bryan, 1973; Orav, Kailas & Jegorova, 2013; Sorour, Hassanen & Ahmed, 2015; Uhlig, Chen & Jen, 1987; Macleod, Macleod & Subramanian, 1988). These compounds impart a "herbal" and "celery-like" aroma (Turner et al, 2021a; Turner et al, 2021b). The proportion of the aroma profile comprised of phthalide compounds varied between years and genotype, with 2018 exhibiting a higher proportion composition compared to 2020. Lund, Wagner, and Bryan (1973) identified sedanenolide, 3-n-butylphthalide, hexahydro-3-n-butylphthalide and β-selinene to exhibit a celery-like odour. Three of these compounds were identified in all eight genotypes in both harvest years but their contribution to the composition varied. Sedanenolide and β-selinene had a higher proportion of the 2018 grown celery and are observed in the highest proportion in genotype 12. van Wassenhove, Dirinck, Vulsteke and Schamp (1990) observed slight differences in the concentration of these compounds between years, however, unlike this study, no significant differences were reported. Furthermore, they presented a similar phthalide content, ranging from 6–11 %, while in this study 19 % and 3 % was comprised of phthalides. The variation in the prominence of sedanenolide found in celery is very apparent not only in this study but in a plethora of studies where the percentage composition ranges from 0.2–39.5 % (Turner et al., 2021a). Genotype 12 exhibited a high proportion of monoterpenes and the highest proportion of sesquiterpenes for both harvest years. In 2018, genotype 10 expressed the highest proportion of phthalides compared to other genotypes, exhibiting a high percentage of 3-n-butylphthalide (9.4 %) and sedanenolide (15 %) and genotype 12 had the highest proportion of sedanenolide (16 %). On the other hand, genotypes 18 and 22 in 2020 exhibited the highest proportion of these compounds including 3-n-butylphthalide (3.1 and 2.6 %, respectively). Turner et al. (2021a) identified 3-n-butylphthalide to be the most reported phthalide (Macleod & Ames, 1989; Kurobayashi et al., 2006; Philippe et al., 2002; van Wassenhove et al., 1990; Turner et al, 2021b; Orav, Kailas & Jegorova, 2013; Macleod, Macleod & Subramanian, 1988). Based on this observation, genotypes 10 and 12 in 2018 and genotype 22 in 2020 would be perceived as the genotypes with the strongest celery odour.

In terms of other compounds, smaller differences in the average composition between the years were observed: alcohols 1.3 % and 0.15 %, esters 0.16 % and 0.5 % and finally alkanes 1.6 % for both

2018 and 2020 harvests, respectively. Limited research has been published about these types of compounds and their contribution to the celery aroma profile. By combining GC/MS and gas chromatography/olfactometry (GC/O), Turner et al. (2021b) identified compounds that contribute to the distinct celery aroma and how the aroma changed and developed throughout maturity. Using two of the same genotypes also used in this study (12 and 22), the aroma development over three time-points was studied: two-weeks before commercial maturity, at commercial maturity and two-weeks after commercial maturity. Monoterpene, sesquiterpene and phthalide compounds identified in the present study reflect those compounds observed by Turner et al. (2021b) and demonstrate that they are strongly influenced by maturity. Once commercial maturity was reached, the relative abundance of these compounds in the overall profile decreased, while alcohol and ester compounds became more abundant. Esters also identified by Turner et al. (2021b), including carveol acetate and hexyl hexanoate, were reported to contribute to green, herbal and damp odours in overmature celery according to GC/O analysis. The ester composition in the present study also varied as a consequence of both genotype and harvest year (Table 3.1) and a higher ester composition was observed from the 2020 harvest; however, methyl butanoate and (E)-pinocarvyl acetate were not significantly influenced by the genotype, only harvest year.

Principal component analysis (PCA) allowed for the visual comparison of the volatile composition of the eight celery genotypes in 2018 and 2020 (Figure 3.1) and the examination of any correlations occurring between genotype, harvest year and chemical compounds. Using only the significant compounds for harvest year, genotype and their interaction, a clear divide between the compounds associated with each year was observed. Principal component one (F1) and two (F2) explained 62.78 % in total of the variation present in the data and it can be observed that the first axis separated samples from the two harvest years (2018 and 2020), while the second axis separated the various genotypes within a harvest year. Differences between the harvest years were apparent as is exhibited by the separation along the F1 component, which accounts for 52.06 % of the variation. Genotypes were consistently separated across the F2 component for both years, which explains 10.81 % of the variation. Metabolic pathways are genetically regulated, leading to the hypothesis that compounds that are important to a particular cultivar should remain constant in their relative abundance

between seasons and any deviations in these compounds are most likely due to external factors rather than genotype (Fellman, Miller & Mattinson, 2000). Genotypes 12, 8 and 5 for both years along with genotype 15 from 2018 were positively correlated with F2. Conversely, genotypes 10, 18, 22 and 25 for both years were negatively associated with F2.

Predominantly, monoterpenes and phthalides were separated across F2 and influenced by genotype, while sesquiterpenes, aldehydes and esters were separated across F1, respectively. Strong significant relationships were also observed between the compound groups, such as with alcohols and aldehydes expressing strong and positive correlations together, while low boiling monoterpenes including delta-3-carene and limonene expressed strong negative correlations with alcohols and aldehydes. Conversely, sesquiterpenes and phthalides had a negative correlation with the above monoterpenes and, instead, expressed a positive correlation with higher boiling monoterpenes including L-carvone, thymol and carvacrol.

(A) (B) (C)

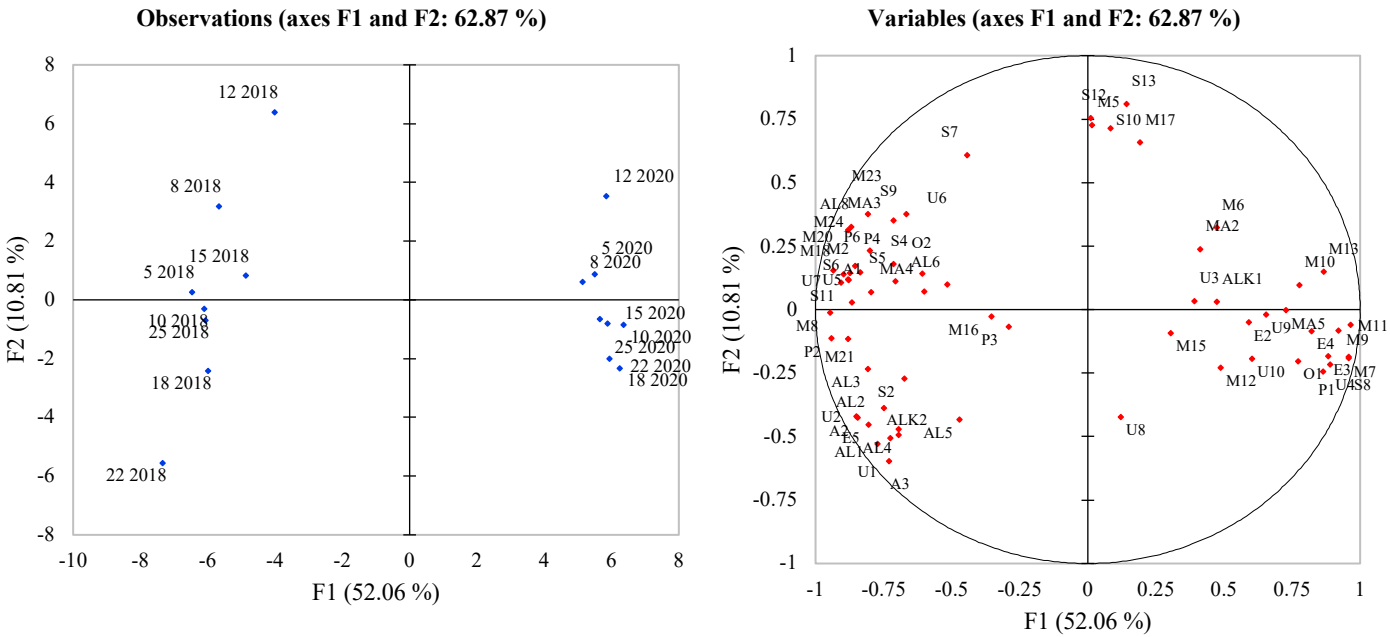


Figure 3.1. Principal component analysis of eight celery samples harvested in 2018 and 2020 showing correlations with volatile compounds. (**A**) Projection of the samples; (**B**) Distribution of variables; (**C**) Compound codes as appear in plot (**B**).

A1	3-methyl-3-buten-1-ol	M24	carvacrol
A2	(E)-2-penten-1-ol	MA2	dihydrolinalool
A3	1-pentanol	MA3	trans-pinocarveol
AL1	hexanal	MA4	terpinen-4-ol
AL2	(E)-2-hexenal	MA5	(E)-8-hydroxylinalool
AL3	heptanal	S2	α-copaene
AL4	(E)-2-heptenal	S4	β-caryophyllene
AL5	n-octanal	S5	(+)-aromadendrene
AL6	m-tolualdehyde	S6	curcumene
AL8	(E,E)-2,6-nonadienal	S7	α-humulene
E2	1-octen-3-yl-acetate	S8	α-gurjunene
E3	(E)-pinocarvyl acetate	S9	β-selinene
E4	carveol acetate	S10	valencene
E5	hexyl hexanoate	S11	α-selinene
ALK 1	nonane	S12	kessane
ALK 2	decane	S13	β-gurjuene
M2	α-pinene	P1	3-butylhexahydro phthalide
M5	β-pinene	P2	3-n-butylphthalide
M6	myrcene	P3	(Z)-3-butylidenephthalide
M7	α -phellandrene	P4	sedanenolide
M8	delta-3-carene	P6	(cis)-ligustilide
M9	α -terpinene	O1	(Z)-limonene oxide
M10	m-cymene	O2	caryophyllene oxide
M11	limonene	U1	unknown 1
M12	β-(E)-ocimene	U2	unknown 2
M13	γ-terpinene	U3	unknown 3
M15	allo-ocimene	U4	unknown 4
M16	p-mentha-1,5,8-triene	U5	unknown 5
M17	pentylcyclohexa-1,3-diene	U6	unknown 6
M18	cis-dihydrocarvone	U7	unknown 7
M20	trans-dihydrocarvone	U8	unknown 8
M21	L-carvone	U9	unknown 9
M23	thymol	U10	unknown 10

In 2018, the genotype had a stronger influence over the volatile composition, and this is reflected through the more noticeable separation between the eight genotypes and a stronger association with aroma compounds. However, genotypes 12, 18, 22 and 25 exhibited similar placements on the observation plot between the two years, albeit on opposing sides of F2. Monoterpenes (M2, 8, 16, 18, 21, 22, 23, 24), monoterpenoid alcohols (MA3, 4), sesquiterpenes (S2, 4, 5, 6, 9) and phthalides (P2, 3, 4,6) were positively correlated with celery samples grown in 2018. Conversely, monoterpenes (M6, 7, 9, 10, 11, 12, 13, 15), sesquiterpenes (S8, 10, 12, 13), monoterpenoid alcohols (MA2, 5) were positively correlated with celery samples grown in 2020. The spread of monoterpene and sesquiterpene compounds across the plot and presence within all genotypes across both years (Table 3.1) proves these are fundamental compounds to celery. As it can be observed from Figure 1, the aroma profile in 2018 consisted of a higher proportion of phthalide compounds than in 2020, where all phthalides, apart from 3-butylhexahydrophthalide (P1), appeared closely associated with the 2018 samples. Due to the odour active nature of sedanenolide and other phthalides and the strong celery odours that these compounds impart, celery genotypes exhibiting a high proportion of these compounds are more likely to possess a strong characteristic celery odour.

The harvest year and genotype both had an influence on the volatile content of celery samples, however, a much stronger influence over the percentage composition for all genotypes and most volatile compounds was observed by harvest year. Genotypes exhibited fewer significant differences over the majority of monoterpenes, aldehydes, sesquiterpenes and phthalides. Although the genotype is known to play a role in predetermining the aroma composition (Fellman, Miller & Mattinson, 2000), the variation caused by harvest year and, therefore, the growing environment possessed a more significant role in determining the aroma composition (Table 3.1, Figure 3.1). Differences in climate during growth are most likely the cause of these compositional changes and will be discussed further in Section 3.5.3. The aroma and flavour quality of certain genotypes such as 12, 18 and 25 were consistent across the two years demonstrating that these genotypes may provide consistent quality crop for celery growers and breeders irrespective of the environmental changes. Carrying out sensory profiling on these cultivars will permit the examination of the impact of the different compositions caused by genotype and harvest year on flavour perception.

3.5.2. Sensory Evaluation of Fresh Celery Samples

The sensory profile of the eight celery samples was generated by a trained panel who came to the consensus of 22 and 24 terms for the quantitative assessment of samples in the 2018 and 2020 samples, respectively. The two additional attributes in 2020 were that of “fresh parsley flavour” and “celery residue in mouth” as an aftereffect. Table 3.2 shows the mean panel scores for these attributes. Out of the 22 attributes that were profiled in 2018, 14 of these were found to be significantly different between the genotypes and in 2020, 18 out of the 24 attributes were found to be significantly different. There were few significant assessor sample interactions identified for both the 2018 and 2020 harvests, which suggests that the panelists scored samples in a consistent manner (Lignou, Parker, Baxter & Mottram, 2014).

Table 3.2. Mean panel scores for sensory attributes of the eight celery samples harvested in 2018 and 2020.

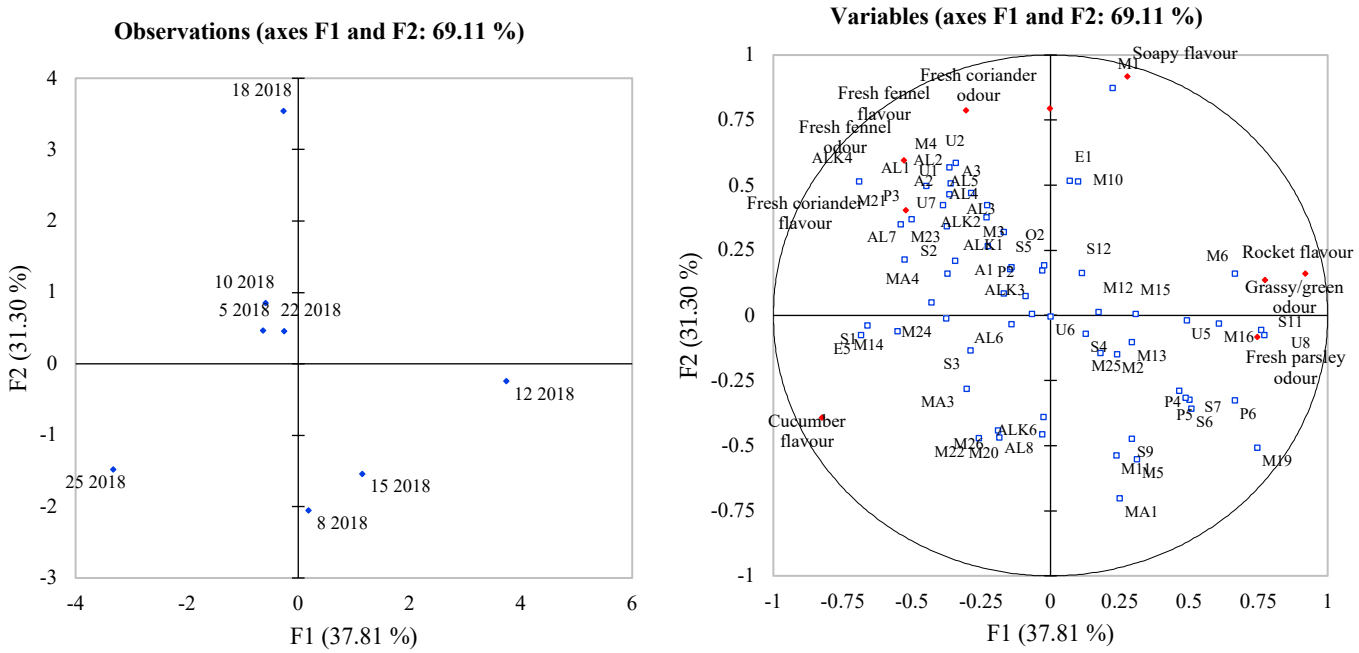
Attribute	Score [A]																	
	2018									2020								
	5	8	10	12	15	18	22	25	P[b]	5	8	10	12	15	18	22	25	*p* [B]
Appearance																		
Colour	56.4 b	63.6 ab	62.6 ab	72.9 a	72.1 a	65.6 ab	70.5 a	26.8 c	***	46.3 cd	53.0 bcd	44.6 d	67.5 ab	61.0 abc	55.6 abcd	70.5 a	14.7 e	***
Stalk thickness	49.8 ab	49.5 ab	55.8 a	20.9 b	58.7 a	62.5 a	61.3 a	55.0 a	***	60.6 abc	47.7 cde	36.2 def	20.7 ee	51.1 cd	74.1 a	72.0 ab	59.8 abc	***
Ribbed	46.6 bc	61.0 ab	61.7 a	65.9 a	35.5 cd	25.4 d	34.2 cd	37.4 cd	***	60.3 ab	65.8 a	66.6 a	68.5 a	45.9 b	50.7 b	56.4 ab	55.6 ab	***
Aroma																		
Fresh fennel	16.5	14.2	18.9	15.5	15.3	18.6	15.4	18.2	ns	32.1	22.1	22.8	21.1	23.6	19.8	30.8	20.3	*
Grassy/green	32.6 a	31.0 ab	32.1 ab	36.3 a	30.7 ab	28.3 ab	35.3 a	21.1 b	***	27.1 ab	33.8 a	25.9 ab	32.8 a	34.5 a	34.6 a	28.5 ab	18.2 b	***
Fresh parsley	14.1	19.7	19.0	19.1	20.6	16.7	16.7	10.8	ns	18.0	19.2	20.8	16.8	20.6	19.4	17.3	16.4	ns
Fresh coriander	12.8	12.1	14.2	11.7	14.2	17.5	15.4	11.1	ns	15.4	13.0	14.8	12.0	14.2	16.6	16.3	7.7	ns
Taste/flavour																		
Bitter	23.1 abc	24.0 abc	24.7 abc	35.9 a	28.2 abc	31.3 ab	24.4 abc	15.5 c	**	33.2 abc	20.6 abc	35.0 ab	38.4 a	35.2 a	34.4 ab	33.0 abc	19.6 c	***
Sweet	15.2 bcd	20.3 ab	21.6 ab	10.6 d	15.6 bcd	12.2 cd	20.0 ab	24.6 a	***	17.3 abc	25.0 abc	20.0 abc	17.1 abc	13.1 c	14.8 bc	18.1 abc	23.7 ab	**
Fresh fennel	11.9	10.3	12.6	11.0	7.7	13.6	11.6	11.3	ns	27.5 a	23.5 ab	23.3 ab	16.9 ab	21.1 ab	13.7 b	23.3 ab	21.3 ab	**
Rocket	11.3 bc	13.4 bc	12.4 bc	23.8 a	16.6 abc	16.9 abc	10.4 bc	7.7 c	***	1.1	1.8	2.7	3.8	4.2	0.7	3.4	1.3	ns
Fresh coriander	17.5	16.3	16.0	9.6	15.0	18.1	18.9	14.1	ns	17.2	18.2	21.2	19.1	16.7	18.2	17.9	11.6	ns
Soapy	18.2 ab	12.4 b	16.4 ab	18.4 ab	15.4 ab	23.7 a	16.3 ab	13.0 ab	*	14.9 ab	14.2 ab	19.1 ab	20.0 a	17.4 ab	22.9 a	14.1 ab	9.3 [b]	***
Watery/cucumber	25.7 ab	33.2 ab	30.4 ab	9.1 c	30.0 ab	22.4 b	27.9 ab	37.7 a	***	19.8 ab	15.7 ab	12.1 b	10.8 b	16.2 ab	20.5 ab	23.2 ab	27.0 a	**
Fresh parsley	nd	nd	nd	nd	nd	nd	nd	nd		15.5	14.7	13.8	16.7	15.2	13.0	11.0	9.7	ns
Mouthfeel																		
Crunchy	65.4 abc	62.6 bc	64.9 abc	56.7 c	70.2 ab	66.4 abc	73.7 a	62.5 bc	***	70.6 ab	65.8 ab	72.9 a	66.7 ab	74.2 a	58.5 b	74.7 a	67.6 ab	**
Stringy	40.8 b	46.6 b	40.1 b	64.1 a	33.2 b	40.6 b	35.1 b	35.2 b	***	53.2 bc	62.8 ab	61.8 ab	74.2 a	54.4 bc	45.7 c	51.1 bc	45.1 c	***
Moist	50.6 a	47.2 a	50.0 a	29.7 b	53.1 a	44.3 a	51.4 a	54.8 a	***	55.0 abc	51.0 bc	44.8 c	28.3 d	49.3 bc	50.3 bc	54.8 bc	57.6 ab	***
Firmness of first bite	63.7	59.9	63.3	59.2	68.9	65.7	67.6	58.6	ns	69.3 ab	65.2 ab	68.1 ab	66.2 ab	72.4 ab	60.6 b	74.9 a	65.1 ab	*
After effects																		
Celery residue in mouth	nd	nd	nd	nd	nd	nd	nd	nd		51.4 ab	51.1 ab	52.5 ab	64.0 a	48.3 b	45.8 b	48.8 ab	39.4 b	***
Soapy	16.9 ab	15.7 ab	16.7 ab	21.2 ab	19.9 ab	24.8 a	18.6 ab	12.9 b	*	15.4 b	14.4 b	21.1 b	23.2 a	18.0 b	21.2 b	14.4 b	14.6 b	**
Grassy/green	27.7	27.0	27.9	27.6	28.4	26.4	31.4	19.0	ns	14.8	20.6	19.0	18.4	21.3	20.1	21.7	15.3	ns
Numbness	13.1	8.6	9.6	11.5	10.0	14.0	9.8	9.0	ns	11.4 a	12.1 a	11.5 a	11.7 a	12.6 a	13.2 a	9.8 [b]	7.3 [b]	**
Bitter	17.4 bc	18.4 bc	18.3 bc	29.0 a	19.1 bc	25.7 ab	16.0 bc	12.0 c	***	18.0 bc	20.9 abc	28.5 a	27.5 ab	25.5 ab	23.0 abc	19.6 abc	13.5 c	***

[A] Mean score of two replicate samples taken from the trained panel (n=12). Means labelled with letters (a,b,c,d,e) are significantly different ($p < 0.05$) according to the Assessor x Sample interaction; Means not labelled with the same letters are significantly different ($p < 0.05$); nd, not detected. [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

The odour and flavour attributes evaluated displayed clear significant differences between both genotypes and harvest year. The attributes "watery/cucumber" and "rocket" flavour along with "grass/green" odour were scored highly in the 2018 harvest, while "fresh fennel and parsley" flavour

were scored highly in the 2020 harvest. “Fresh coriander” aroma and flavour along with “soapy” flavour were scored similarly for both years. Genotype 25 was scored low for both years for flavour and aroma attributes apart from the “watery/cucumber” flavour, while genotype 12 was scored as the most bitter for both years. Combining these attributes with the volatile compounds identified through GC/MS (Table 3.1) provided a deeper understanding in the differences within the aroma composition and its impact on flavour perception. Principal component analysis was used to visualise the sensory and chemical differences across the eight genotypes and the volatile compounds identified (Table 3.1) and the attributes related to odour and flavour were used as variables (Figures 3.2 and 3.3).

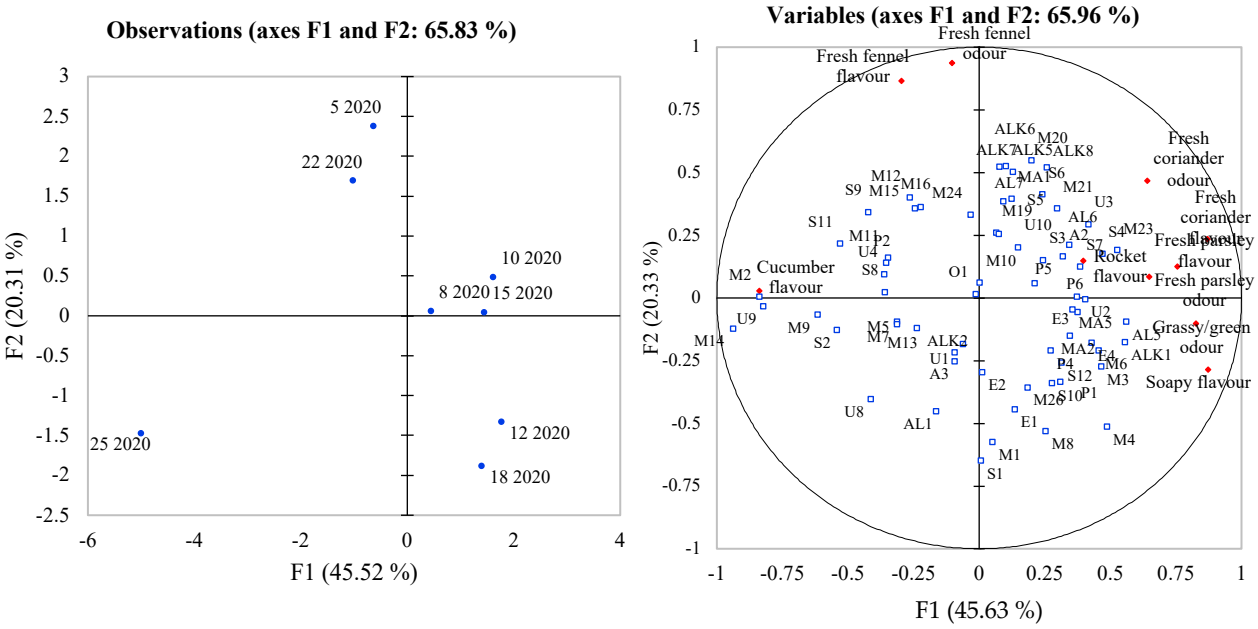
(A) (B) (C)



A1	3-methyl-3-buten-1-ol	M20	cis- dihydrocarvone
A2	(E)-2-penten-1-ol	M21	trans carveol
A3	1-pentanol	M22	trans-dihydrocarvone
AL1	hexanal	M23	L-carvone
AL2	(E)-2-hexenal	M24	D-carvone
AL3	heptanal	M25	thymol
AL4	(E)-2-heptenal	M26	carvacrol
AL5	n-octanal	MA1	p-mentha-2,8-dien-1-ol
AL6	m-tolualdehyde	MA3	trans-pinocarveol
AL7	nonanal	MA4	terpinen-4-ol
AL8	(E,E)-2,6-nonadienal	S1	α-ylangene
E1	methyl butanoate	S2	α-copaene
E5	hexyl hexanoate	S3	(E)-β-caryophyllene
ALK1	nonane	S4	β-caryophyllene
ALK2	decane	S5	(+)-aromadendrene
ALK3	undecane	S6	curcumene
ALK4	dodecane	S7	α-humulene
ALK6	tetradecane	S9	β-selinene
M1	α-thujene	S11	α-selinene
M2	α-pinene	S12	kessane
M3	camphene	P2	3-n-butylphthalide
M4	sabinene	P3	(Z)-3-butylidenephthalide
M5	β-pinene	P4	sedanenolide
M6	myrcene	P5	trans-neocnidilide
M10	m-cymene	P6	(cis)-ligustilide
M11	limonene	O2	caryophyllene oxide
M12	β-(E)-ocimene	U1	unknown 1
M13	γ-terpinene	U2	unknown 2
M14	terpinolene	U5	unknown 5
M15	allo-ocimene	U6	unknown 6
M16	*p*-mentha-1,5,8-triene	U7	unknown 7
M19	pentylcyclohexa-1,3-diene	U8	unknown 8

Figure 3.2. Principal component analysis of eight celery samples harvested in 2018 showing correlations with volatile compounds and sensory attributes. Projection of the samples; (**B**) Distribution of variables; (**C**) Compound codes as they appear in plot (**B**).

(**A**) (**B**) (**C**)



A2	(E)-2-penten-1-ol	M19	pentylcyclohexa-1,3-diene
A3	1-pentanol	M20	cis-dihydrocarvone
AL1	hexanal	M21	trans carveol
AL5	n-octanal	M23	L-carvone
AL6	m-tolualdehyde	M24	D-carvone
AL7	nonanal	M26	carvacrol
E1	methyl butanoate	MA1	p-mentha-2,8-dien-1-ol
E2	1-octen-3-yl-acetate	MA2	dihydrolinalool
E3	(E)-pinocarvyl acetate	MA5	(E)-8-hydroxylinalool
E4	carveol acetate	S1	α-ylangene
ALK1	nonane	S2	α-copaene
ALK2	decane	S3	(E)-β-caryophyllene
ALK5	tridecane	S4	β-caryophyllene
ALK6	tetradecane	S5	(+)-aromadendrene
ALK7	pentadecane	S6	curcumene
ALK8	hexadecane	S7	α-humulene
M1	α-thujene	S8	α-gurjunene
M2	α-pinene	S9	β-selinene
M3	camphene	S10	valencene
M4	sabinene	S11	α-selinene
M5	β-pinene	S12	kessane
M6	myrcene	P1	3-butylhexahydro phthalide
M7	α -phellandrene	P2	3-n-butylphthalide
M8	delta-3-carene	P4	sedanenolide
M9	α -terpinene	P5	trans-neocnidilide
M10	m-cymene	P6	(cis)-ligustilide
M11	limonene	O1	(Z)-limonene oxide
M12	β-(E)-ocimene	U1	unknown 1
M13	γ-terpinene	U2	unknown 2
M14	terpinolene	U3	unknown 3
M15	allo-ocimene	U4	unknown 4
M16	*p*-mentha-1,5,8-triene	U8	unknown 8
		U9	unknown 9

Figure 3.3. Principal component analysis of eight celery samples harvested in 2020 showing correlations with volatile compounds and sensory attributes. (**A**) Projection of the samples; (**B**) Distribution of the variables; (**C**) Compound codes as they appear in plot (**B**).

Firstly, a clear variation between the genotype was observed in 2018 (Figure 3.2) whereby principal component one (F1) and two (F2) explained 69.11 % of the total variation within the data. The first axis separates genotypes 5, 10, 18 and 22 from other genotypes, whereas the second axis separates genotypes 8, 15 and 12. Genotype 25 had low scores for most of the flavour attributes and only scored high in the watery and cucumber flavour. On the other hand, genotype 12 negatively correlated with genotype 25 and was associated with a parsley and grass-like odour with a rocket aftertaste. Genotype 18 was positively correlated to the fresh fennel flavour with the soapy characteristics that accompany many members of the Apiaceae family, such as coriander. A grouping of aroma compounds in the centre of the PCA was observed, whereas the sensory characteristics were positioned in the outer rim of the biplot with genotypes 5, 10 and 22 grouped in the middle of the observation plot. Apart from genotype 10, these exhibited an average volatile content (Table 3.1) compared to genotype 12 along with no strong association with sensory attributes (Figure 3.2). Many of the phthalides were associated with genotypes 12 and 10.

Overall, it seems that most monoterpenes were negatively correlated with the first principal component (F1) and compounds belonging to classes such as alcohols, sesquiterpenes and phthalides were positively associated with F1 along with most of the flavour attributes. Samples harvested in 2018 exhibited a lower proportion of monoterpenes but a higher proportion of alcohols and aldehydes, thus, explaining the low association with many of the flavour and aroma attributes from the sensory analysis.

In 2020, principal component one (F1) and two (F2) explained 65.96 % of the total variation present and it can be observed that the first axis separates genotypes 5, 8, 10, 15 and 22, whereas the second axis separates genotypes 12, 18 and 25. According to the data presented in Figure 3.3, the genotype appears to express a weaker influence over the volatile composition than in 2018, which explains 20.31 % of the variation present within the data. Differences in the volatile composition for the celery samples harvested in 2020 resulted in differences in the flavour perception. Compared to 2018 where genotypes 12, 18 and 25 were reported as the most distinctive, genotypes 5, 10, 12, 18, 22 and 25 became more distinguished from the remainder genotypes and displayed close associations with individual attributes. “Fresh fennel” was shown to be closely associated with genotype 18 in 2018 but became more strongly associated with genotypes 5 and 22 in 2020. In 2020, “fresh coriander”, “parsley”

and “grass green” positively correlated with F1 were associated with genotypes 8, 10, 12, 15 and 18, while the “fresh fennel” odour and flavour attributes in the top left quadrant (Figure 3.3) were associated with genotypes 5 and 22. The cucumber flavour remained in a similar position for both years, showing a close association to genotype 25. The most consistent genotype out of the eight was genotype 25 in terms of sensory and volatile profile; in both harvests, it appeared to be the least aromatic reflected by its close association to the cucumber flavour. Celery samples harvested in 2020 exhibited a higher proportion of monoterpenes which contribute to the herbal sensory attributes. Within the correlation matrix, fresh fennel exhibited many positive correlations with compounds that contribute to warm, herbal, sweet and spearmint odours such as trans-dihydrocarvone (M20), L-carvone (M24), (E)-β-caryophyllene (S3) and α-humulene (S7) as well as sedanenolide (P4) and (cis)-ligustilide (P6). Afifi, El-Mahis, Heiss and Farag (2021) classified 12 fennel varieties based on their aroma profile and similarities can be observed when comparing the monoterpene profile of celery in this study with the aroma profiles of the fresh fennel used by Afifi et al. (2021).

According to the results presented so far, samples harvested in 2020 had a more complex aroma profile leading to more flavourful genotypes compared to those harvested in 2018. Genotypes such as 10, 12 and 15 had a strong association with odour active compounds such as phthalides and, thus, associated with herbal flavour attributes such as fennel, coriander, and parsley. However, genotypes grown in 2018 expressed a higher proportion of phthalides, which suggests that the typical celery odour would be more noticeable in these celery genotypes. Thappa et al. (2003) investigated the variation of major components of genetically improved celery and reported that celery with a high phthalide content, such as those harvested in 2018, led to higher quality celery. The confirmation of whether this statement remains true for the celery used in this study requires the completion of consumer acceptability and preference trials.

3.5.3. Environmental Differences between Harvest Years and Influence on the Aroma Profile

In this study, clear differences in the volatile and sensory profile of the same genotypes grown in the same region of the United Kingdom across two different years were observed. Environmental

data including climatic variances in temperature, rainfall and relative humidity were collected at the nearest weather station to the farm of growth and provided by G's Fresh (Table 3.3). These environmental differences were hypothesised to influence the chemical composition within the crop. The daily air temperatures in 2018 (average 18 °C) were much higher than those in 2020 (average 14 °C). This change in temperature may have led to a warmer soil temperature in 2018, with a daily average presented to be over 7 °C warmer than in 2020. Although no differences in the volume of precipitation between years were observed, a large difference can be seen between the relative humidity. The impact of different growing conditions, such as temperature, on the flavour composition in celery is inadequately investigated and, within this experiment, only two growing seasons have been used; therefore, any conclusions that are drawn here can only be hypothesised. The utilisation of multiple years would generate more data and information about how celery responds to different climates and environments, which would produce a robust and vast dataset that will indicate more significant relationships between the plant's response towards the environment and confirm or disprove any of the theories discussed in this section.

Table 3.3. Environmental data recorded at the nearest weather station to the farm of celery growth and provided by G's Fresh.

Weeks after Field Transplant	2018				2020			
	Air Temp (°C)	Soil Temp (°C)	Rainfall (mm)	Relative Humidity (%)	Air Temp (°C)	Soil Temp (°C)	Rainfall (mm)	Relative Humidity (%)
1	17.0	17.1	0.0	73.0	9.8	9.6	0.1	82.0
2	14.7	17.3	0.0	81.3	11.4	10.7	0.0	74.6
3	16.4	18.1	0.1	66.1	9.4	9.9	0.0	67.9
4	17.0	24.4	0.0	94.8	16.7	16.9	0.0	63.3
5	18.9	27.9	0.0	98.5	15.7	17.3	0.0	62.3
6	19.8	28.6	0.0	99.7	14.4	16.1	0.0	71.1
7	18.2	25.5	0.0	99.4	12.0	12.6	0.0	86.4
8	20.4	29.0	0.0	99.0	17.2	18.3	0.2	80.7
9	21.4	26.7	0.1	70.5	19.6	21.5	0.0	69.1
10	20.9	27.7	0.0	71.8	16.0	18.6	0.0	78.9
11	17.3	20.7	0.2	99.9	16.0	17.6	0.2	86.6
12	18.4	28.6	0.0	98.6				
13	15.8	17.5	0.0	93.9				
Average	18.2	23.8	0.2	88.1	14.3	15.4	0.05	74.8

Being such a widely grown and consumed crop, it was expected that certain celery cultivars have been developed to grow under a range of temperatures. For example, cultivars EC 99249-1, RRL

85-1 and NRCSS-A have been identified as suitable for growth under the Indian climate, producing excellent essential oil content and high yield (Farooqui & Sreeramu, 2001; Malhotra & Vashishtha, 2008). However, climates with long growing seasons with temperatures between 16 °C and 21 °C, with light rainfall and suitable irrigation, are thought to be optimal growing conditions for celery (Malhotra, 2012). Kader (2008) identified that preharvest factors including environmental conditions (temperatures, rainfall, and wind speed) and agricultural techniques (planting density, irrigation, and pesticide regimes) resulted in a decline in flavour quality. For other crops, such as apples, that are dependent on ester formation for flavour, Fellman, Miller and Mattinson (2000) stressed the importance of genotype along with abiotic factors such as growing temperatures and cultural practices and they stated that these are "critical factors" involved in the synthesis of precursors involved in ester formation. Esters comprised a higher proportion of the aroma profile of celery grown in 2020 than celery grown in 2018 (Table 3.1), contributing to aroma such as fruity, apple and green and are shown to be associated with a grassy/green odour (Figure 3.3). With respect to celery, the lower temperatures exhibited in 2020 were preferable for ester formation.

The influence of temperature on isoprene formation, the smallest terpene unit and building block for more complex monoterpenes, has been discussed by Sharkey, Wiberley and Donohue (2008), whereby isoprene expresses a relationship with temperature and light and provides plant protection in the form of thermotolerance. Light and temperature have an influence in controlling the monoterpene and sesquiterpene plant emission as reported by Ibrahim et al. (2010), where the total monoterpene and sesquiterpene emissions in silver birch (*Betula pendula*) and European aspen (*Populus tremula*) trees increased at higher temperatures and peaked at 18 °C. Sesquiterpene content was positively correlated to temperature whilst monoterpenes expressed the opposite and was identified at higher abundances at lower temperatures. These findings support the volatile results from celery presented in Table 3.1, where the total sesquiterpene content was higher in 2018 when higher temperatures were recorded and, by contrast, monoterpenes comprised most of the aroma profile in 2020 when lower temperatures were observed. From these findings it can be hypothesised that sesquiterpenes act as a protective mechanism from heat stress within celery.

How phthalide compounds, the characteristic compounds imparting celery odour, react to different environmental stimuli have not previously been studied. Although existing research discusses the importance of their presence in celery samples, there is a poor understanding of how they are synthesised and what the factors that influence the abundance of these compounds are (Turner et al. 2021a). Sedanenolide made up the highest proportion of the phthalide profile in both 2018 and 2020, albeit much higher in 2018. Overall, samples harvested in 2018 had a higher total phthalide content than celery grown in 2020, which mimics a similar pattern to sesquiterpenoid compounds (Table 3.1) and thus, acting as a protective mechanism in response to the heat stress. Synthesising aromatic compounds is a standard response to abiotic stresses, such as temperature, in order to protect the crop (Yan, Li, Xu, Gu & Zhu, 2014). Possessing a lower total phthalide content in 2020 explained why aromas and flavours such as fresh coriander and parsley were revealed and are becoming more apparent to human assessors (Table 3.2).

3.6. Conclusions

Harvest year showed a stronger influence over the aroma composition of eight celery genotypes compared to genotypes, leading to differences in the aroma profile and, thus, creating sensory differences between two different years. Completing volatile analysis and sensory evaluation of the eight genotypes of celery demonstrated that the celery genotypes harvested in 2018 were perceived as being less herbal and associated with green aroma and cucumber flavour compared to the samples harvested in 2020. Samples harvested in 2020 imparted herbal flavour notes such as parsley, fennel and coriander, which are all members of the Apiaceae family potentially because these flavour notes were revealed when dominant aromas derived from phthalides were less abundant.

Although the genotypes were observed to play less of a role than the harvest year, the genetic make-up of the crop undoubtedly plays a role in predetermining the flavour profile as well as the capacity to synthesise aroma compounds in response to stress (Fellman, Miller & Mattinson, 2000; Kader, 2008; Sharkey, Wiberley & Donohue, 2008; Ibrahim et al., 2010), as shown by a high proportion of compounds expressing significant differences according to genotype, the variation caused by genotype and the variation in genotype perception from sensory evaluation. The eight genotypes used

in this study all exhibited clear differences within the aroma composition; however, less variation between years was apparent for genotype 25, which imparted a cucumber flavour and was less associated with aromatic compounds. Similarly genotype 12, with a strong fresh parsley odour, had a constant aroma profile over the two harvest years and expressed a high proportion of sesquiterpenes and phthalide compounds according to the volatile composition.

The influence of the environment on the aroma composition was also evident in this study with most of the compounds identified as significantly different between the two harvest years. The chemical composition was different in each year, with alcohol (including monoterpenoid alcohols), aldehyde, sesquiterpene and phthalide content all being in higher proportions in 2018. The warmer and dryer climates experienced in 2018 explain these compositional differences, particularly with sesquiterpene and phthalide compounds, which have been previously observed to act as a crop protective mechanism in response to heat stress. Taking into consideration these observations, the celery grown in 2018 would have a strong celery flavour but whether this would be preferred by the consumers requires consumer acceptability and preference trials for confirmation.

There is currently limited research to support the impact of the environment on the volatile composition and sensory profile of celery and, to confirm the environmental role, further work using controlled growth combined with sensory and chemical analysis needs to be carried out to provide a deeper understanding of the environmental relationship and how it affects volatile composition. Additionally, growing celery in alternative geographical locations would elucidate this relationship and provide more evidence as to how different environments affect the volatile composition. Providing explanations concerning the causes of aroma composition variation within celery, as well as other Apiaceae crops, will aid breeders to focus breeding programs on temperature resistant crops or steer fresh produce growers to utilise crops that are more resilient to the geographical climate of growth. These considerations, combined with regular inhouse taste panels and quality testing, will ultimately lead to better tasting crops with more stable flavour qualities.

3.7. Relative abundance

Table 3.4. Relative abundance of volatile compounds identified in the headspace of eight celery genotypes using SPME GC/MS and harvested in 2018 and 2020.

A)

		Relative abundance (mg/L)																		
		2018								2020								P-value		
	Identfied compound	5	8	11	12	15	18	22	25	5	8	11	12	15	18	22	25	G	E	Gx E
Codes	Alcohols																			
A1	3-methyl-3-butenol	1.91	2.09	4.28	2.66	1.43	1.19	1.26	2.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
A2	(*E*)-2-pentenol	3.20	2.59	2.62	1.65	2.32	3.31	4.57	2.39	0.34	0.39	0.73	0.03	0.99	0.35	0.48	0.24	***	***	***
A3	pentanol	1.00	0.83	1.54	1.14	1.57	2.27	3.11	1.55	0.24	0.16	0.30	0.29	0.66	0.77	0.69	0.53	ns	**	ns
	Aldehydes																			
AL1	hexanal	40.64	8.48	10.07	4.57	15.45	42.40	47.82	28.48	1.41	1.13	1.53	1.68	1.56	1.72	1.35	1.83	**	**	**
AL2	(*E*)-2-hexenal	1.78	1.99	2.00	1.32	0.79	1.60	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*	*	*
AL3	heptanal	0.46	0.00	0.13	0.41	0.18	0.31	0.00	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*	*	*
AL4	*(E)*-2-heptenal	10.26	2.43	1.36	1.70	2.39	2.63	2.81	1.30	1.57	2.37	1.96	4.48	3.60	2.96	2.79	0.21	ns	ns	ns
AL5	octanal	0.45	0.00	0.27	2.16	0.73	0.40	0.68	0.75	1.60	1.62	1.47	3.19	1.21	1.33	1.50	1.05	ns	ns	ns
AL6	*meta*-tolualdehyde	2.91	6.19	2.25	3.21	2.18	4.02	2.48	4.28	0.24	0.17	0.06	0.29	0.20	0.27	0.35	0.00	***	***	***
AL7	nonanal	1.27	1.86	1.39	2.41	1.40	2.46	1.69	1.36	0.92	0.54	1.37	0.61	0.48	0.60	0.85	0.54	***	***	***
AL8	(*E*,*Z*)-2,6-nonadienal	1.17	1.80	1.16	1.99	1.47	1.27	1.38	1.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**	**	**
	Esters																			
E1	methyl butanoate	0.22	0.10	0.24	0.21	0.23	0.21	0.22	0.14	0.00	0.04	0.00	0.09	0.19	0.22	0.13	0.05	ns	ns	ns

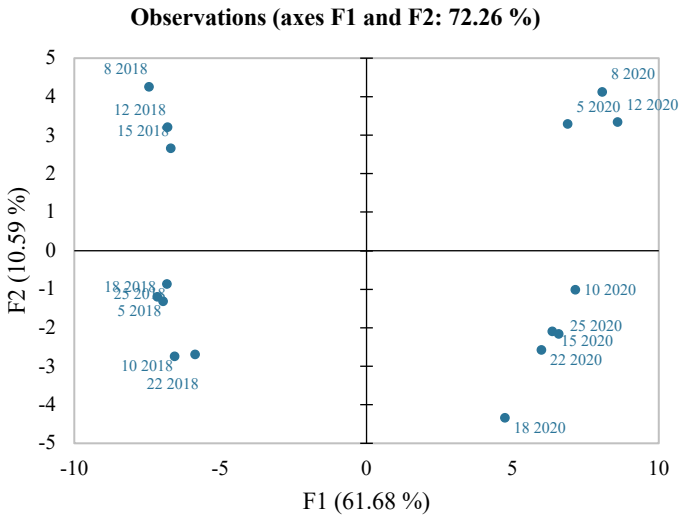
E2	1-octen-3-yl-acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.12	0.72	0.53	0.06	0.13	0.00	0.43	***	***	***
E3	*(E)*-pinocarvyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.18	3.95	2.86	1.75	2.70	3.20	1.32	1.72	***	***	***
E4	carveol acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.59	1.31	1.37	0.96	1.13	0.56	0.21	0.61	***	***	***
E5	hexy isobutanoate	1.19	1.77	0.74	0.35	1.12	1.04	0.88	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
	Alkanes																			
ALK 1	nonane	0.33	0.00	0.93	1.10	1.80	1.12	1.23	1.15	1.80	4.00	4.82	4.43	3.37	2.29	1.78	1.97	**	**	**
ALK 2	decane	0.48	0.00	1.93	2.03	2.87	2.66	2.11	2.40	1.27	1.37	0.66	0.74	1.20	1.67	1.18	1.03	*	*	*
ALK 3	undecane	1.47	0.78	0.79	0.63	1.13	0.79	0.73	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
ALK 4	dodecane	1.76	2.45	1.29	1.46	1.86	0.95	0.82	1.36	3.51	3.56	1.10	1.37	0.75	0.61	0.60	0.54	ns	ns	ns
ALK 5	tridecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.49	5.45	1.34	1.92	0.93	0.62	0.64	0.49	ns	ns	ns
ALK 6	tetradecane	0.14	0.35	0.00	0.00	0.14	0.00	0.00	0.00	4.46	4.57	1.68	2.98	0.34	0.80	0.89	0.82	ns	ns	ns
ALK 7	pentadecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83	2.63	1.13	1.97	1.13	0.65	0.84	0.84	***	***	***
ALK 8	hexadecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.87	0.47	0.86	0.30	0.21	0.24	0.24	***	***	***
ALK 9	heptadecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.51	0.22	0.63	4.11	3.51	0.31	0.26	ns	ns	ns
	Monoterpenes																			
M1	α-thujene	2.84	6.39	2.37	4.96	6.60	5.13	2.45	3.73	1.00	0.87	0.63	1.74	0.75	1.31	0.90	0.99	**	**	**
M2	α-pinene	1.00	10.52	6.31	3.91	11.48	16.07	17.73	8.15	2.30	1.36	1.31	0.61	0.69	0.80	0.74	2.84	***	***	***
M3	camphene	1.96	2.30	2.12	2.08	1.95	2.45	2.32	1.82	0.99	1.43	1.14	1.94	1.38	2.43	1.68	0.56	ns	ns	ns

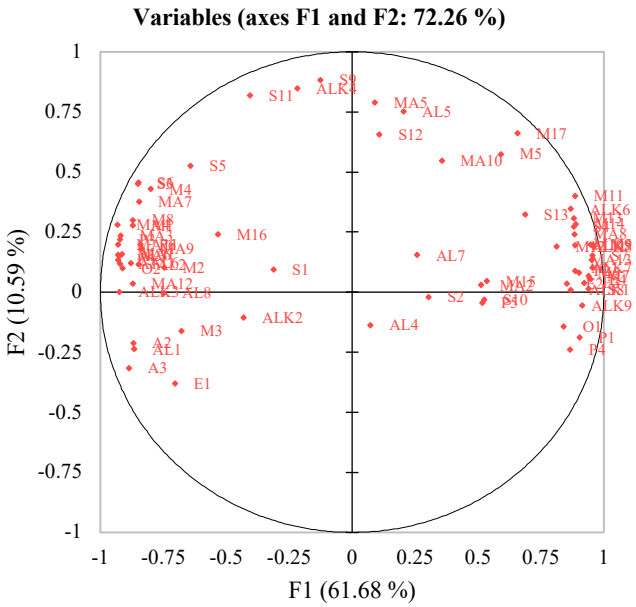
M4	sabinene	13.43	38.92	4.45	42.54	25.92	15.12	4.31	28.20	2.34	2.64	2.19	4.95	1.57	2.71	1.75	1.67	**	**	**
M5	β-pinene	5.19	14.21	9.65	19.95	10.73	11.95	4.72	6.70	25.14	40.63	11.38	68.91	24.85	0.67	18.83	34.53	***	***	***
M6	myrcene	3.56	3.25	0.00	2.89	4.44	5.76	7.81	4.04	16.80	28.37	49.96	100.36	13.81	10.78	10.44	14.75	***	***	***
M7	α-phellandrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.95	3.22	2.64	3.78	2.72	2.86	3.25	3.10	***	***	***
M8	delta-3-carene	19.66	25.91	15.23	28.91	24.42	28.18	14.45	20.47	0.36	0.34	0.09	0.64	0.00	0.70	0.00	0.20	***	***	***
M9	α-terpinene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.06	4.39	2.43	4.44	2.19	2.04	1.81	3.48	***	***	***
M10	m-cymene	1.49	1.75	16.52	8.15	6.46	1.02	1.21	7.92	77.70	67.88	36.50	100.01	28.18	39.59	35.70	42.82	***	***	***
M11	limonene	180.86	301.91	143.22	238.96	268.23	183.07	159.60	180.68	475.99	602.75	394.18	583.48	433.43	319.34	372.79	420.52	***	***	***
M12	β-trans-ocimene	0.89	1.31	0.69	1.87	1.20	0.85	2.11	0.94	3.18	2.55	2.05	5.83	2.35	1.54	7.33	3.00	***	***	***
M13	γ-terpinene	19.35	31.50	15.64	44.77	38.07	31.93	11.21	31.77	145.98	164.49	69.58	189.27	53.88	71.46	56.81	103.43	***	***	***
M14	terpinolene	1.24	1.09	0.77	0.61	1.71	0.86	0.37	0.61	6.66	7.94	5.16	8.80	5.41	4.47	5.06	6.20	***	***	***
M15	*allo*-ocimene	1.20	0.68	0.92	4.37	1.77	0.80	2.71	1.10	2.92	1.40	1.54	7.23	1.92	1.44	10.47	2.93	***	***	***
M16	*p*-mentha-1,5,8-triene	2.61	4.41	1.11	1.63	1.92	1.93	0.27	2.47	0.57	0.30	0.34	1.51	0.38	0.28	2.07	0.62	***	***	***
M17	pentylcyclohexa-1,3-diene	0.95	2.10	0.71	1.40	1.14	0.89	0.31	1.25	3.21	3.60	1.53	4.38	1.83	0.96	1.37	1.79	***	***	***
	Monoterpenoid alcohols																			
MA1	(+)-*cis*-p-mentha-2,8-dienol	0.52	0.81	0.44	2.36	1.67	0.75	3.33	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA2	dihydrolinalool	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.40	0.00	0.00	0.05	0.00	***	***	***
MA3	Pinocarveol trans	0.99	1.76	1.03	3.48	2.06	0.38	1.56	1.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***

MA4	Terpinen-4-ol	2.24	2.64	1.82	2.34	2.41	2.27	2.20	2.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA5	*cis*-dihydrocarvone	0.17	0.87	0.26	0.75	0.97	0.36	0.31	0.24	0.48	0.65	0.48	0.46	0.43	0.18	0.37	0.29	*	*	*
MA6	*trans*-carveol	0.52	0.54	0.45	0.30	0.54	0.51	0.41	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA7	*trans*-dihydrocarvone	3.56	5.27	2.61	3.03	3.98	2.35	1.75	3.11	0.77	1.37	1.30	0.76	0.94	0.33	0.57	0.60	***	***	***
MA8	*cis*-p-mentha-1(7),8-diene-2-ol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.34	0.34	0.27	0.25	0.00	0.18	0.15	***	***	***
MA9	*cis*-carveol	4.42	4.12	5.92	5.26	5.92	3.27	3.68	5.28	0.00	0.29	0.39	0.33	0.27	0.00	0.16	0.00	***	***	***
MA10	L-carvone	0.68	0.88	0.28	1.03	1.56	0.63	0.77	0.48	1.95	1.43	0.55	1.06	0.69	1.10	0.90	0.73	ns	ns	ns
MA11	thymol	2.40	2.64	1.80	4.52	2.02	2.10	0.84	2.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA12	carvacrol	1.11	2.09	0.76	0.72	1.09	0.55	0.80	0.73	0.00	0.16	0.09	0.21	0.08	0.21	0.23	0.11	***	***	***
MA13	(E)-8-hydroxylinalool	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.72	0.64	0.48	0.34	0.16	0.05	0.20	***	***	***
	Sesquiterpenes																			
S1	*α*-ylangene	4.74	6.13	2.72	0.68	1.10	2.60	3.29	4.06	0.73	3.43	1.84	3.30	1.01	1.31	0.96	1.97	***	***	***
S2	α-copaene	0.49	0.45	0.26	0.27	0.72	0.55	0.21	0.37	0.22	4.63	1.95	0.36	0.08	0.93	1.84	2.98	*	*	**
S3	(E)-β-caryophyllene	20.07	38.08	16.68	18.43	31.05	21.53	10.76	10.89	0.15	0.32	0.06	0.08	0.00	0.00	0.00	0.00	***	***	***
S4	β-caryophyllene	0.78	1.52	0.58	0.40	0.95	0.76	0.32	0.54	13.97	31.71	15.80	17.62	11.44	10.37	5.45	6.97	***	***	***
S5	(+)-aromadendrene	0.80	1.59	0.77	0.70	1.05	1.13	0.37	0.63	0.33	1.01	0.48	0.45	0.23	0.22	0.19	0.29	***	***	***
S6	curcumene	1.43	3.90	1.23	3.29	3.07	1.92	0.54	0.99	0.19	0.48	0.23	0.21	0.00	0.00	0.00	0.00	***	***	***
S7	α-humulene	1.92	4.87	1.50	3.80	3.80	2.12	0.83	1.32	1.42	5.43	1.55	3.91	2.68	0.76	0.73	1.01	***	***	***

S8	α-gurjunene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.97	0.58	0.62	0.47	0.51	0.61	0.65	***	***	***
S9	β-selinene	13.70	18.09	5.88	33.78	16.18	9.71	13.98	14.96	22.83	17.18	6.58	18.26	8.18	4.50	6.54	12.10	***	***	***
S10	valencene	0.09	0.00	0.30	0.00	0.74	0.37	0.16	0.50	0.00	1.33	0.45	33.58	0.50	0.38	0.71	1.29	***	***	***
S11	α-selinene	2.75	4.14	1.79	4.70	3.90	2.31	2.93	2.97	2.94	3.45	1.95	2.93	1.46	0.74	1.41	2.39	**	**	**
S12	kessane	0.20	0.83	0.96	0.35	0.56	0.46	0.13	0.00	1.84	1.13	0.06	21.82	0.42	0.06	0.21	0.11	***	***	***
S13	β-gurjuene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.10	0.00	0.57	0.06	0.04	0.00	0.00	***	***	***
	Phthalides																			
P1	3-butylhexahydrophthalide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.13	0.07	0.03	0.15	0.12	0.06	**	**	**
P2	Butylphthalide 3	0.66	1.22	1.41	1.18	1.53	0.95	1.26	1.01	8.25	5.74	6.60	10.83	4.76	4.94	9.44	7.32	***	***	***
P3	Butylidene phthalide 3Z	21.87	64.53	60.20	126.32	93.92	56.00	56.80	72.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
P4	Sedanenolide	1.14	1.61	7.27	1.18	2.02	4.35	4.94	4.76	12.85	8.22	16.06	23.39	10.21	16.50	15.98	10.22	***	***	***
P5	trans-neocnidilide	0.54	0.94	1.01	1.76	1.62	0.85	0.86	1.03	3.45	1.30	0.98	0.94	10.10	3.09	3.05	1.74	ns	ns	ns
P6	(E)-ligustilide	0.52	0.91	0.97	1.70	1.56	0.82	0.83	1.00	0.27	0.17	0.07	0.20	0.55	0.21	0.07	0.07	***	***	***
	Oxides																			
O1	(Z)-limonene oxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.52	5.98	8.33	7.07	3.56	10.30	5.23	***	***	***
O2	Caryophyllene oxide	22.25	34.61	37.98	50.25	48.93	37.34	47.84	37.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***

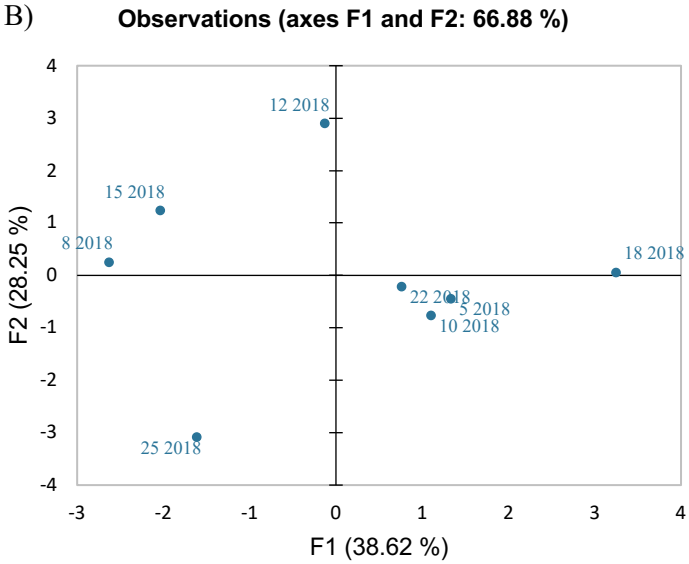
Linear retention indices can be found in table 3.1. Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level

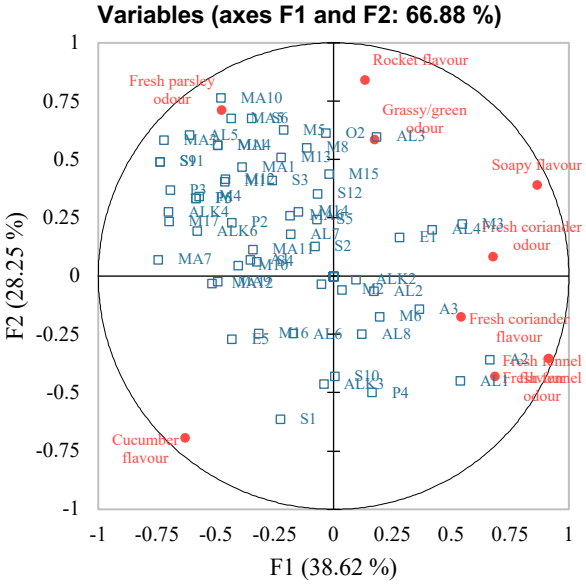

Observations (axes F1 and F2: 72.26 %)
F1 (61.68 %)
F2 (10.59 %)
8 2018
12 2018
15 2018
8 2020
5 2020
12 2020
10 2020
25 2020
15 2020
22 2020
18 2020
10 2018
22 2018
18 2018
25 2018
5 2018


Variables (axes F1 and F2: 72.26 %)
F1 (61.68 %)
F2 (10.59 %)

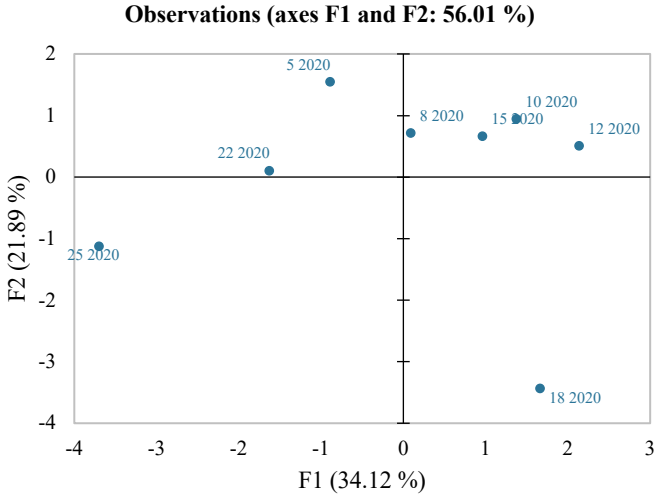
2398

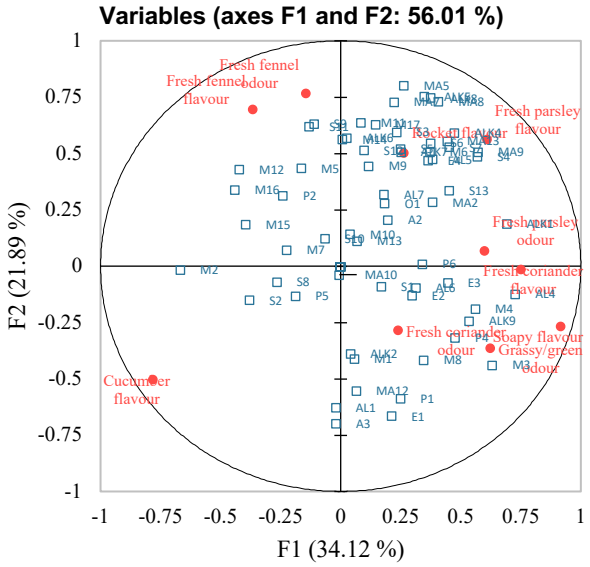
B)


Observations (axes F1 and F2: 66.88 %)
F1 (38.62 %)
F2 (28.25 %)
12 2018
15 2018
8 2018
18 2018
22 2018
5 2018
10 2018
25 2018


Variables (axes F1 and F2: 66.88 %)
F1 (38.62 %)
F2 (28.25 %)
Rocket flavour
Fresh parsley odour
Grassy/green odour
Soapy flavour
Fresh coriander odour
Fresh coriander flavour
Fresh fennel flavour
Fresh fennel odour
Cucumber flavour

2399


Observations (axes F1 and F2: 56.01 %)
F1 (34.12 %)
F2 (21.89 %)
5 2020
8 2020
10 2020
15 2020
12 2020
22 2020
25 2020
18 2020


Variables (axes F1 and F2: 56.01 %)
F1 (34.12 %)
F2 (21.89 %)
Fresh fennel flavour
Fresh fennel odour
Fresh parsley flavour
Fresh parsley odour
Fresh coriander flavour
Fresh coriander odour
Soapy flavour
Grassy/green odour
Cucumber flavour

C)

Figure 3.4. Principal component analysis of the 2018 and 2020 UK harvest using relative abundance (A) volatile components (B) volatile components of 2018 harvest with sensory attributes (C) volatile components of 2020 harvest with sensory attributes.

An alternative method of observing changes in the volatile profile in celery can be done by calculating approximate abundances in accordance with the internal standard. Where the results that were presented as percentage composition in Figures 3.1, 3.2 and 3.3, by adding 50 µl of 100 mg/L propyl propanoate (internal standard) and using the peak area, absolute quantities can be calculated. The findings observed in Table 3.4 and biplots A, B and C, form similar conclusions as those presented in Figures 3.1, 3.2 and 3.3. The separation between years remains clear when presenting the data as relative abundance however, the spread of the data is different to Figure 3.1, where the data points in 2020 was observed to be placed much closer together, exhibiting less variation than the 2018 data. However, the spread of data for 2018 and 2020 presented in biplot A are very much mirrored with both genotypes 5 and 12 in the same position but on the opposite sides of the plot, like genotypes 10 and 22, also. Overall, the harvest conditions of 2018 displayed a stronger influence over the volatile profile of the eight celery genotypes displayed by the higher number of compounds that were positively associated with this harvest year. Monoterpenes (M16, M22, M25, M26), monoterpenoid alcohols (MA1, MA3, MA4) and phthalides (P3, P4) were positively associated with 2018 whereas sesquiterpenes (S2, S9, S11, S12) and monoterpenes (M6, M7, M12, M13, M15) were positively associated with 2020. This confirms the findings stated within this chapter using percentage composition.

On first glance, the sensory plot presented in B appear to have changed when using relative abundance, however, this is not the case. The association of genotypes to sensory attributes remains the same with genotype 12 a rocket and fresh parsley flavour, genotype 18 associated with a fresh coriander odour, genotype 25 associated with a cucumber flavour and genotypes 5, 10 and 22 not displaying any strong association to any sensory attribute. Perhaps the biggest change is observed in the 2020 harvest where the distribution of genotypes and their association differs. There is a significant separation of genotype 18 from the other data points, expressing a close association to grass/green odour and soapy flavour, like what was displayed in Figure 3.3, however, genotype 12, which was originally situated close to genotype 18 no longer displays the same association. Genotype 12 now reflects a positive association with rocket and fresh parsley odour, as it did in 2018. Genotypes 5, 22 and 25 display the

same associations as they did with percentage composition (Figure 3.3) but genotypes 8, 10 and 15 display a similar flavour profile to genotype 12.

3.8. References

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CHAPTER 4: Investigating the relationship of genotype and geographical location on volatile composition and sensory profile of celery (*Apium graveolens*)

4.1. Introduction to paper (As published in the International Journal of Molecular Sciences, Special issue - Breeding Next Generation Vegetables: Improving Flavour and Functional Quality, Nov 2021, 6;22(21):12016)

Once comparison between harvest seasons (2018 and 2020) using the same location (UK) was complete and observed significant differences in the volatile content in all eight genotypes were linked to significant differences in the sensory characteristics, it was decided that the same eight genotypes would be grown in a different geographical location. Spain was chosen as a suitable geographical location for growth, offering vast environmental differences including weather conditions, agricultural practices, water and soil composition and field placement in comparison to the UK harvest. Furthermore, during the winter months, where celery cannot be grown in the UK, celery is grown in Spain and then transported to the UK for consumers, ensuring availability all year round. As used in Chapter 3 and put forward in Chapter 1, the minimum information for a plant aroma experiment (MIAPAE) was used to provide details of the harvest, postharvest and analysis to ensure the experiment is repeatable and interpreted correctly.

Analysis of the aroma profile of celery was previously studied by others, as observed in chapter 1, however these experiments have their limitations with no multisite or multiyear investigation occurring using the same celery cultivars. Completing an experiment whereby the compositional differences in the same cultivar/s are examined over a period and in different locations can help us gain a better understanding of how abiotic and biotic factors influence the aroma profile. Furthermore, utilising a trained sensory panel will determine whether these significant differences observed in the volatile composition will influence the perceived flavour. By combining the data collected in chapter 2 with the data collected in the current chapter, we would have completed a multi-year and multi-site experiment using the same eight celery genotypes and therefore, identify what has not been determined in celery before: (1) what the biggest influencers on the celery aroma composition are (2) the impact of changing the aroma composition upon the sensory profile (3) whether compositional changes are

observed within all genotypes and (4) whether all genotypes respond to these stresses in a similar manner. We aim to identify compound groups that respond differently, either occurring significantly higher or lower in the volatile composition according to geographical location and then link this with differences in the sensory profile. The information collected in this chapter will help educate UK and Spanish celery growers on the influencers of celery aroma and the impact they have on the sensory characteristics and by examining the environmental differences and assessing their influence on the volatile composition, we can guide growers in producing a consistent, high-quality product.

Sections 4.2 – 4.7 have been published in International Journal of Molecular Sciences (See Appendix VII for pdf version of the submitted manuscript).

4.2. Abstract

Numerous varieties of celery are grown in multiple countries to maintain supply, demand, and availability for all seasons. Therefore, there is an expectation for a consistent celery product in terms of taste, flavour, and overall quality. Differences in climate, agronomy and soil composition will all contribute to inconsistencies. The study investigated the volatile and sensory profile of eight celery genotypes grown in UK (2018) and Spain (2019). Solid phase microextraction followed by gas chromatography/mass spectrometry determined the volatile composition of eight genotypes followed by assessment of the sensory profile using a trained panel. Significant differences in the volatile composition and sensory profile were observed, genotype and geographical location both exerted influence. Two genotypes exhibited similar aroma composition and sensory profile in both locations, making them good candidates to drive breeding programmes aimed at producing varieties that consistently display these distinctive sensory properties. Celery samples harvested in the UK exhibited a higher proportion of sesquiterpenes and phthalides, whereas samples harvested in Spain expressed a higher aldehyde and ketone content. We hypothesise that genotype, along with the differences in the availability of micronutrients, will alter the production of secondary metabolites in response to abiotic stresses, leading to a change in the volatile composition. Studying the relationship between growing

environment and genotype will provide information to guide growers in how to consistently produce a high-quality crop.

4.3. Introduction

Apium graveolens, commonly known as celery, is a vegetable with long fibrous stalks belonging to the Apiaceae or Umbelliferae family characterised by its discoid or 'umbrella' shaped flowers known as umbels. Like other members of the Apiaceae family, including carrots, coriander and parsley, celery possesses a strong, distinct flavour profile, placing it as a key component in soups, stocks and sauces (Rozėk, 2007; Malhotra, 2012). Compounds that constitute the aroma profile include a range of monoterpenes (myrcene, limonene, β-pinene and γ-terpinene), sesquiterpenes (β-caryophyllene, α-humulene, α- and β-selinene) and phthalides (sedanenolide, neocnidilide and 3-n-butylphthalide) (Malhotra, 2012; Uhlig, Chang & Jen, 1987; Orav, Kailas & Jegorova, 2003; Sellami, Bettaieb, Bourgou, Dahmani, Limam & Marzouk, 2012; Macleod & Ames, 1989; Turner, Lignou, Gawthrop & Wagstaff, 2021). The latter compounds have been displayed throughout literature to be the characteristic odour compounds to celery (Turner et al., 2021a), with odour characteristics identified by Turner, Dawda, Gawthrop, Wagstaff and Lignou (2021b) of 'celery', 'cooked celery' and 'herbal'. Celery has long been grown and consumed globally and for this reason, the aroma profile has been studied by using a range of cultivars, grown in a variety of years and geographical locations and analysed using extraction methods including solvent assisted flavour extraction (SAFE) and solid phase microextraction (SPME) and most typically followed by gas chromatography/mass spectrometry (GCMS) (Uhlig, Chang & Jen, 1987; Orav, Kailas & Jegorova, 2003; Sellami et al., 2012; Macleod & Ames, 1989; Turner et al., 2021b). Possibly the earliest investigation completed by Gold and Wilson (1963), determined the volatile composition of celery juice using distillation followed by gas chromatography. This identified a collection of compounds ranging from aldehydes, esters, alcohols and most importantly, phthalides. More recent work completed, not only confirms the compounds identified by Gold and Wilson (1963) but displays the complex aroma profile of celery and the variety of compound groups that comprise the aroma profile (Turner et al., 2021a)

As a commonly used vegetable, there is an expectation for celery to be available continuously for consumers, however, in countries such as the United Kingdom this is not possible due to the unfavourable winter temperatures and conditions. During the summer months, celery can be grown in the UK as weather conditions are suitable for growth and often celery can continue to be grown on the East Coast through Autumn. Nevertheless, the annual consumer demand for celery is not met. To combat this issue, celery is grown in warmer locations, such as southern Spain where they are packaged and processed and then transported to UK retailers. Although offering a solution to meet the demand, utilising seasons in Spain means growing in arid and semi-arid conditions, requiring different agronomy compared to that needed for the UK's growing environment and thus creating inconsistencies within the aroma quality of the celery produce available. While not thoroughly understood within celery, the influence of abiotic and biotic factors upon the aroma of crops in general has been investigated by others and differences have been observed (Turner et al., 2021a; Turner, Lignou Gawthrop & Wagstaff, 2021c; Marongui et al., 2013; Rożek, Nurzyńska-Wierdak & Kosior, 2013; van Wassenhove, Dirinck, Schamp & Vulsteke, 1990). Exposure to different stresses such as temperature, relative humidity, soil, and water compositions have been shown to influence the production of primary and secondary metabolites, ultimately leading to variation within the volatile composition (Turner et al., 2021a; Turner et al. 2021c). Previously, Turner et al. (2021c) observed significant differences in the volatile composition and sensory profile of eight celery genotypes grown in the same geographical location in 2018 and 2020. Despite genotype displaying significant interactions, it was the differences in environment over the two seasons that had a stronger influence over the volatile composition of celery. The review recently completed by the authors (Turner et al., 2021a), combined data from previously published experiments that investigated the aroma profile of celery, identifying missing data through the exclusion of information including cultivar name, origin, location of growth, harvest year, and conditions of growth. Exposing variation in the presence or absence of compounds and their composition within celery, the authors concluded that without stating all experimental information, the data becomes unrepeatable. To overcome this, the authors put forward Minimum Information About a Plant Aroma Experiment (MIAPAE), inviting authors to include parameters used during preharvest, harvest and

postharvest as well as extraction and analysis methods, allowing for the building of a repository whereby aroma data for plants can repeated and interpreted correctly (Turner et al., 2021a).

Albeit limited, investigations exploring the impact of geographical locations on celery have been completed; Marongiu et al. (2013) compared the volatile composition of wild celery grown and collected in Portugal and Italy as well as using different extraction methods (super critical fluid extraction and hydrodistillation). Differences in the composition caused by both the geographical location and extraction method were observed. Phthalide compounds including sedanenolide and neocnidilide expressed significant differences according to these factors. Ultimately concluding that environmental differences between Portugal and Italy were the main cause of observed compositional differences. The cultivar of the wildtype celery used in this study was not included or differences in agricultural techniques and growing environments. However, observed variances in the aroma composition in celery caused by these factors has previously been displayed. Rożek et al. (2013) identified drought stress led to an increase in essential oil due to an increase in the production of secondary metabolites whereas van Wassenhove et al. (1990) observed changes in the phthalide and terpene content when nitrogenous fertiliser (organic and/or inorganic) was applied to celery.

This study aims to investigate the relationship between genotype and geographical location of cultivation site upon the volatile composition of eight celery varieties grown in Ely, UK in 2018 and Águilas, Spain in 2019. By growing eight genotypes in the UK as well as Spain, the influence of geographical location and its environmental conditions over the aroma profile of celery can be investigated. Sensory evaluation using a trained panel was completed to understand how chemical and physiological changes lead to differences in the organoleptic perception and to identify interactions between compound groups and geographical location. Ultimately, this information can be used to assist breeders and growers to develop and select cultivars that are optimal for specific growing environments, to produce a consistently flavoured product. Although factors such as temperature and relative humidity are uncontrollable, growers can apply organic/inorganic fertilisers, herbicides/fungicides, and supplementary irrigation to aid optimal conditions for celery growth

4.4. Materials and Methods

4.4.1. Celery material and MIAPAE standard

4.4.1.1. Sample Information

The eight varieties used in these field trials were chosen due to their differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each line used in this paper, the origins of these parental breeding lines and their image postharvest can be found in Appendix IX. Prior to GC/MS analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis. As expected, volatile loss was observed between fresh and freeze-dried samples however, consistency in relative amount was observed throughout repetitions and the most reported compounds were also identified. Freeze-drying is a method that has been used previously to preserve the volatile content of herbs (Lisiewska & Kmiecik, 1998; Diaz-Maroto, Palomo, Castro, González Viñas, & Pérez-Coello, 2004; Rołson, Osińska & Wajs-Bonikowska, 2013) and furthermore, Hoffman (2007) identified freeze-drying as a preservation method that best retains a typical aroma at a strong intensity.

4.4.1.2. Timing, Location and Environment

Celery seed (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd (Cobham, United Kingdom) were grown in commercial conditions and harvested in Cambridgeshire (United Kingdom) by G's Fresh Ltd (Ely, United Kingdom (52°21'12.9"N 0°17'15.6"E) during spring/summer 2018. In 2019, the same eight parental varieties of celery were grown and harvested in Águilas, Spain by G's España Ltd (37°25'43.2"N 1°39'56.2"W).

Celery grown in the UK was grown on sandy loam soils with naturally high groundwater and a peaty surface, whereas celery grown in Spain was grown on Calcisol soils. Both harvests were grown in a randomised block design, using commercial celery products as border plants to remove edge effects and subject to commercial conditions including application of agronomic techniques, fertilizer, and irrigation as commercial celery. For both years, 20 – 25 mm of overhead irrigation was used every four days, and standard commercial fertiliser, pest and disease control regimes were applied. In 2018, plugs were transplanted mid-June after 22 days growing in the nursery then harvested 91 days later. The average daily air temperature was 18.2 °C, 0.2 mm of rainfall daily and an average relative humidity of

88.1 %. Average wind speed was 1.9 m/s and dew point was 15.5 °C. In 2019, plugs were transplanted early January after 20 days growing in the nursery then harvested late-March, 87 days later. The average daily air temperature was 17.6 °C, 0.4 mm of average rainfall and an average relative humidity of 77.3 %. Average wind speed was 1.7 m/s and dew point was 6.0 °C. Prior to harvest, the celery was subject to regular in-field assessment to ensure standards for commercial quality are met, including visual and taste tests. These celeries were harvested within a close timeframe of the commercial produce also being grown in the field, which acted as an indicator for the appropriate commercial harvest maturity.

4.4.1.3 Raw material collection, processing storage

The celery was grown at a density of 10 plants per m^2 and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles and sealed in labelled bags for transportation to the University of Reading (United Kingdom). Harvesting in Spain followed the same procedure; however, celery was packed into cool boxes and transported to the UK in refrigerated conditions using G's Fresh Ltd courier. Transportation took two days and samples were collected from G's Fresh (Ely, Cambridgeshire) before transportation back to the University of Reading.

Celery samples used for sensory evaluation were refrigerated for one day before presenting to the trained panel whereas samples for aroma analysis were immediately frozen at -80 °C for one week and subsequently freeze-dried for five days. Samples were then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ) and stored in an airtight container for a maximum of two weeks before analysis with gas chromatography/mass spectrometry (GC/MS).

4.4.2. Chemicals Reagents

For GC/MS analysis, calcium chloride and the alkane standard C6-C25 (100 μg/mL) in diethyl ether were obtained from Merck (Poole, UK).

4.4.3. Volatile analysis using SPME GCMS

The celery sample (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution, and filled to 5 mL using HPLC-grade water in a 15 mL SPME vial fitted with a screw cap. Samples were analysed by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m 250 µm 0.25 µm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of the injector, interface and detector was 250 °C and the sample injection mode was splitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions.

4.4.4. Sensory Profiling

Sensory evaluation was carried out using quantitative descriptive analysis (QDA^{TM}) to determine the sensory characteristics of the eight celery samples, and the characteristics were estimated quantitatively. The trained sensory panel at the Sensory Science Centre (University of Reading, n=12; 11 female and 1 male) was used to develop a consensus vocabulary to describe the sensory characteristics of the eight celery genotypes. The terms were discussed by the panellists as a group, facilitated by a panel leader, and this led to a consensus of 22 and 23 attributes for the UK and Spanish

harvest respectively. The sensory assessment of the samples was carried out according to Turner et al (2021c) at the Sensory Science Centre (University of Reading) using Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Guelph, ON, Canada) to acquire the data.

4.4.5. Statistical analysis

The percentage composition was calculated from the peak area data collected by SPME GC/MS analysis and quantitative data for each compound identified in the SPME GC/MS analysis were analysed by both one- and two-way analysis of variance (ANOVA) and principal component analysis using Spearman's correlation (PCA) on XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($P<0.05$) between geographical location and the celery genotypes. Only those compounds exhibiting significant differences between geographical location (G), genotype (E), and their interaction (GxE) were included in the PCA. To compose the PCA plots that combine both sensory and instrumental data, the volatile data was added as supplementary data on top of the flavour and aroma attributes.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out ANOVA of sensory panel data. The means from sensory data were taken over two sessions for all assessors and correlated with the percentage composition means from the instrumental data via PCA using XLSTAT

4.5. Results and Discussion

4.5.1. Volatile composition

In total, 118 compounds were detected in the headspace of the eight celery genotypes in both geographical locations (UK and Spain) (Table 4.1). Sixty-five compounds were identified in 2018 across eight genotypes, including: 22 monoterpenes, ten sesquiterpenes, eight aldehydes, five alcohols (three of which are classified as monoterpenoid alcohols) and five phthalides. Additional compounds were identified in the headspace of the same genotypes from the Spanish harvest including: 27 monoterpenes, 17 aldehydes, 11 sesquiterpenes and alcohols (six of which are classified as monoterpenoid alcohols), nine ketones and six phthalides. Quantitative differences were observed

between the two geographical locations as well as the eight genotypes in this study and two-way ANOVA revealed significant differences in aroma difference caused by both factors. Where Spanish grown celery displays higher alcohol, aldehyde and ketone content, UK grown celery expresses a much higher monoterpene, sesquiterpene and phthalide content. Seventeen compounds expressed no significant difference in relative amount by these factors and seven of these came from lower boiling compounds including camphene, sabinene and β-pinene along with D-carvone and carvacrol. These low boiling monoterpenes were not observed to differ significantly when harvested in 2018 and 2020 in the UK (Turner et al., 2021c), suggesting that monoterpenes are fundamental to the crop and factors including genotype and climate hold limited influence over the abundance of these compounds.

Table 4.1. Percentage composition of volatile compounds identified in the headspace of eight celery genotypes using SPME GC/MS and harvested in UK and Spain

				Percentage Composition (%)[C]																	
				UK								Spain								P-value[D]	
Code	Compound	LRI$_{exp}$ [A]	ID[B]	5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G[E]	E[F]	GxE G
	Alcohols																					
A1	3-methyl-3-buten-1-ol	730	A	0.42± 0.08^{abc}	0.31± 0.04^{ab}	0.94± 0.27^{c}	0.35± 0.14^{abc}	0.22± 0.07^{a}	0.23± 0.06^{a}	0.30± 0.12^{ab}	0.39± 0.06^{abc}	0.60± 0.35^{abc}	0.40± 0.06^{ahc}	0.91± 0.27^{bc}	0.59± 0.13^{abc}	0.36± 0.05^{abc}	0.57± 0.22^{abc}	0.54± 0.02^{abc}	0.49± 0.13^{abc}	**	**	**
A2	2-methyl-1-butanol	742	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.01^{ab}	0.10± 0.03^{ab}	0.12± 0.02^{b}	0.11± 0.01^{ab}	nd^{a}	0.10± 0.04^{ab}	0.10± 0.05^{ab}	0.10± 0.02^{ab}	***	**	***
A3	(E)-2-pentenol	758	A	0.73± 0.28^{ab}	0.42± 0.16^{ab}	0.64± 0.04^{ab}	0.23± 0.08^{a}	0.32± 0.09^{ab}	0.65± 0.23^{ab}	1.2± 0.54^{ab}	0.50± 0.22^{ab}	0.72± 0.34^{ab}	1.3± 0.25^{b}	1.1± 0.18^{ab}	0.71± 0.09^{ab}	0.60± 0.09^{ab}	0.81± 0.31^{ab}	0.87± 0.24^{ab}	0.52± 0.06^{ab}	**	*	*
A4	pentanol	763	A	0.21± 0.06^{a}	0.11± 0.04^{a}	0.31± 0.20^{a}	0.13± 0.10^{a}	0.23± 0.15^{a}	0.39± 0.14^{ab}	0.63± 0.25^{ab}	0.28± 0.08^{a}	1.6± 0.27^{b}	0.50± 0.11^{a}	0.76± 0.28^{ab}	0.49± 0.06^{a}	1.1± 0.13^{ab}	0.87± 0.34^{ab}	1.5± 0.51^{b}	0.88± 0.22^{ab}	***	**	***
A5	hexanol	862	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.53± 0.19^{ab}	0.44± 0.27^{ab}	0.79± 0.44^{b}	0.40± 0.21^{ab}	0.33± 0.08^{ab}	0.40± 0.10^{ab}	0.48± 0.14^{ab}	0.47± 0.23^{ab}	***	**	***
	Total			**1.4**	**0.84**	**1.9**	**0.71**	**0.77**	**1.3**	**2.1**	**1.2**	**3.5**	**2.7**	**3.7**	**2.3**	**2.4**	**2.7**	**3.5**	**2.5**			
	Aldehydes																					
AH1	2-methyl-2-butenal	739	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.16± 0.07^{bc}	0.15± 0.08^{bc}	0.14± 0.06^{bc}	0.13± 0.02^{abc}	0.23± 0.03^{c}	0.19± 0.04^{bc}	0.19± 0.05^{bc}	0.10± 0.03^{ab}	***	**	***
AH2	(E)-2-pentenal	753	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.78± 0.04^{c}	0.13± 0.08^{a}	0.34± 0.14^{ab}	nd^{a}	0.78± 0.08^{c}	0.80± 0.36^{c}	0.77± 0.09^{bc}	0.38± 0.11^{abc}	***	**	***
AH3	hexanal	800	A	9.7± 0.8^{a}	1.3± 0.46^{a}	2.6± 0.32^{a}	0.65± 0.29^{a}	2.0± 0.39^{a}	8.9± 2.7^{a}	13± 5.5^{a}	6.3± 1.2^{a}	25± 7.8^{a}	24± 6.2^{a}	14± 5.2^{a}	8.6± 3.6^{a}	22± 7.5^{a}	24± 4.9^{a}	25± 7.0^{a}	22± 6.3^{a}	**	**	**
AH4	(E)-2-hexenal	849	A	0.18± 0.11^{abc}	tr ± 0.02^{a}	tr ± 0.02^{a}	0.04± 0.01^{ab}	0.03± 0.03^{a}	0.15± 0.11^{abc}	0.20± 0.08^{abc}	0.11± 0.05^{abc}	0.56± 0.13^{c}	0.57± 0.24^{c}	0.30± 0.10^{abc}	0.30± 0.07^{abc}	0.55± 0.11^{c}	0.54± 0.19^{c}	0.57± 0.15^{c}	0.51± 0.20^{bc}	***	**	***
AH5	heptanal	901	A	tr ± 0.03^{ab}	nd^{a}	0.28± 0.15^{ab}	0.16± 0.13^{ab}	0.25± 0.16^{ab}	0.23± 0.14^{ab}	0.29± 0.08^{ab}	0.25± 0.15^{ab}	0.68± 0.18^{b}	0.58± 0.18^{ab}	0.51± 0.13^{ab}	0.48± 0.10^{ab}	0.49± 0.35^{ab}	0.57± 0.13^{ab}	0.61± 0.20^{ab}	0.72± 0.12^{b}	**	**	**
AH6	(E)-2-heptenal	954	A	0.19± 0.22^{a}	1.6± 0.55^{ab}	1.6± 0.23^{ab}	0.52± 0.04^{a}	1.5± 0.10^{ab}	3.2± 1.5^{abc}	4.2± 1.3^{abc}	1.8± 0.97^{ab}	6.4± 0.75^{bcd}	8.1± 0.23^{cd}	6.0± 0.36^{bcd}	6.1± 0.64^{bcd}	11± 0.55^{d}	7.8± 0.33^{cd}	7.3± 0.45^{cd}	7.5± 0.40^{cd}	***	**	***
AH7	benzaldehyde	969	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	3.3± 1.8^{b}	1.7± 0.50^{ab}	1.9± 0.14^{b}	1.9± 0.26^{b}	1.7± 0.10^{ab}	1.6± 0.48^{ab}	1.7± 0.22^{ab}	1.9± 0.22^{b}	***	**	***
AH8	octanal	1007	A	0.10± 0.10^{ab}	nd^{a}	0.49± 0.06^{abcd}	0.27± 0.06^{abc}	0.39± 0.19^{abcd}	0.51± 0.26^{abcd}	0.51± 0.17^{abcd}	0.51± 0.23^{abcd}	0.86± 0.19^{cd}	0.95± 0.22^{cde}	0.56± 0.10^{abcd}	0.63± 0.13^{abcd}	1.6± 0.35^{e}	0.78± 0.21^{bcd}	0.54± 0.04^{abcd}	1.0± 0.22^{de}	***	**	***
AH9	phenylacetaldehyde	1049	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.31± 0.13^{bc}	0.24± 0.04^{bc}	0.26± 0.06^{bc}	0.42± 0.06^{c}	0.26± 0.02^{bc}	0.24± 0.06^{bc}	0.23± 0.98^{b}	0.29± 0.05^{bc}	***	**	***
AH10	2-E-octenal	1057	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	3.3± 1.3^{b}	2.2± 1.5^{ab}	1.5± 0.39^{ab}	1.4± 0.39^{ab}	3.4± 0.89^{b}	3.5± 1.2^{b}	2.8± 0.96^{b}	3.5± 1.0^{b}	***	**	***
AH11	m-tolualdehyde	1086	B[1]	0.33± 0.07^{a}	0.24± 0.02^{a}	4.0± 0.28^{c}	1.1± 0.28^{ab}	0.95± 0.02^{ab}	0.19± 0.02^{a}	0.26± 0.05^{a}	1.6± 0.29^{b}	0.72± 0.57^{ab}	0.66± 0.26^{ab}	0.71± 0.17^{ab}	0.91± 0.19^{ab}	0.64± 0.06^{ab}	0.68± 0.32^{ab}	0.57± 0.10^{a}	0.97± 0.08^{ab}	***	**	***
AH12	nonanal	1105	A	0.33± 0.14^{abc}	0.12± 0.02^{ab}	0.20± 0.03^{abc}	0.10± 0.01^{a}	0.17± 0.03^{abc}	0.16± 0.10^{abc}	0.22± 0.17^{abc}	0.19± 0.09^{abc}	0.68± 0.11^{c}	0.59± 0.18^{abc}	0.39± 0.10^{b}	0.35± 0.13^{abc}	0.57± 0.16^{abc}	0.64± 0.35^{bc}	0.61± 0.08^{abc}	0.59± 0.11^{abc}	***	**	***

AH13	(E,E)-2,4-octadienal	1110	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.15± 0.05[b]	0.13± 0.04[b]	0.11± 0.01[b]	0.13± 0.03[b]	0.16± 0.02[b]	0.15± 0.03[b]	0.14± 0.05[b]	0.20± 0.02[b]	***	**	***
AH14	(E,Z)-2,6-nonadienal	1162	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.10± 0.06[ab]	0.15± 0.03[ab c]	0.11± 0.02[ab c]	0.12± 0.02[abc]	0.29± 0.10[c]	0.23± 0.02[bc]	0.23± 0.16[bc]	0.28± 0.05[c]	***	**	***
AH15	(E)-2-nonenal	1165	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.10± 0.03[ab]	0.10± 0.02[ab]	tr ± 0.03[ab]	0.14± 0.02[b]	0.10± 0.01[ab]	0.10± 0.01[ab]	tr ± 0.05[ab]	0.12± 0.10[b]	***	**	***
AH16	myrtenal	1207	B[2]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.19± 0.02[ab]	0.14± 0.02[a]	0.10± 0.03[a]	0.11± 0.01[a]	0.16± 0.04[ab]	0.15± 0.04[ab]	0.10± 0.06[a]	0.37± 0.21[b]	***	**	***
AH17	(E,E)-2,6-nonadienal	1156	A	0.21± 0.04[ab]	0.30± 0.03[ab]	0.18± 0.02[ab]	0.18± 0.04[ab]	0.17± 0.03[ab]	0.16± 0.08[ab]	tr ± 0.03[a]	0.22± 0.08[ab]	0.36± 0.11[ab]	0.48± 0.24[b]	0.20± 0.03[ab]	0.16± 0.05[ab]	0.41± 0.11[ab]	0.35± 0.11[ab]	0.46± 0.22[ab]	0.20± 0.17[ab]	*	*	*
	Total			**11**	**3.6**	**9.4**	**3.0**	**5.5**	**14**	**19**	**11**	**44**	**41**	**28**	**23**	**44**	**44**	**43**	**41**			
	Esters																					
E1	methyl butanoate	717	A	tr ± 0.03[ab c]	tr ± 0.01[a]	tr ± 0.02[ab c]	tr ± 0.01[ab]	tr ± 0.02[ab]	tr ± 0.04[ab]	tr ± 0.05[ab]	tr ± 0.01[ab]	0.22± 0.14[cd]	0.18± 0.01[ab cd]	0.25± 0.04[d]	0.17± 0.01[abc d]	0.18± 0.04[ab cd]	0.18± 0.04[ab cd]	0.16± 0.02[abcd]	0.19± 0.03[bc d]	***	**	***
E2	methyl pentanoate	837	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.34± 0.23[b]	0.24± 0.02[ab]	0.37± 0.13[b]	0.40± 0.09[b]	0.23± 0.07[ab]	0.39± 0.18[b]	0.27± 0.05[ab]	0.30± 0.05[ab]	***	**	***
E3	Methyl hexanoate	921	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.25± 0.12[ab]	0.29± 0.16[ab]	0.12± 0.01[ab]	0.10± 0.03[ab]	0.25± 0.09[ab]	0.38± 0.10[b]	0.28± 0.10[bc]	0.24± 0.11[ab]	***	**	***
E4	carveol acetate	1343	B[3]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.21± 0.05[bc]	0.14± 0.02[ab]	0.22± 0.04[bc]	0.17± 0.04[bc]	0.20± 0.04[bc]	0.27± 0.08[bc]	0.20± 0.05[a]	0.29± 0.10[c]	***	**	***
E5	hexyl isobutanoate	1378	B[4]	0.10± 0.03	0.10± 0.04	0.14± 0.02	tr ± 0.03	0.10± 0.05	0.16± 0.04	0.32± 0.06	0.12± 0.03	0.15± 0.12	0.15± 0.12	0.40± 0.04	0.22± 0.11	0.18± 0.13	0.11± 0.16	0.36± 0.23	0.13± 0.11	ns	ns	ns
	Total			**0.14**	**0.10**	**0.20**	**0.07**	**0.11**	**0.19**	**0.36**	**0.14**	**1.2**	**1.0**	**1.4**	**1.0**	**1.0**	**1.3**	**1.3**	**1.2**			
	Ketones																					
K1	2-methyl-3-pentanone	746	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.10± 0.05[ab]	0.10± 0.02[ab]	0.19± 0.02[b]	0.10± 0.01[ab]	0.10± 0.01[a]	0.10± 0.02[ab]	0.10± 0.01[ab]	0.10± 0.02[ab]	***	**	***
K2	3-heptanone	884	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.14± 0.05[a]	0.13± 0.08[a]	0.12± 0.08[a]	tr ± 0.02[a]	0.10± 0.03[a]	0.13± 0.01[a]	0.13± 0.03[a]	0.13± 0.04[a]	***	**	**
K3	2-heptanone	889	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.49± 0.14[b]	0.48± 0.15[b]	0.31± 0.08[ab]	0.17± 0.12[ab]	0.39± 0.08[ab]	0.49± 0.12[b]	0.44± 0.16[b]	0.56± 0.18[b]	***	**	**
K4	1-octen-3-one	976	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	3.0± 0.55[b]	3.9± 1.7[b]	2.9± 0.17[b]	2.3± 0.35[ab]	4.4± 0.61[b]	3.3± 0.73[b]	3.5± 1.3[b]	3.9± 0.95[b]	***	**	**
K5	(E,E)-3,5-octadien-2-one	1070	B[5]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.79± 0.14[b]	1.1± 0.29[b]	0.60± 0.14[ab]	0.81± 0.23[b]	1.3± 0.15[b]	0.82± 0.19[b]	1.3± 0.41[b]	0.63± 0.45[ab]	***	**	***
K6	acetophenone	1073	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.30± 0.16[b]	0.25± 0.16[b]	0.27± 0.05[b]	0.31± 0.04[b]	0.25± 0.01[b]	0.26± 0.07[b]	0.28± 0.07[b]	0.29± 0.02[b]	***	**	***
K7	3,5-octadien-2-one	1092	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	2.2± 0.65[b]	2.4± 1.1[b]	0.92± 0.38[ab]	0.81± 0.32[ab]	2.1± 0.77[b]	2.2± 1.0[b]	2.2± 0.81[b]	2.1± 0.91[ab]	***	**	***
K8	p-methyl-acetophenone	1179	B[6]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.11± 0.04[ab]	0.10± 0.01[a]	tr ± 0.03[a]	0.10± 0.04[a]	0.10± 0.04[ab]	nd[a]	0.10± 0.05	0.22± 0.10[b]	***	**	*
K9	dihydrojasmone	1378	A	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.62± 0.33[ab]	0.69± 0.38[b]	0.06± 0.04[ab]	0.17± 0.13[ab]	0.71± 0.36[b]	0.63± 0.26[ab]	0.30± 0.21[ab]	0.57± 0.15[ab]	***	**	***
	Total			**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7.8**	**9.1**	**5.4**	**4.8**	**9.4**	**7.9**	**8.3**	**8.5**			
	Alkanes																					

ALK 1	nonane	900	A	0.41± 0.15^{ab}	0.32± 0.11^{ab}	0.43± 0.19^{ab}	0.14± 0.18^{a}	0.13± 0.10^{a}	0.28± 0.11^{ab}	nd^{a}	0.17± 0.02^{a}	0.84± 0.44^{ab}	0.62± 0.36^{ab}	0.69± 0.21^{ab}	0.27± 0.14^{a}	1.7± 0.34^{b}	0.41± 0.06^{ab}	0.36± 0.16^{ab}	0.90± 0.35^{ab}	*	*	*
ALK 2	decane	1000	A	0.80± 0.24$^{ab cd}$	0.49± 0.13^{ab}	nd^{a}	0.37± 0.11^{ab}	0.60± 0.26^{abc}	1.1± 0.21$^{bcd e}$	1.7± 0.29^{ef}	0.83± 0.33$^{ab cd}$	1.6± 0.18^{def}	1.7± 0.33^{ef}	1.5± 0.36$^{cd ef}$	1.6± 0.05^{def}	2.2± 0.21^{f}	1.9± 0.05^{ef}	1.9± 0.18^{ef}	1.6± 0.19^{def}	***	***	***
ALK 3	undecane	1100	A	0.26± 0.15$^{ab cd}$	0.14± 0.09	0.19± 0.11$^{ab cd}$	0.04± 0.05^{a}	0.24± 0.06^{abc}	0.14± 0.10^{abc}	0.07± 0.08^{a}	0.11± 0.06^{ab}	0.60± 0.31^{cd}	0.27± 0.10$^{ab cd}$	0.57± 0.04$^{bc d}$	0.63± 0.02^{f}	0.55± 0.03$^{bc d}$	0.33± 0.03$^{ab cd}$	0.43± 0.12^{abcd}	0.52± 0.05$^{ab cd}$	***	***	***
ALK 4	dodecane	1199	A	0.48± 0.08	0.37± 0.03	0.46± 0.05	0.31± 0.10	0.33± 0.10	0.44± 0.13	0.46± 0.10	0.44± 0.12	0.48± 0.23	0.20± 0.03	0.37± 0.10	0.31± 0.05	0.26± 0.03	0.29± 0.03	0.27± 0.04	0.34± 0.08	ns	ns	ns
ALK 5	tridecane	1299	A	nd	nd	nd	nd	nd	nd	nd	nd	0.16± 0.03	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
ALK 6	tetradecane	1399	A	0.11± 0.02	tr ± 0.03	tr ± 0.02	tr ± 0.03	0.10± 0.06	0.10 ± 0.03	tr ± 0.03	0.10± 0.02	0.16± 0.12	tr ± 0.03	tr ± 0.01	tr ± 0.01	tr ± 0.01	tr ± 0.03	tr ± 0.02	0.10± 0.06	ns	ns	ns
ALK 7	pentadecane	1499	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.15± 0.02^{a}	nd^{a}	tr ± 0.05^{a}	nd^{a}	0.18± 0.02^{a}	0.14± 0.01^{a}	0.14± 0.02^{a}	nd^{a}	**	**	**
	Total			**2.1**	**1.4**	**1.1**	**0.94**	**1.4**	**2.1**	**2.3**	**1.6**	**4.0**	**2.8**	**3.2**	**2.8**	**4.9**	**3.1**	**3.1**	**3.4**			
	Monoterpenes																					
M1	α-thujene	933	B[7]	0.27± 0.09	0.24± 0.08	0.29± 0.13	0.30± 0.11	0.22± 0.10	0.41± 0.19	0.32± 0.14	0.22± 0.13	0.64± 0.31	0.52± 0.19	1.1± 0.17	0.78± 0.20	0.42± 0.02	0.58± 0.14	0.64± 0.06	0.72± 0.22	ns	ns	ns
M2	α-pinene	943	A	0.62± 0.05	0.85± 0.22	0.52± 0.19	0.62± 0.18	1.0± 0.42	0.89± 0.20	0.43± 0.20	0.62± 0.31	0.83± 0.14	0.49± 0.26	1.0± 0.30	0.81± 0.16	0.77± 0.33	0.69± 0.10	1.1± 0.58	0.75± 0.46	ns	ns	ns
M3	camphene	960	A	2.5± 0.5	0.33± 0.07	0.29± 0.12	0.21± 0.08	0.35± 0.10	0.48± 0.05	0.66± 0.26	0.22± 0.08	0.73± 0.21	0.57± 0.05	0.93± 0.05	0.94± 0.13	0.73± 0.12	0.45± 0.32	0.96± 0.11	0.68± 0.14	ns	ns	ns
M4	sabinene	981	A	0.44± 0.13	0.33± 0.04	0.66± 0.39	0.27± 0.04	0.28± 0.05	0.45± 0.03	0.53± 0.13	0.36± 0.06	0.37± 0.25	0.29± 0.08	0.34± 0.19	0.32± 0.09	0.31± 0.08	0.38± 0.15	0.30± 0.07	0.34± 0.07	ns	ns	ns
M5	β-pinene	989	A	3.0± 0.64	5.2± 1.6	0.96± 0.36	5.4± 1.6	3.8± 1.6	2.7± 0.99	0.79± 0.24	4.5± 1.1	2.3± 0.63	2.1± 1.1	1.5± 0.38	2.6± 0.65	3.5± 1.4	1.1± 0.18	2.5± 1.3	2.9± 1.9	ns	ns	ns
M6	myrcene	992	A	1.1± 0.26$^{ab c}$	1.9± 0.64$^{ab c}$	2.6± 0.74^{bc}	2.6± 0.22^{bc}	1.6± 0.37^{abc}	2.1± 0.61^{abc}	0.84± 0.34^{ab}	1.1± 0.45$^{ab c}$	0.51± 0.03^{a}	0.54± 0.19^{ab}	1.8± 0.46$^{ab c}$	1.4± 0.06^{abc}	0.48± 0.10^{a}	1.1± 0.25$^{ab c}$	0.56± 0.18^{ab}	0.51± 0.05^{a}	***	***	***
M7	α -phellandrene	1013	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.37± 0.16^{bc}	0.31± 0.03^{b}	0.52± 0.06^{c}	0.40± 0.06^{bc}	0.33± 0.04^{b}	0.39± 0.03^{bc}	0.39± 0.07^{bc}	0.37± 0.03^{bc}	***	***	***
M8	delta-3-carene	1019	A	0.24± 0.10	0.23± 0.18	0.25± 0.04	0.25± 0.12	0.22± 0.11	0.21± 0.10	0.32± 0.09	0.23± 0.05	0.72± 0.33	0.69± 0.39	0.94± 0.74	0.63± 0.44	0.54± 0.30	0.58± 0.30	0.77± 0.38	0.77± 0.46	ns	ns	ns
M9	m-cymene	1032	A	4.3± 0.61	3.6± 0.41	3.5± 0.69	3.8± 0.43	3.4± 0.78^{a}	5.0± 0.71	2.8± 0.61	3.7± 0.55	3.8± 0.94	3.7± 1.1	4.6± 1.3	3.4± 0.67	2.3± 0.94	3.9± 0.82	3.4± 1.5	3.3± 1.1	ns	ns	ns
M10	limonene	1034	A	39± 8.2^{bc}	43± 0.56^{c}	33± 5.1^{abc}	32± 2.3^{abc}	39± 3.1^{bc}	32± 4.5^{abc}	29± 3.9^{abc}	33± 3.1^{abc}	11± 4.9^{a}	19± 1.9^{abc}	24± 7.6^{abc}	21± 2.1^{abc}	11± 6.1^{a}	12± 5.1^{a}	15± 5.3^{ab}	11± 5.3^{a}	***	***	***
M11	β-(E)-ocimene	1049	B[8]	0.19± 0.01^{a}	0.18± 0.07^{a}	0.17± 0.02^{a}	0.24± 0.03^{a}	0.17± 0.02^{a}	0.16± 0.02^{a}	0.42± 0.08^{a}	0.18± 0.02^{a}	1.3± 0.91^{ab}	0.71± 0.32^{a}	nd^{a}	nd^{a}	1.7± 0.29^{ab}	1.1± 0.28^{a}	nd^{a}	3.1± 0.43^{b}	***	***	***
M12	γ-terpinene	1066	A	4.2± 1.2^{bcd}	4.3± 1.2^{bcd}	3.6± 0.60$^{ab cd}$	5.9± 0.28^{d}	5.6± 0.27^{cd}	5.5± 1.4^{cd}	2.1± 0.90^{ab}	5.6± 1.4^{d}	0.72± 0.12^{a}	2.6± 1.4^{abcd}	2.2± 0.36$^{ab c}$	2.0± 0.35^{ab}	1.2± 0.24^{ab}	1.1± 0.24^{ab}	1.1± 0.20^{ab}	1.1± 0.36^{ab}	***	***	***
M13	terpinolene	1097	A	0.62± 0.19$^{ab c}$	0.89± 0.07^{c}	0.53± 0.09$^{ab c}$	0.43± 0.01$^{ab c}$	0.36± 0.22^{abc}	0.73± 0.20^{bc}	0.57± 0.14$^{ab c}$	0.90± 0.31^{c}	0.35± 0.08^{abc}	0.25± 0.18$^{ab c}$	0.13± 0.08^{ab}	0.20± 0.14^{ab}	0.38± 0.14$^{ab c}$	0.34± 0.14$^{ab c}$	nd^{a}	0.25± 0.18$^{ab c}$	***	***	**
M14	allo-ocimene	1132	B[9]	0.11± 0.06^{ab}	0.10± 0.01^{ab}	0.10± 0.05^{ab}	0.31± 0.03^{b}	0.24± <0.01$^{a b}$	0.13± 0.04^{ab}	0.31± 0.27^{b}	0.13± 0.08^{ab}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	***	**
M15	β-thujone	1124	B[2]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.02^{ab}	tr ± 0.02^{a}	0.10±	0.20± 0.04^{c}	tr ± 0.02^{ab}	0.10± 0.02^{ab}	0.17± 0.12^{bc}	0.10± 0.02^{ab}	***	***	***

												0.01^{abc}							
M16	*p*-mentha-1,5,8-triene	1135	B[10]	0.26± 0.05^{ab}	0.10± 0.01^{ab}	0.22± 0.02^{ab}	0.56± 0.09^{b}	0.26± 0.07^{ab}	0.13± 0.09^{ab}	0.49± 0.17^{ab}	0.19± 0.08^{ab}	0.10± 0.02^{ab}	tr ± 0.02^{a}	0.16± 0.04^{ab}	0.55± 0.15^{ab}	0.10± 0.01^{ab}	0.17± 0.05^{ab}	0.50± 0.27^{ab}	0.10± 0.06^{ab}	**	**	**
M17	*trans*-carveol	1147	B[2]	0.48± 0.13^{bcd}	0.57± 0.17^{cd}	0.23± 0.08^{abc}	0.18± 0.08^{ab}	0.24± 0.02^{ab}	0.31± 0.21^{abc}	tr ± 0.03^{a}	0.13± 0.10^{ab}	0.51± 0.07^{cd}	0.45± 0.21^{bcd}	0.65± 0.09^{d}	0.44± 0.02^{bcd}	0.34± 0.07^{abcd}	0.51± 0.14^{cd}	0.26± 0.09^{abcd}	0.60± 0.23^{d}	***	**	***
M18	pentylcyclohexa-1,3-diene	1166	B[2]	0.20± 0.05^{ab}	0.23± 0.08^{ab}	0.25± 0.03^{ab}	0.46± 0.11^{abc}	0.31± 0.03^{ab}	0.10± 0.04^{a}	0.26± 0.16^{ab}	0.20± 0.01^{ab}	0.20± 0.06^{ab}	0.13± 0.09^{a}	0.19± 0.08^{ab}	0.20± 0.02^{ab}	0.16± 0.05^{ab}	0.19± 0.02^{ab}	0.12± 0.09^{a}	0.30± 0.14^{ab}	*	*	*
M19	*cis*-dihydrocarvone	1208	A	0.39± 0.09^{b}	0.36± 0.05^{b}	0.35± 0.08^{b}	0.19± 0.06^{ab}	0.27± 0.05^{ab}	0.18± 0.04^{ab}	0.20± 0.08^{ab}	0.26± 0.02^{ab}	0.35± 0.03^{b}	0.28± 0.02^{ab}	0.30± 0.05^{b}	0.25± 0.06^{ab}	0.23± 0.12^{ab}	0.20± 0.14^{ab}	nd^{a}	0.39± 0.06^{b}	**	**	**
M21	camphor	1157	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.27± 0.15^{bc}	0.17± 0.04^{abc}	0.22± 0.06^{abc}	0.17± 0.05^{abc}	0.18± 0.08^{abc}	0.23± 0.06^{bc}	0.15± 0.03^{ab}	0.38± 0.13^{c}	**	**	***
M22	isoborneol	1173	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.25± 0.14^{b}	0.17± 0.03^{ab}	0.16± 0.06^{ab}	0.17± 0.04^{ab}	0.19± 0.04^{ab}	0.25± 0.04^{b}	0.18± 0.05^{ab}	0.23± 0.12^{b}	**	**	***
M23	*trans*-dihydrocarvone	1240	B[10]	0.79± 0.12^{f}	0.79± 0.14^{f}	0.67± 0.10^{ef}	0.41± 0.08^{cde}	0.57± 0.09^{ef}	0.43± 0.05^{de}	0.38± 0.06^{bcde}	0.59± 0.03^{ef}	0.10± 0.03^{ab}	0.10± 0.04^{a}	0.10± 0.02^{ab}	0.10± 0.01^{ab}	0.10± 0.02^{a}	0.11± 0.03^{abc}	tr ± 0.04^{a}	0.14± 0.09^{abcd}	**	**	***
M24	β-cyclocitral	1230	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.04^{b}	0.12± 0.02^{b}	0.11± 0.03^{b}	0.18± 0.02^{b}	0.15± 0.01^{b}	0.12± 0.02^{b}	0.10± 0.01^{b}	0.14± 0.06^{b}	**	**	***
M25	L-carvone	1248	A	0.96± 0.19^{bcd}	0.57± 0.11^{abc}	1.5± 0.05^{d}	0.71± 0.06^{abc}	0.81± 0.13^{abcd}	0.61± 0.14^{abc}	0.75± 0.17^{abcd}	1.1± 0.12^{cd}	0.38± 0.22^{abc}	0.26± 0.11^{ab}	0.18± 0.06^{ab}	0.14± 0.02^{a}	0.23± 0.08^{ab}	0.36± 0.03^{abc}	0.17± 0.08^{ab}	0.45± 0.23^{abc}	**	**	***
M26	D-carvone	1262	A	0.43± 0.19	0.36± 0.10	0.24± 0.02	0.18± 0.03	0.23± 0.08	0.34± 0.15	0.44± 0.07	0.29± 0.06	0.33± 0.13	0.27± 0.06	0.60± 0.13	0.36± 0.17	0.30± 0.10	0.48± 0.11	0.52± 0.11	0.47± 0.18	ns	ns	ns
M27	thymol	1290	A	0.17± 0.05^{b}	0.11± 0.14^{ab}	0.12± 0.04^{ab}	0.15± 0.09^{ab}	0.11± 0.08^{ab}	0.10± 0.03^{ab}	nd^{a}	0.14± 0.11^{ab}	0.15± 0.09^{ab}	0.12± 0.07^{ab}	0.15± 0.01^{ab}	0.16± 0.01^{ab}	0.12± 0.01^{ab}	0.19± 0.08^{b}	0.10± 0.03^{ab}	0.16± 0.05^{ab}	*	*	*
M28	carvacrol	1317	A	0.54± 0.08	0.42± 0.09	0.45± 0.03	0.60± 0.02	0.29± 0.03	0.39± 0.03	0.18± 0.04	0.52± 0.04	0.44± 0.21	0.36± 0.27	0.45± 0.05^{a}	0.53± 0.08	0.31± 0.12	0.56± 0.23	0.19± 0.07	0.39± 0.14	ns	ns	ns
	Total			**61**	**64**	**50**	**56**	**59**	**53**	**42**	**54**	**27**	**34**	**42**	**38**	**26**	**27**	**29**	**30**			
	Monoterpenoid Alcohols																					
MA1	p-mentha-2,8-dien-1-ol	1122	A	0.10± 0.03	0.15± 0.01	tr ± 0.03	0.28± 0.03	0.10± 0.02	0.10± 0.03	tr ± 0.03	0.14± 0.01	0.15± 0.03	0.16± 0.01	0.15± 0.03	0.13± 0.02	0.12± 0.07	0.13± 0.02	0.12± 0.03	0.19± 0.13	ns	ns	ns
MA2	dihydrolinalool	1142	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.75± 0.31^{abc}	0.33± 0.26^{abc}	0.93± 0.08^{bc}	1.2± 0.06^{c}	0.78± 0.18^{abc}	0.64± 0.30^{abc}	0.29± 0.11^{ab}	0.48± 0.24^{abc}	**	**	***
M20	*trans*-pinocarveol	1147	B[11]	0.59± 0.13^{a}	0.63± 0.17^{a}	0.30± 0.08^{a}	0.20± 0.08^{a}	0.28± 0.02^{a}	0.35± 0.21^{a}	tr ± 0.06^{a}	0.45± 0.10^{a}	0.29± 0.09^{a}	0.21± 0.10^{a}	0.11± 0.06^{a}	0.10± 0.01^{a}	0.20± 0.10^{a}	0.47± 0.32^{a}	0.15± 0.03^{a}	0.57± 0.42^{a}	*	*	*
MA3	terpinen-4-ol	1184	A	0.10± 0.01^{ab}	nd^{a}	tr ± 0.03^{a}	tr ± 0.03^{ab}	tr ± 0.03^{a}	0.10± 0.07^{ab}	nd^{a}	0.13± 0.03^{ab}	0.10± 0.09^{ab}	0.15± 0.04^{ab}	0.13± 0.03^{ab}	0.18± 0.02^{b}	0.10± 0.04^{ab}	0.15± 0.06^{ab}	nd^{a}	0.20± 0.04^{b}	**	**	***
MA5	α-terpineol	1211	A	nd	nd	nd	nd	nd	nd	nd	nd	0.10± 0.04	nd	0.10± 0.01	0.10± 0.01	tr ± 0.03	0.10± 0.01	tr ± 0.03	0.13± 0.09	ns	ns	ns
MA4	(E)-8-hydroxylinalool	1349	B[2]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.19± 0.05^{b}	0.15± 0.06^{b}	0.10± 0.04^{ab}	0.10± 0.01^{ab}	0.10± 0.02^{ab}	0.18± 0.03^{b}	0.10± 0.06^{ab}	0.18± 0.05^{b}	**	**	***
MA6	caryophylladienol II	1665	B[2]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.05^{b}	nd^{a}	0.10± 0.01^{b}	0.10± 0.02^{b}	0.10± 0.01^{b}	0.11± 0.03^{b}	0.10± 0.02^{b}	0.10± 0.03^{b}	**	**	***
	Total			**0.79**	**0.78**	**0.38**	**0.53**	**0.39**	**0.48**	**0.06**	**0.72**	**1.6**	**1.0**	**1.6**	**1.9**	**1.4**	**1.8**	**0.77**	**1.7**			
	Sesquiterpenes																					

S1	α-ylangene	1384	B[10]	0.26± 0.11^{c}	0.24± 0.07^{c}	0.17± 0.11^{c}	tr ± 0.01^{ab}	0.16± 0.05^{bc}	0.19± 0.10^{c}	0.20± 0.26^{c}	0.20± 0.14^{c}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	***	***
S2	α-copaene	1390	A	1.1± 0.02^{e}	0.86± 0.01^{de}	0.62± 0.03^{cde}	0.10± 0.02^{ab}	0.15± 0.05^{ab}	0.49± 0.03^{bcd}	0.78± 0.04^{de}	0.77± 0.05^{de}	0.14± 0.04^{ab}	0.09± 0.06^{ab}	0.06± 0.02^{ab}	nd^{a}	nd^{a}	0.12± 0.05^{ab}	0.24± 0.07^{abc}	0.22± 0.18^{abc}	***	***	***
S3	(E)-β-caryophyllene	1430	B[12]	tr ± 0.03	tr ± 0.02	nd	nd	tr ± 0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
S4	β-caryophyllene	1445	A	4.4± 0.61^{bc}	5.5± 0.32^{c}	4.1± 0.43^{bc}	2.5± 0.39^{ab}	4.3± 1.3^{bc}	4.1± 1.2^{bc}	2.4± 0.29^{ab}	2.2± 0.50^{ab}	0.67± 0.52^{a}	0.60± 0.40^{a}	1.4± 0.73^{a}	1.0± 0.15^{a}	0.46± 0.17^{a}	1.2± 0.13^{a}	0.55± 0.28^{a}	0.69± 0.28^{a}	***	***	***
S5	(+)-aromadendrene	1452	A	0.17± 0.04^{de}	0.21± 0.01^{e}	0.15± 0.04^{cde}	tr ± 0.07^{abc}	0.13± 0.03^{cde}	0.15± 0.08^{cde}	0.10± 0.06^{abc}	0.10± 0.01^{bcd}	tr ± 0.01^{ab}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	***	***
S6	curcumene	1472	B[13]	0.18± 0.09^{abcd}	0.23± 0.11^{b}	0.19± 0.06^{b}	0.09± 0.05^{a}	0.15± 0.22^{b}	0.22± 0.19^{b}	tr ± 0.03^{bcde}	0.12± 0.05^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	ns	***
S7	α-humulene	1479	A	0.42± 0.16^{abcd}	0.70± 0.58^{d}	0.38± 0.29^{abcd}	0.49± 0.10^{bcd}	0.51± 0.76^{cd}	0.40± 0.65^{abcd}	0.18± 0.01^{abc}	0.26± 0.91^{abcd}	0.11± 0.02^{ab}	0.10± 0.06^{a}	0.10± 0.05^{a}	0.10± 0.02^{a}	0.19± 0.04^{abc}	0.10± 0.06^{a}	tr ± 0.03^{a}	0.13± 0.05^{abc}	***	***	***
S8	β-selinene	1508	B[14]	3.0± 0.05^{cd}	2.7± 0.06^{bcd}	1.5± 0.02^{abc}	4.6± 0.15^{d}	2.2± 0.19^{abcd}	1.9± 0.12^{abc}	3.3± 0.26^{cd}	3.0± 0.14^{bcd}	0.35± 0.25^{ab}	0.31± 0.16^{ab}	0.31± 0.17^{ab}	1.3± 0.29^{abc}	0.17± 0.06^{a}	0.40± 0.26^{ab}	0.36± 0.15^{ab}	0.50± 0.12^{ab}	***	***	***
S9	valencene	1514	A	nd^{a}	nd^{a}	nd^{a}	2.9± 0.44^{c}	nd^{a}	nd^{a}	nd^{a}	0.20± 0.07^{a}	nd^{a}	nd^{a}	tr ± 0.02^{a}	2.1± 0.16^{b}	tr ± 0.02^{a}	tr ± 0.01^{a}	tr ± 0.02^{a}	0.36± 0.05^{a}	***	***	***
S10	α-selinene	1515	B[15]	0.61± 0.02^{c}	0.60± 0.02^{c}	0.43± 0.05^{abc}	0.63± 0.44^{c}	0.54± 0.04^{bc}	0.44± 0.03^{abc}	0.71± 0.02^{c}	0.59± 0.07^{c}	0.10± 0.04^{a}	tr ± 0.03^{a}	tr ± 0.03^{a}	0.14± 0.03^{ab}	tr ± 0.02^{a}	tr ± 0.05^{a}	tr ± 0.04^{a}	0.10± 0.02^{a}	***	***	***
S11	kessane	1557	B[2]	nd^{a}	0.12± 0.02^{a}	nd^{a}	2.8± 0.05^{c}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	tr ± 0.03^{a}	tr ± 0.01^{a}	nd^{a}	2.0± 0.13^{b}	nd^{a}	tr ± 0.02^{a}	nd^{a}	0.36± 0.05^{a}	***	***	***
S12	cuparene[$]	1530	B[7]	nd	nd	nd	nd	nd	nd	nd	nd	tr ± 0.02	nd	nd	nd	tr ± 0.01	tr ± 0.01	nd	tr ± 0.04	ns	ns	ns
S13	(E)-nerolidol	1540	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	tr ± 0.02^{a}	tr ± 0.02^{a}	nd^{a}	nd^{a}	0.10± 0.02^{a}	tr ± 0.04^{a}	tr ± 0.03^{a}	tr ± 0.03^{a}	**	**	**
S14	liguloxide [$]	1560	B[16]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	tr ± 0.01^{a}	nd^{a}	tr ± 0.05^{a}	nd^{a}	tr ± 0.01^{a}	**	*	*
	Total			**10**	**11**	**7.5**	**14**	**8.2**	**7.9**	**7.7**	**7.4**	**1.4**	**1.2**	**1.9**	**6.7**	**0.95**	**2.0**	**1.3**	**2.4**			
	Phthalides																					
P1	3-butylhexahydrophthalide	1662	B[2]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	tr ± 0.04^{abc}	tr ± 0.02^{ab}	tr ± 0.01^{abc}	nd^{a}	0.10± 0.01^{bc}	0.10± 0.02^{c}	tr ± 0.01^{abc}	0.10± 0.01^{bc}	***	***	***
P2	3-n-butylphthalide	1676	B[17,18]	5.0± 0.01^{abc}	5.2± 0.03^{abc}	9.4± 0.05^{cd}	6.6± 0.01^{abcd}	7.1± 0.03^{abcd}	6.7± 0.01^{abcd}	9.8± 0.06^{d}	7.0± 0.03^{abcd}	4.2± 1.1^{ab}	3.6± 0.81^{a}	5.6± 1.1^{abcd}	8.5± 0.86^{bcd}	4.9± 0.93^{ab}	5.6± 1.4^{abcd}	5.2± 1.3^{abc}	4.6± 0.87^{ab}	***	***	***
P3	(Z)-3-butylidenephthalide	1685	B[2]	0.15± 0.06^{ab}	0.22± 0.05^{abc}	0.36± 0.09^{b}	0.16± 0.02^{ab}	0.25± 0.02^{ab}	0.17± 0.07^{ab}	0.25± 0.34^{ab}	0.18± 0.25^{ab}	0.22± 0.20^{ab}	0.10± 0.04^{a}	0.13± 0.01^{ab}	0.13± 0.01^{ab}	0.25± 0.06^{ab}	0.17± 0.06^{ab}	0.10± 0.01^{a}	0.14± 0.04^{ab}	*	*	*
P4	sedanenolide	1748	B[17,18]	4.8± 0.30^{abcd}	9.7± 2.3^{bcde}	15± 1.9^{e}	16± 1.6^{e}	14± 3.0^{e}	9.5± 2.9^{abcde}	11± 3.0^{cde}	13± 2.2^{de}	1.1± 0.30^{ab}	0.96± 0.03^{a}	3.7± 1.1^{abc}	9.2± 1.1^{abcde}	1.5± 0.49^{ab}	2.0± 0.89^{ab}	0.92± 0.52^{a}	1.3± 1.1^{ab}	***	***	***
P5	*trans*-neocnidilide	1755	B[2]	0.26± 0.03^{a}	0.13± 0.03^{a}	1.8± 0.02^{c}	0.16± 0.04^{a}	0.30± 0.06^{ab}	0.78± 0.06^{abc}	0.99± 0.04^{abc}	0.94± 0.04^{abc}	1.4± 1.1^{abc}	0.45± 0.24^{abc}	1.2± 0.24^{abc}	0.14± 0.01^{a}	0.37± 0.15^{ab}	1.7± 0.55^{bc}	1.0± 0.23^{abc}	1.1± 0.19^{abc}	***	***	***
P6	(E)-ligustilide	1764	B[17,18]	0.12± 0.02^{a}	0.15± 0.10^{a}	0.24± 0.01^{a}	0.23± 0.03^{a}	0.25± 0.05^{a}	0.14± 0.01^{a}	0.18± 0.09^{a}	0.18± 0.05^{a}	tr ± 0.02^{a}	tr ± 0.02^{a}	0.10± 0.03^{a}	0.11± 0.03^{a}	0.25± 0.04^{a}	tr ± 0.02^{a}	tr ± 0.01^{a}	tr ± 0.02^{a}	*	*	*
	Total			**10**	**16**	**27**	**23**	**22**	**17**	**22**	**21**	**7.0**	**5.1**	**11**	**18**	**7.3**	**9.6**	**7.3**	**7.2**			

	Aromatic Hydrocarbons																			
AHC 1	toluene	769	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.24± 0.11^{bc}	0.23± 0.11^{bc}	0.38± 0.10^{c}	0.25± 0.07^{bc}	0.17± 0.01^{ab}	0.19± 0.04^{abc}	0.29± 0.06^{bc}	0.27± 0.08^{bc}	***	***	***
AHC 2	p-xylene	876	B[2]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.11± 0.08^{ab}	0.12± 0.06^{b}	0.14± 0.05^{b}	0.09± 0.01^{ab}	0.11± 0.01^{ab}	0.17± 0.05^{b}	0.15± 0.03^{b}	0.15± 0.03^{b}	***	***	***
	Total			**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.35**	**0.35**	**0.52**	**0.34**	**0.28**	**0.36**	**0.44**	**0.42**			
	Oxides																			
O1	caryophyllene oxide	1610	A	tr ± 0.01^{abc}	0.13± 0.04^{abcdef}	0.25± 0.05^{cdef}	tr ± 0.02^{abcd}	0.10± 0.07^{abcde}	0.10± 0.02^{abcde}	tr ± 0.01^{ab}	nd^{a}	0.25± 0.06^{cdef}	0.27± 0.08^{cdef}	0.28± 0.04^{ef}	0.24± 0.09^{bcdef}	0.26± 0.03^{cdef}	0.33± 0.11^{f}	0.22± 0.03^{abcdef}	0.27± 0.11^{def}	***	***	***
	Lactone																			
L1	γ-nonalactone	1372	A	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.01^{bcd}	0.10± 0.02^{bcd}	tr ± 0.01^{abc}	tr ± 0.01^{ab}	0.10± 0.01^{bcde}	0.10± 0.01^{cde}	0.10± 0.03^{de}	0.10± 0.01^{e}	***	***	***
L2	dihydroactinolide	1557	B[19]	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	tr ± 0.06^{ab}	0.10± 0.05^{abc}	0.10± 0.02^{abc}	nd^{a}	0.16± 0.01^{c}	0.10± 0.06^{abc}	0.10± 0.03^{bc}	tr ± 0.02^{ab}	***	***	***
	Total			**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.10**	**0.13**	**0.11**	**0.03**	**0.32**	**0.15**	**0.19**	**0.13**			
	Unknowns																			
U1	unknown 1	n/a		0.57± 0.09^{abc}	0.31± 0.03^{ab}	0.43± 0.06^{ab}	0.19± 0.02^{ab}	0.27± 0.01^{ab}	0.71± 0.20^{bc}	1.2± 0.47^{c}	0.51± 0.29^{abc}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	***	***
U2	unknown 2	n/a		2.3± 0.63^{bc}	1.7± 0.03^{abc}	2.1± 0.06^{abc}	0.84± 0.02^{ab}	1.0± 0.01^{ab}	2.7± 0.20^{bc}	3.4± 0.47^{c}	1.5± 0.29^{abc}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	***	***
U3	unknown 3	735		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.19± 0.08^{b}	0.17± 0.05^{b}	0.25± 0.01^{b}	0.25± 0.05^{b}	0.14± 0.01^{b}	0.16± 0.04^{b}	0.23± 0.02^{b}	0.18± 0.03^{b}	***	***	***
U4	unknown 4	766		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.17± 0.08^{b}	0.15± 0.03^{b}	0.23± 0.03^{b}	0.17± 0.01^{b}	0.12± 0.02^{ab}	0.11± 0.09^{ab}	0.15± 0.01^{b}	0.19± 0.02^{b}	***	***	***
U5	unknown 5	787		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.23± 0.11^{b}	0.20± 0.07^{b}	0.23± 0.09^{b}	0.23± 0.05^{b}	0.16± 0.02^{ab}	0.18± 0.06^{ab}	0.28± 0.06^{b}	0.22± 0.05^{b}	***	***	***
U6	unknown 6	896		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.22± 0.09^{b}	0.16± 0.04^{b}	0.25± 0.07^{b}	0.22± 0.05^{b}	0.17± 0.01^{b}	0.22± 0.03^{b}	0.22± 0.05^{b}	0.16± 0.06^{b}	***	***	***
U7	unknown 7	971		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.64± 0.04^{bc}	0.52± 0.06^{ab}	1.1± 0.01^{c}	0.78± 0.17^{bc}	0.42± 0.04^{ab}	0.58± 0.02^{bc}	0.64± 0.05^{bc}	0.73± 0.03^{b}	***	***	***
U8	unknown 8	1249		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.54± 0.18^{b}	0.46± 0.06^{b}	0.65± 0.06^{b}	0.59± 0.02^{b}	0.55± 0.03^{b}	0.56± 0.13^{b}	0.52± 0.05^{b}	0.49± 0.02^{b}	***	***	***
U9	unknown 9	1279		0.16± 0.06^{ab}	0.08± 0.01^{a}	0.10± 0.01^{a}	0.13± 0.03^{a}	0.24± 0.01^{ab}	0.11± 0.01^{a}	0.17± 0.03^{ab}	0.10± 0.04^{ab}	0.29± 0.12^{ab}	0.18± 0.06^{ab}	0.19± 0.07^{ab}	0.18± 0.02^{ab}	0.17± 0.05^{ab}	0.22± 0.05^{ab}	0.14± 0.04^{ab}	0.50± 0.19^{bc}	*	*	*
U10	unknown 10	1362		0.10± 0.02^{ab}	0.09± 0.03^{ab}	nd^{a}	0.16± 0.01^{b}	0.03± 0.04^{a}	0.10± 0.01^{ab}	0.08± 0.01^{ab}	0.07± 0.4^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	***	**	***
U11	unknown 11	1506		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.05^{ab}	0.10± 0.01^{ab}	0.13± 0.04^{b}	0.10± 0.05^{ab}	0.10± 0.03^{a}	0.13± 0.05^{b}	0.13± 0.03^{b}	0.13± 0.06^{b}	**	***	***
U12	unknown 12	1539		0.25± 0.02^{ab}	0.33± 0.04^{b}	0.19± 0.02^{ab}	0.13± 0.01^{a}	0.10± 0.04^{ab}	0.10± 0.01^{a}	0.18± 0.01^{ab}	0.12± 0.04^{ab}	0.10± 0.04^{a}	0.10± 0.07^{a}	0.17± 0.04^{ab}	0.20± 0.02^{ab}	0.11± 0.02^{a}	0.17± 0.07^{ab}	0.10± 0.01^{a}	0.13± 0.06^{ab}	**	**	**
U13	unknown 13	1684		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	tr ± 0.06^{a}	tr ± 0.02^{a}	tr ± 0.02^{a}	tr ± 0.03^{a}	tr ± 0.02^{a}	0.10± 0.01^{a}	tr ± 0.02^{a}	tr ± 0.01^{a}	*	**	*
U14	unknown 14	1706		nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.10± 0.09^{ab}	tr ± 0.02^{ab}	0.10± 0.02^{ab}	0.11± 0.01^{b}	0.10± 0.04^{ab}	0.13± 0.02^{b}	0.10± 0.03^{ab}	0.10± 0.05^{ab}	***	***	***

U15	unknown 15	1799	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	nd[a]	0.13± 0.03[b]	0.13± 0.05[b]	0.18± 0.01[b]	0.13± 0.04[b]	0.10± 0.01[b]	0.18± 0.04[b]	0.12± 0.02[b]	0.13± 0.05[b]	** *	** *	***
	Total		**3.4**	**2.5**	**2.9**	**1.4**	**1.8**	**3.8**	**5.1**	**2.4**	**2.7**	**2.2**	**3.5**	**3.0**	**2.2**	**2.7**	**2.6**	**3.0**			

[a] Linear retention index on a HP-5MS column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; [1]Radulovic et al. (2010); [2] Andriamaharavo, (2014); [3] Stashenko et al. (2003); [4] Lucero et al. (2006); [5] Beaulieu et al. (2001); [6] Lucero et al. (2003); [7] Adams et al. (2005); [8] Sabulal et al. (2007); [9] Havlik et al. (2006); [10] Bylaite & Meyer, (2006); [11] Block et al. (2006); [12] Boulanger et al. (1999); [13] Cao et al. (2011); [14] Yu et al. (2007); [15] Zeng et al. (2007); [16] Pripdeevech & Saansoomchai, (2013); [17] Turner et al. (2021b); [18] Turner et al. (2021c); [19] Ansorena et al. (2001); [$] tentatively identified, spectral quality value of 70 was used for this compound. [c] Percentage composition of total peak area divided by compound peak area; means labelled with letters are significantly different ($p < 0.05$) according to the GxE interaction; means of three replicate samples; tr, trace amounts <0.10%; nd, not detected. [d] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [e] Geographical location. [f] Genotype. [g] geographical location x genotype interaction. Cells are colour coded; red expresses the genotype with the higher value compared to location; green expresses the genotype with the lower value compared to location; no colour expresses no difference in percentage composition for both location

As observed in various studies, monoterpenes, sesquiterpenes and phthalides are the most reported compound groups to contribute to celery's aroma profile (Orav et al, 1987; Sellami et al., 2012; Macleod & Ames, 1989; Turner et al., 2021b; Philippe, Suvarnalatha, Sankar & Suresh, 2002; van Wassenhove, Dirinck, Vulsteke, & Schamp, 1990). The composition of celery grown in UK expressed an average of 55 % monoterpenes, 20 % phthalides and 9.2 % sesquiterpenes, whereas genotypes grown in Spain had an average of 32 %, 2.2 % and 9 % respectively. Monoterpenes comprised most of the composition of the aroma profile of all celery genotypes grown in the UK, with limonene, γ-terpinene, β-pinene and m-cymene exhibiting the highest proportion of monoterpenes (Orav et al., 2003; Turner et al., 2021a). A lower proportion of monoterpenes comprised Spanish-grown celery, however, genotypes 10 and 12 displayed over 10 % more than the other genotypes (Table 4.1). The authors previously carried out gas chromatography-olfactometry (GC/O) on two celery genotypes (12 and 25) and reported that these compounds contribute citrus, fresh, pine, and mint odours to celery (Turner et al., 2021b). Although these compounds comprised much of the aroma profile, their odour activity remains high and therefore, they would not be considered characteristic compounds to celery. By completing aroma extraction dilution analysis (AEDA), Kurobayashi, Kouno, Fujita, Morimitsu, and Kubota (2006) identified the flavour dilution (FD) factor of volatile compounds of raw and boiled celery. Phthalides including 3-n-butylphthalide and ligustilide were found to have the highest FD factor of 3,125, whereas myrcene, a monoterpene also identified within the current study had a FD value of 625. Uhlig, Chang and Jen (1987) investigated the effect of phthalides on celery flavour using eight celery cultivars of varying origins, observing a positive correlation with total phthalide content and the intensity of the 'celery flavour' attribute. Significant variation between celery cultivars and phthalide content was also observed, most obviously in the concentration of sedanenolide. This is reflected in the current study.

The prominence of phthalides and their contribution to celery aroma is apparent throughout literature. A review completed by the authors (2021a) identified 3-n-butylphthalide and sedanenolide to be the most reported phthalides in celery, with odour descriptors such as celery, herbal, and cooked celery. These compounds have been identified as characteristic compounds to celery aroma and when authors (2021b) completed GC/O upon two celery genotypes also used in this study (12, 22) and the

average odour intensity of these compounds was high throughout maturity. Growing celery in UK 2018 produced genotypes with a higher phthalide composition, particularly high in 3-n-butylphthalide and sedanenolide, comprising an average percentage of 7.1 % and 11.6 % respectively. The average percentage of these compounds was lower in celery growing in Spain 2019, with 3-n-butylphthalide and sedanenolide contributing an average of 5.3 % and 2.6 % respectively. However, trans-neocnidilide was expressed at a higher composition in Spanish celery, comprising an average of 0.92 % of the aroma profile. Pino, Rosado, and Fuentes (1997) identified sedanenolide to comprise much of the volatile profile of celery leaf oil, comprising 32.1 % of the composition. The significantly higher abundance of these phthalide compounds reflected in Table 4.1, will allow assumptions to be drawn that these genotypes have a stronger typical celery aroma (Uhlig et al., 1987).

A similar pattern was observed within sesquiterpenes, whereby celery grown in the UK exhibited a significantly higher proportion of sesquiterpenes compared to Spanish grown celery. β-Caryophyllene and β-selinene comprised the highest proportion of the sesquiterpene profile for both geographical locations and these two are the most reported sesquiterpenes in celery (Turner et al., 2021a; Philippe et al., 2002; van Wassenhove et al., 1990; Shojaei, Ebrahimi & Salimi, 2011). A similar sesquiterpene trend was observed in another study (Turner et al., 2021c) between two harvest years (2018 and 2020) for the same eight genotypes whereby the sesquiterpene content comprised a higher proportion of the volatile profile of celery grown in 2018, a significantly warmer season than 2020 (Turner et al., 2021c). Pino, Rosado, and Fuentes (1997) identified β-caryophyllene to comprise 13.5 % of the volatile profile of Cuban celery leaf oil whereas Lund, Wagner, and Bryan (1973) identified β-caryophyllene and β-selinene to comprise an average of 1.5 % and 3.4 % respectively. Lund et al., also identified β-selinene to have a celery-like odour.

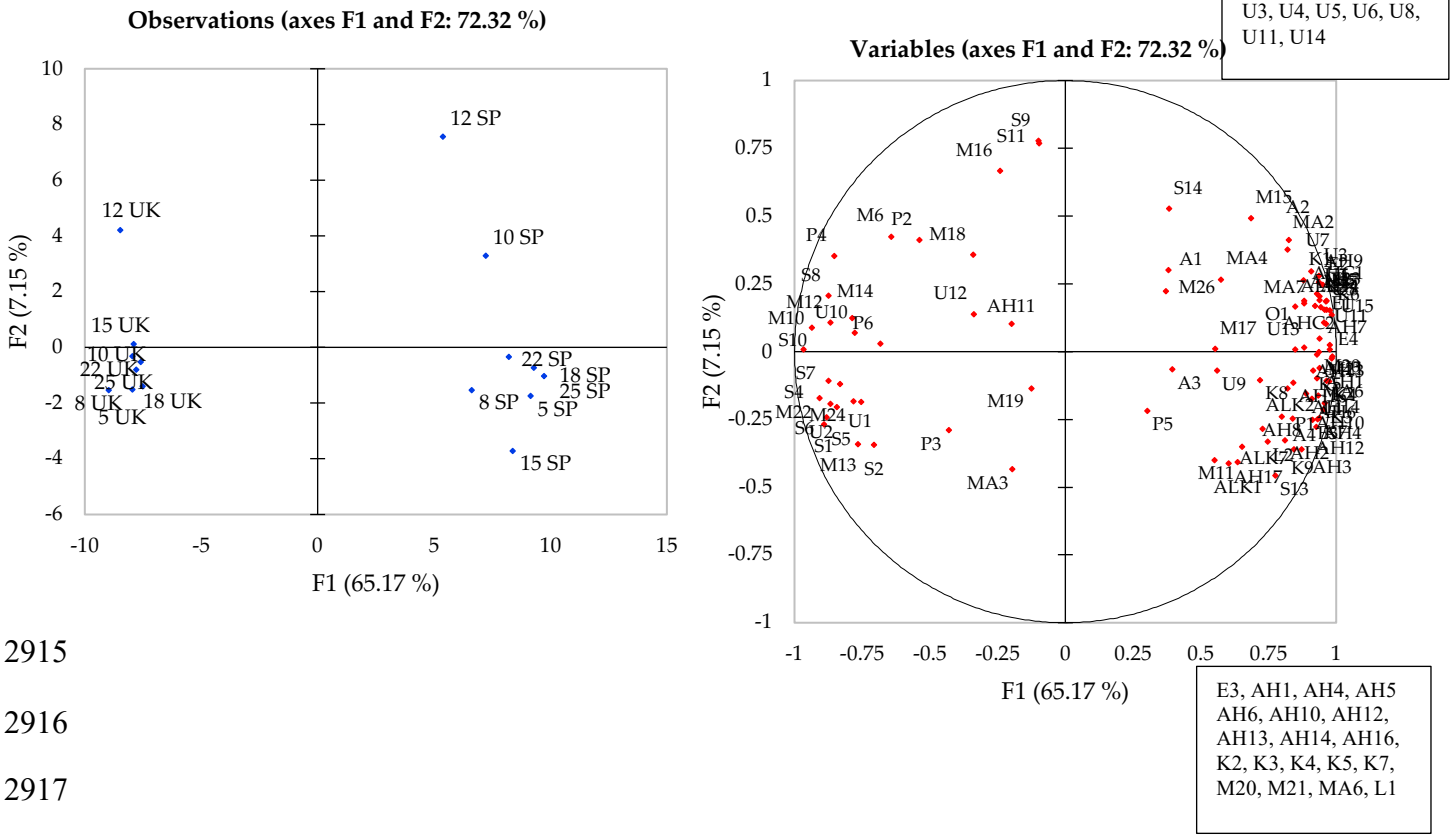
Whilst monoterpenes formed much of the composition of UK grown celery, aldehydes were observed to contribute a high proportion in Spanish-grown celery for all genotypes except genotypes 10 and 12. Comprising an average of 38.5 % of the aroma composition. Hexanal and (E)-2-heptenal were the most abundant compounds in this group for both geographical locations and genotypes, with odour characteristics of fresh, green, and fatty. Although not identified in UK grown celery, benzaldehyde and (E)-2-octenal composed a high proportion of the volatile composition with odour

characteristics of almond, cherry, and cucumber, green, averaging to comprise 2.0 % and 2.7 % respectively. Aldehyde content within celery has not been discussed thoroughly, with only few studies detecting the compound group. Gold and Wilson (1963) identified a range of aldehydes including hexanal, octanol and heptanal yet Shojaei et al. (2011) only identified phenylacetaldehyde and nonanal within three ecotypes of wild celery. A large proportion of aldehydes that were identified in the current study, were detected using GC/O to be prominent throughout celery maturity (Turner et al., 2021b). Hexanal was one of the compounds contributing the most to the aldehyde content in celery for all genotypes across both locations, with odour characteristics including fresh, green and apple, as well as identified throughout celery maturity (Turner et al., 2021b).

Similarly, the ketone content of celery has rarely been discussed and only few studies have reported these compounds (Turner et al., 2021b, Gold & Wilson, 1963; Lund et al., 1973). Accompanying the identification of aldehydes, Shojaei et al., (2011) further detected p-methyl acetophenone and 2-undecanone within the three wild celery ecotypes. An explanation for the variation in ketone content between geographical location would involve investigating the formation of phthalides. The metabolic pathway involved in the synthesis of phthalides has yet to be confirmed and currently, there are multiple suggestions looking into how phthalides are synthesised (Turner et al., 2021a). Phan, Kim, and Dong (2009) identified a method of synthesising phthalides through ketone hydroacylation. Here, the hydroacylation of ketones led to the formation of five-membered lactones, inducing the synthesis of 1(3H)-isobenzofuranone, the simplest phthalide structure. From here, various phthalides can be formed according to the substitution at C3 (Turner et al., 2021a; Phan et al., 2009). The large variety of ketones identified (Table 1) may be an indication of the potential for the Spanish crop to synthesis phthalides. Many ketones were identified by the authors (Turner et al., 2021b) to be important to celery aroma when using GC/O to measure the change in aroma during celery maturity. 3-Pentanone, 2-hexanone and 3-octen-2-one were detected at higher intensities in immature celery, displaying the crop's potential to synthesis phthalide compounds whereas 1-octen-3-one was identified by GC/MS with a relative abundance of 6.7 and 4.7 mg/L, respectively, in post-mature celery.

4.5.1.1 Principal Component Analysis of volatile compounds in UK and Spanish celery samples

Principal component analysis allowed for the visual comparison of the volatile composition of the eight celery genotypes grown in UK and Spain (Figure 4.1) and to examine any correlations occurring between genotype, geographical location, and chemical compounds. Using only the significant compounds for geographical location (G), genotype (E) and their interaction (GxE), a clear divide between the compounds associated with each year was observed. Principal component one (F1) and two (F2) explained 72.32 % of the total variation present in the data and it can be observed that the first axis separated samples from the geographical location (UK and Spain), whereas the second axis separated the various genotypes within a location. Differences between geographical location were apparent as they separated along F2 component.



A1	3-methyl-3-buten-1-ol	M16	p-mentha-1,5,8-triene
A2	2-methyl-1-butanol	M17	*trans* carveol
A3	(E)-2-penten-1-ol	M18	pentylcyclohexa-1,3-diene
A4	1-pentanol	M21	isoborneol
A5	hexanol	M22	trans-dihydrocarvone
AH1	2-methyl-2-butenal	M24	L-carvone
AH2	(E)-2-pentenal	M25	D-carvone
AH3	hexanal	M27	carvacrol
AH4	(E)-2-hexenal	MA1	p-mentha-2,8-dien-1-ol
AH5	heptanal	MA2	dihydrolinalool
AH6	(E)-2-heptenal	MA3	trans pinocarveol
AH7	(E,E)-2,6-nonadienal	MA4	terpinen-4-ol
AH8	n-octanal	MA6	E)-8-hydroxylinalool
AH9	phenylacetaldehyde	S1	α-ylangene
AH10	2-E-octen-1-al	S2	α-copaene
AH11	m-tolualdehyde	S3	(E)-β-caryophyllene
AH12	nonanal	S4	β-caryophyllene
AH13	(E,E)-2,4-octadienal	S5	(+)-aromadendrene
AH14	(E,Z)-2,6-nonadienal	S6	curcumene
AH15	(E)-2-nonenal	S7	α-humulene
AH16	myrtenal	S8	β-selinene
AH17	(E,E)-2,6-nonadienal	S9	valencene
E1	methyl butanoate	S10	α-selinene
E2	methyl pentanoate	S11	kessane
E4	carveol acetate	P1	3-butylhexahydro phthalide
K1	2-methyl-3-pentanone	P2	3-n-butylphthalide
K2	3-heptanone	P3	(Z)-3-butylidenephthalide
K3	2-heptanone	P4	sedanenolide
K4	1-octen-3-one	P5	trans neocnidilide
K5	(E,E)-3,5-octadien-2-one	AHC1	toluene
K6	acetophenone	AHC2	p-xylene
K7	3,5-octadien-2-one	O1	caryophyllene oxide
K8	p-methyl-acetophenone	L1	γ-nonalactone
K9	dihydrojasmone	L2	dihydroactinolide
ALK1	nonane	U1	unknown 3
ALK2	decane	U2	unknown 4
ALK3	undecane	U3	unknown 5
ALK7	pentadecane	U4	unknown 6
M6	myrcene	U5	unknown 7
M7	α-phellandrene	U6	unknown 8
M10	limonene	U7	unknown 9
M11	β-(E)-ocimene	U8	unknown 10
M12	γ-terpinene	U9	unknown 11
M13	terpinolene	U11	unknown 12
M14	allo-ocimene	U13	unknown 13
M15	β-thujone	U14	unknown 14

Figure 4.1. Principal component analysis of eight celery samples harvested in 2018 and 2020 showing correlations with volatile compounds. (A) Projection of the samples; (B) Distribution of variables; (C) Compound codes as appear in plot (B)

Genotype expressed a significant influence over both the UK- and Spanish-grown celery (Table 4.1) yet a more noticeable separation was observed in the Spanish-grown celery between genotypes in addition to a strong association with more aroma compounds than UK celery (Figure 4.1). Genotype expressed significant differences (Table 4.1) but genotypes 12, 22 and 25 for Spain were positioned in a similar place on the opposite quadrant in the observation plot. Genotype 12 in both locations took the appearance of an outlier, displayed as the most significantly different from other genotypes used within this experiment. This was caused by the high abundance of sesquiterpene compounds present in the UK harvest, especially from β-selinene, and the high phthalide content within the Spanish harvest, with 3-n-butylphthalide and sedanenolide comprising 8.5 % and 9.2 % of the total volatile content. Significant compound associations with Spanish grown celery were expressed within Figure 4.1 including all aldehydes (except AH11) and ketones accompanied by monoterpenes (M11, 15, 17, 20, 26), sesquiterpenes (S13, 14), phthalides (P1, 5) and alcohols (A1, 2, 3). This was further reflected in Table 4.1. Conversely, less noticeable separation between the eight celery genotypes was observed by celery grown in the UK, in addition to fewer compound associations. Monoterpenes (M6, 10, 12, 13, 14, 16, 18, 22, 24), sesquiterpenes (S1, 2, 4, 5, 6, 7, 8, 10, 12) and phthalides (P2, 3, 4, 6) were positively correlated with samples grown in the UK. The spread of monoterpenes, sesquiterpenes and phthalides across the plot, together with ubiquity within all celery genotypes regardless of location of growth, harvest year (Turner et al., 2021c) and maturity (Turner et al., 2021b) confirmed the importance of these compound groups to celery and celery aroma. This was originally concluded by the authors (Turner et al., 2021c), where eight genotypes of celery grown in the UK in 2018 and 2020 both exhibited these compounds and in a similar pattern. Aldehydes and ketones appeared to be more strongly influenced by geographical location rather than genotype, explaining why these compounds are not commonly reported within the celery volatile composition.

Genotype and geographical location both expressed a significant influence over the volatile content of celery (Table 4.1), however, geographical location expressed a much stronger influence upon the composition (Figure 4.1). Differences within the growing climate and agronomy applied to the celery increased the risk of variation, as similarly expressed between harvest years (Turner et al., 2021c) whereby differences in air temperatures were likely the cause for the large variation expressed between

years 2018 and 2020, altering the sensory profile of the crop. The differences in composition observed between the eight celery genotypes grown in the UK and Spain (Figure 4.1) and the impact that these have upon the sensory characteristics were investigated through sensory profiling.

4.5.2. Sensory evaluation of fresh celery samples

The sensory profile of the eight celery samples was generated by a trained panel who came to the consensus of 22 and 23 terms for the quantitative assessment of samples grown in the UK in 2018 and samples grown in 2019, Spain, respectively. The additional attribute for the samples grown in Spain 2019 was salty taste and we hypothesised that this was because of the saline soils present in this part of the country as observed in other studies such as tomato (Moya et al., 2017), pepper (Marin, Rubio, Martinez & Gil, 2009) and cauliflower (Giuffrida, Cassaniti, Malvuccio & Leonardi, 2017). Mean panel scores for these attributes are presented in Table 4.2. Out of the 22 attributes that were profiled from the UK harvest, 14 of these were found to be significantly different between the genotypes and seven out of 23 attributes were significantly different for the Spanish trial in 2019 respectively. Few significant assessor x sample interactions were identified for both UK and Spanish harvests, suggesting that the panellists scored samples in a consistent manner (Lignou, Parker, Baxter & Mottram, 2014). Statistical comparison of sensory differences between location could not be completed due to the one-year difference between harvests, however, general trends will be discussed.

Appearance attributes for both locations displayed significant differences caused by genotype and similarities were observed between scoring for stalk thickness and colour attributes. A significant difference ($P<0.001$) for ribbed appearance was apparent between locations for all genotypes. The genotype variation between ribbed appearance was more apparent for those harvested in UK than those harvested in Spain, with scores ranging from 25.4 to 65.9. Mouthfeel attributes displayed a positive correlation with appearance attributes and these attributes were the highest scoring attributes in all genotypes across both locations, apart from stringiness. Stringiness was scored higher in Spanish celery, with the Spanish celery all genotypes recording an increase of at least 10 apart from genotype 22. Genotype 22 was scored significantly lower for stringiness when comparing other genotypes in both locations. Although not significantly different, grassy after-effect was scored higher within UK celery

and exhibited a positive correlation between grassy odour, an attribute that was significantly different in both locations.

Significant differences in the odour and flavour attributes evaluated in both genotypes and geographical location were observed but more significantly different attributes were identified in UK celery. The attributes cucumber and rocket flavour with grass odour were scored higher in the UK harvest whereas Spanish-grown celery scored higher for fresh coriander odour, fennel, and soapy flavour. The flavour attribute fresh coriander was scored alike for both locations, however genotype 12 displayed a higher score in coriander flavour when grown in Spain, going from a score of 9.6 to 17.4. Furthermore, genotype 12 was scored as most bitter with genotype 8 and 18 for both locations but scored sweeter when grown in Spain. Genotype 18 was scored with the strongest soapy flavour, which expressed a positive correlation with fresh fennel. Where genotype 12 scored high for flavour/odour attributes (apart from cucumber), genotype 25 scored low for flavour/odour attributes, only scoring high in the cucumber flavour attribute in both locations.

Table 4.2. Mean panel scores for sensory attributes of the eight celery samples harvested in UK 2018 and Spain 2019.

	Score[A]																	
	UK									Spain								
Attribute	5	8	10	12	15	18	22	25	P[B]	5	8	10	12	15	18	22	25	P[B]
Appearance																		
Colour	56.4^{b}	63.6^{ab}	62.6^{ab}	72.9^{a}	72.1^{a}	65.6^{ab}	70.5^{a}	26.8^{c}	***	45.6^{c}	51.2^{c}	50.0^{c}	69.9^{ab}	71.8^{a}	56.0^{bc}	71.6^{a}	26.7^{d}	***
Stalk thickness	49.8^{ab}	49.5^{ab}	55.8^{a}	20.9^{b}	58.7^{a}	62.5^{a}	61.3^{a}	55.0^{a}	***	42.4^{ab}	46.8^{ab}	38.2^{bc}	27.3^{c}	55.5^{a}	55.9^{a}	58.4^{a}	54.4^{a}	***
Ribbed	46.6^{bc}	61.0^{ab}	61.7^{a}	65.9^{a}	35.5^{cd}	25.4^{d}	34.2^{cd}	37.4^{cd}	***	66.7^{a}	64.0^{ab}	67.9^{a}	76.1^{a}	48.4^{c}	42.1^{c}	49.6^{bc}	49.5^{bc}	***
Odour																		
Fresh fennel	16.5	14.2	18.9	15.5	15.3	18.6	15.4	18.2	ns	19.5	18.4	16.8	15.4	24.8	19.9	15.8	13.7	ns
Grassy/green	32.6^{a}	31.0^{ab}	32.1^{ab}	36.3^{a}	30.7^{ab}	28.3^{ab}	35.3^{a}	21.1^{b}	***	11.6^{b}	19.4^{ab}	24.3^{a}	25.6^{a}	23.5^{a}	20.1^{ab}	23.2^{a}	19.2^{ab}	**
Fresh parsley	14.1	19.7	19.0	19.1	20.6	16.7	16.7	10.8	ns	11.5	15.5	16.8	16.1	18.5	16.6	14.1	11.4	ns
Fresh coriander	12.8	12.1	14.2	11.7	14.2	17.5	15.4	11.1	ns	17.9	18.9	21.5	15.1	22.8	22.7	17.7	14.3	ns
Taste/flavour																		
Bitter	23.1^{abc}	24.0^{abc}	24.7^{abc}	35.9^{a}	28.2^{abc}	31.3^{ab}	24.4^{abc}	15.5^{c}	ns	24.4^{ab}	30.9^{ab}	29.4^{ab}	30.9^{ab}	28.4^{ab}	36.4^{a}	26.1^{ab}	18.1^{b}	**
Salt	nd	nd	nd	nd	nd	nd	nd	nd	**	26.4	22.6	27.3	31.3	23.4	31.2	24.8	18.7	ns
Sweet	15.2^{bcd}	20.3^{ab}	21.6^{ab}	10.6^{d}	15.6^{bcd}	12.2^{cd}	20.0^{ab}	24.6^{a}	***	18.3	19.8	21.4	18.2	20.0	14.5	16.1	22.8	ns
Fresh fennel	11.9	10.3	12.6	11.0	7.7	13.6	11.6	11.3	ns	15.0	15.7	10.4	13.2	17.4	13.6	8.0	10.8	ns
Rocket	11.3^{bc}	13.4^{bc}	12.4^{bc}	23.8^{a}	16.6^{abc}	16.9^{abc}	10.4^{bc}	7.7^{c}	***	1.8	2.0	3.2	1.8	1.4	1.0	0.8	0.2	ns
Fresh coriander	17.5	16.3	16.0	9.6	15.0	18.1	18.9	14.1	ns	17.2	21.0	18.1	17.4	18.0	21.4	15.7	13.8	ns
Soapy	18.2^{ab}	12.4^{b}	16.4^{ab}	18.4^{ab}	15.4^{ab}	23.7^{a}	16.3^{ab}	13.0^{ab}	*	19.1	20.5	25.1	22.0	20.0	27.5	19.7	15.0	ns
Cucumber	25.7^{ab}	33.2^{ab}	30.4^{ab}	9.1^{c}	30.0^{ab}	22.4^{b}	27.9^{ab}	37.7^{a}	***	12.8	14.1	9.9	5.8	15.3	11.8	11.8	14.8	ns
Mouthfeel																		
Crunchy	65.4^{abc}	62.6^{bc}	64.9^{abc}	56.7^{c}	70.2^{ab}	66.4^{abc}	73.7^{a}	62.5^{bc}	***	64.0	67.4	67.8	61.9	70.5	66.2	70.3	65.5	ns
Stringy	40.8^{b}	46.6^{b}	40.1^{b}	64.1^{a}	33.2^{b}	40.6^{b}	35.1^{b}	35.2^{b}	***	60.2^{ab}	58.2^{ab}	59.9^{ab}	71.9^{a}	47.2^{bc}	57.3^{abc}	38.5^{c}	52.4^{abc}	***
Moist	50.6^{a}	47.2^{a}	50.0^{a}	29.7^{b}	53.1^{a}	44.3^{a}	51.4^{a}	54.8^{a}	***	49.9	55.8	45.1	35.5	58.6	47.8	52.1	56.2	ns
Firmness of first bite	63.7	59.9	63.3	59.2	68.9	65.7	67.6	58.6	ns	64.8	66.1	65.6	63.5	67.2	63.2	69.9	63.2	ns
Aftereffects																		
Numbness	13.1	8.6	13.8	11.5	10.0	14.0	9.8	9.0		17.0	19.3	20.9	16.4	21.1	23.1	16.0	11.4	ns
Bitter	17.4^{bc}	18.4^{bc}	18.3^{bc}	29.0^{a}	19.1^{bc}	25.7^{ab}	16.0^{bc}	12.0^{c}	***	16.7^{ab}	19.4^{ab}	24.3^{a}	21.8^{ab}	19.2^{ab}	25.0^{a}	17.2^{ab}	12.0^{b}	*
Soapy	16.9^{ab}	15.7^{ab}	16.7^{ab}	21.2^{ab}	19.9^{ab}	24.8^{a}	18.6^{ab}	12.9^{b}	*	18.3	21.5	22.7	20.8	21.7	25.5	18.8	11.7	ns
Grassy/green	27.7	27.0	30.3	27.6	28.4	26.4	31.4	19.0	ns	12.3	13.3	15.8	19.9	15.8	14.3	15.7	13.6	ns

[A] Means are from two replicate samples; differing small letters (a, b, c, d, e, f) represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$); nd, not detected. [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

4.5.1.1 Principal component analysis of flavour attributes and volatile compounds

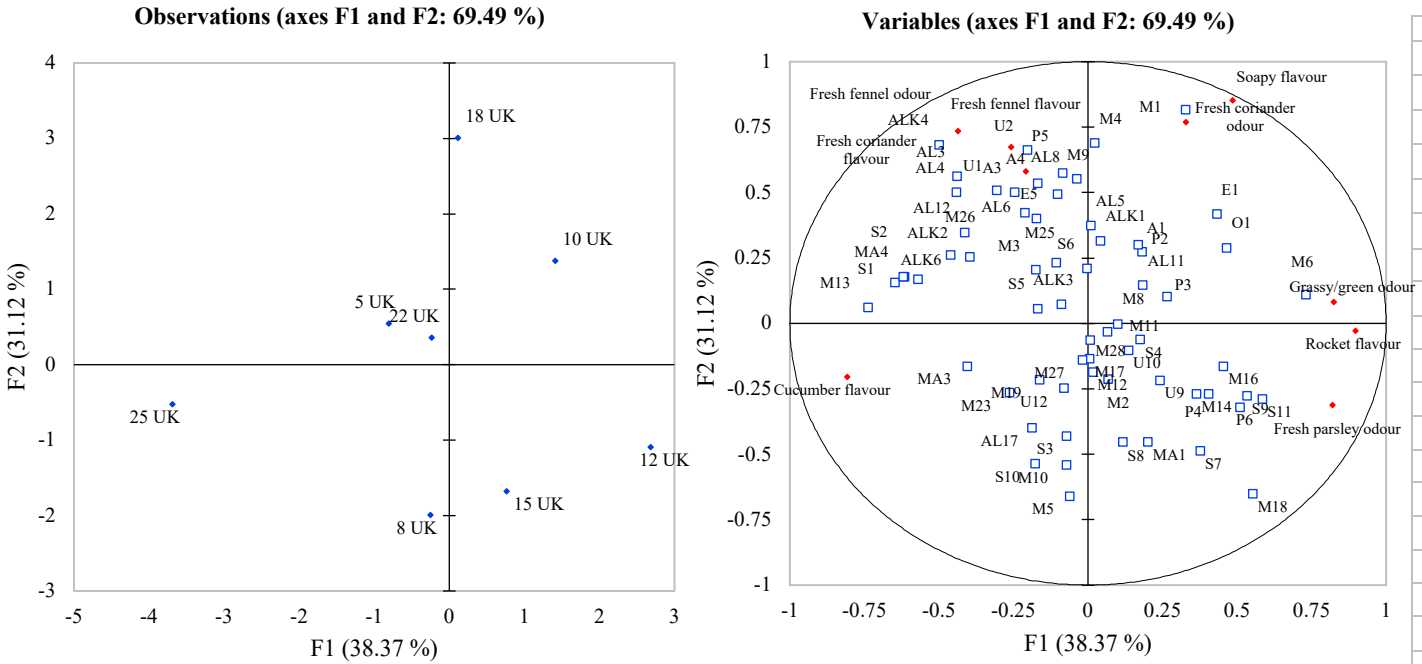
PCA was used to visualise the sensory and chemical differences observed across the eight genotypes with the volatile compounds identified (Table 4.1) and the sensory attributes related to odour and flavour used as variables (Figure 4.2 and Figure 4.3). Celery grown in the UK expressed a large variation between the eight genotypes (Figure 4.2) whereby principal component one (F1) and two (F2) explained 69.49 % of the total variation within the data. The first axis separated genotypes 5, 10, 18 and 22 from other genotypes, whereas the second axis separated genotypes 10, 12, 15 and 18. Genotype 25 was scored the lowest for all flavour attributes, only scoring high in cucumber flavour (Table 4.2), whereas genotype 12 opposed genotype 25 (Figure 4.2) and displayed strong association with a fresh parsley and grass odour along with a rocket flavour. Genotype 18 was positively correlated to fresh fennel and coriander flavour with the soapy characteristics that accompany many members of the Apiaceae family (Eriksson et al., 2012). A grouping of aroma compounds in the centre of the PCA was observed whereas the sensory characteristics remained positioned on the outer rim of the biplot with genotypes 5 and 22 grouped in the middle of the observation plot accompanied with no strong associations with any flavour/odour attribute (Figure 4.2). These genotypes exhibited a lower volatile content to genotype 12 (Table 4.1). Predominantly, monoterpenes and sesquiterpenes were negatively correlated with the first principal component (F1) and compounds belonging to compound classes such as alcohols and aldehydes were positively associated with F1. Phthalides were distributed around the plot, with trans-neocnidilide (P5) displaying positive association to fresh fennel whereas sedanenolide and (E)-ligustilide (P4 and P6) express a positive correlation with fresh parsley.

Principal component one (F1) and two (F2) explained 71.26 % of total variation observed within the dataset for the samples grown in Spain and the first axis separated genotypes 10, 12 and 22, whereas genotypes 5, 12, 22 and 25 are separated along the second axis (Figure 4.3). Genotype 25 Spain exhibited a low association to all attributes apart from cucumber flavour, observed in UK 25 and genotype 12 Spain expressed a significant association to grass odour as observed in UK. Furthermore, genotype 18 displayed a positive association with fresh coriander and fennel odour and flavour attributes when grown in Spain and UK. The perception of genotypes 5, 8, 10, 15 and 22 were observed to change significantly between locations caused by the chemical compositional changes.

The flavour attribute of cucumber displayed no significant correlations in UK compounds (Figure 2) yet significant correlations between compounds and this attribute was observed with multiple aldehydes (AH3, AH5, AH10, AH12 and AH13) that express odour characteristics such as fatty, cucumber and green (Figure 4.3). These compounds were not identified in UK harvest. Compounds identified in UK celery (Figure 4.2) all displayed association with a flavour/odour attribute of sorts; however, this was not reflected within Spanish-grown celery. Plotto, Margaría, Goodner, Goodrich and Baldwin (2004) calculated the retronasal and orthonasal activity values for selected terpenes and aldehydes in an orange juice matrix, identifying limonene, β-pinene and γ-terpinene to have the highest thresholds in water and orange juice whereas hexanal, octanal and nonanal, all aldehydes identified in celery (Table 4.1), expressed a much lower threshold. Due to the lower proportions of monoterpenes identified in Spanish-grown celery, the flavour characteristics contributed by these aldehydes (green, waxy, cucumber, honey (Turner et al, 2021b)), allowed the panel to detect these more easily. This explains the differences observed in the sensory panel between the celery grown in the UK and in Spain. Furthermore, observed on the factor plot in the bottom left quadrant (Figure 4.3), a large group of compounds displayed no significant associations with any sensory attribute.

Celery harvested in Spain expressed a different aroma profile when compared to samples harvested in the UK as observed in the significant difference of the aroma composition (Table 4.1) and although we cannot compare statistically UK and Spanish genotypes, differences in the scoring of attributes were observed. Genotypes 5, 8 and 15 displayed no association with herbal odour and flavour attributes in UK (Figure 4.2) but were scored higher after growing in Spain, where strong associations to fresh fennel, coriander, and parsley were displayed (Figure 4.3). Genotype 12 expressed close association with grass and fresh parsley odours in addition to sedanenolide and 3-n-butylphthalide, compounds known for their celery odours and displayed significant positive correlations with grass and parsley odour. On the other hand, genotype 25 expressed the lowest relative content of volatile compounds identified apart from aldehyde compounds and was scored with a significantly higher cucumber flavour than any other genotype in both locations. Here, this genotype does not exhibit a strong characteristic odour in comparison to genotype 12. As both these genotypes performed in a similar manner across location, we would recommend these genotypes to breeders and fresh produce

growers who plan to use the same cultivar across different locations as they have expressed stability in volatile composition.



A1	3-methyl-3-buten-1-ol	M18	pentylcyclohexa-1,3-diene
A3	(E)-2-penten-1-ol	M19	*cis*-dihydrocarvone
A4	1-pentanol	M23	trans-dihydrocarvone
AL3	hexanal	M25	L-carvone
AL4	(E)-2-hexenal	M26	D-carvone
AL5	heptanal	M27	thymol
AL6	(E)-2-heptenal	M28	carvacrol
AL8	n-octanal	MA1	p-mentha-2,8-dien-1-ol
AL11	m-tolualdehyde	MA3	trans-pinocarveol
AL12	nonanal	MA4	terpinen-4-ol
AL17	(E,E)-2,6-nonadienal	S1	α-ylangene
E1	methyl butanoate	S2	α-copaene
E5	hexyl hexanoate	S3	(E)-β-caryophyllene
ALK1	nonane	S4	β-caryophyllene
ALK2	decane	S5	(+)-aromadendrene
ALK3	undecane	S6	curcumene
ALK4	dodecane	S7	α-humulene
ALK6	tetradecane	S8	β-selinene
M1	α-thujene	S9	valencene
M2	α-pinene	S10	α-selinene
M3	camphene	S11	kessane
M4	sabinene	P2	3-n-butylphthalide
M5	β-pinene	P3	(Z)-3-butylidenephthalide
M6	myrcene	P4	sedanenolide
M8	delta-3-carene	P5	*trans* neocnidilide
M9	m-cymene	P6	(E)-ligustilide
M10	limonene	O1	caryophyllene oxide
M11	β-(E)-ocimene	U1	unknown 1
M12	γ-terpinene	U2	unknown 2
M13	terpinolene	U9	unknown 9
M14	allo-ocimene	U10	unknown 10
M16	*p*-mentha-1,5,8-triene	U12	unknown 12
M17	*trans* carveol		

Figure 4.2. Principal component analysis of eight celery samples harvested in UK 2018 showing correlations with volatile compounds and sensory attributes. (A) Projection of the samples; (B) Distribution of variables; (C) Compound codes as appear in plot (B).

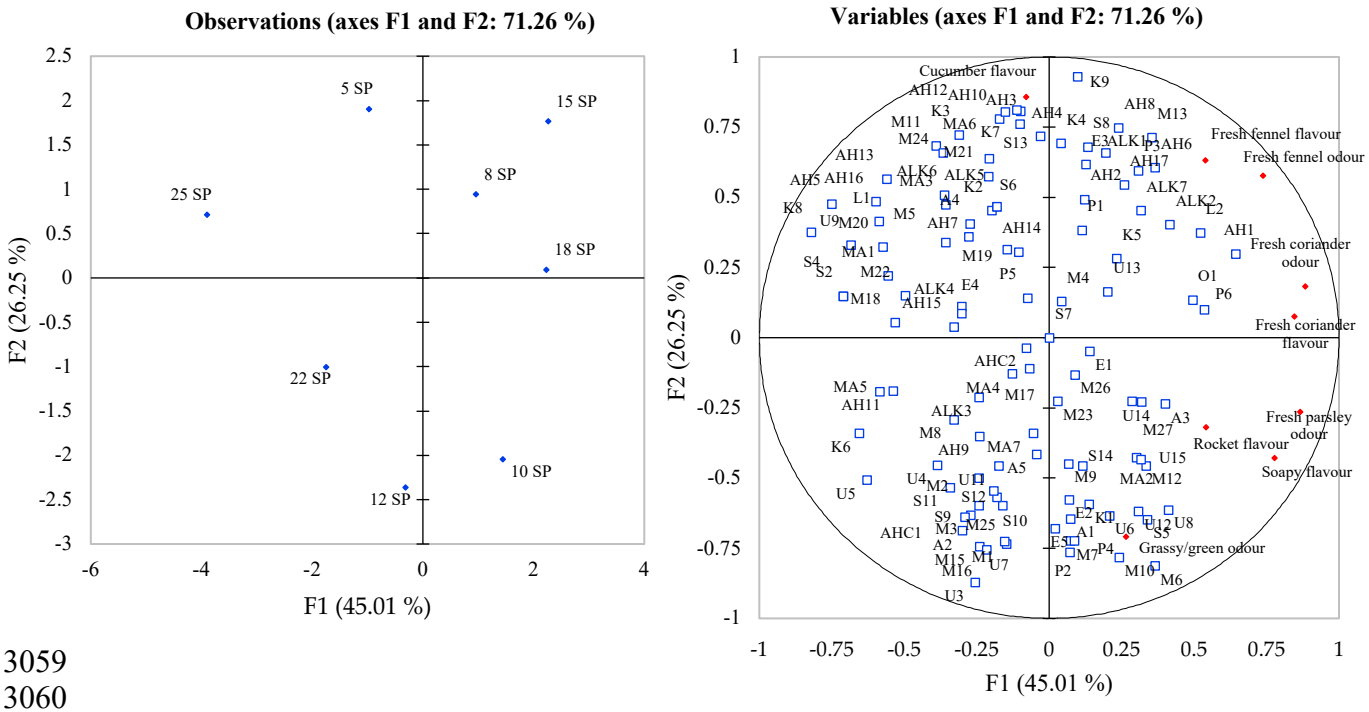


Figure 4.3. Principal component analysis of eight celery samples harvested in Spain 2019 showing correlations with volatile compounds and sensory attributes. (A) Projection of the samples; (B) Distribution of variables; (C) Compound codes as appear in plot (B).

A1	3-methyl-3-butenol	M13	terpinolene
A2	2-methyl-1-butanol	M14	*allo*-ocimene
A3	(E)-2-penten-1-ol	M15	β-thujone
A4	1-pentanol	M16	p-mentha-1,5,8-triene
A5	hexanol	M17	*trans* carveol
AH1	2-methyl-2-butenal	M18	pentylcyclohexa-1,3-diene
AH2	(E)-2-pentenal	M19	*cis*- dihydrocarvone
AH3	hexanal	M20	camphor
AH4	(E)-2-hexenal	M21	isoborneol
AH5	heptanal	M22	(trans--dihydrocarvone
AH6	(E)-2-heptenal	M23	β-cyclocitral
AH7	(E,E)-2,6-nonadienal	M24	L-carvone
AH8	n-octanal	M25	D-carvone
AH9	phenylacetaldehyde	M26	thymol
AH10	2-E-octenal	M27	carvacrol
AH11	m-tolualdehyde	MA1	p-mentha-2,8-dien-1-ol
AH12	nonanal	MA2	dihydrolinalool
AH13	(E,E)-2,4-octadienal	MA3	trans pinocarveol
AH14	(E,Z)-2,6-nonadienal	MA4	terpinen-4-ol
AH15	(E)-2-nonenal	MA5	α-terpineol
AH16	myrtenal	MA6	(E)-8-hydroxylinalool
AH17	(E,E)-2,6-nonadienal	MA7	caryophylladienol II
E1	methyl butanoate	S2	α-copaene
E2	methyl pentanoate	S4	β-caryophyllene
E3	Methyl hexanoate	S7	α-humulene
E4	carveol acetate	S8	β-selinene
E5	hexyl hexanoate	S9	valencene
K1	2-methyl-3-pentanone	S10	α-selinene
K2	3-heptanone	S11	kessane
K3	2-heptanone	S12	cuparene
K4	1-octen-3-one	S13	(E)-nerolidol
K5	(E,E)-3,5-octadien-2-one	S14	liguloxide
K6	acetophenone	P1	3-butylhexahydro phthalide
K7	3,5-octadien-2-one	P2	3-n-butylphthalide
K8	p-methyl-acetophenone	P3	(Z)-3-butylidenephthalide
K9	dihydrojasmone	P4	sedanenolide
ALK1	nonane	P5	*trans* neocnidilide
ALK2	decane	P6	(E)-ligustilide
ALK3	undecane	AHC1	toluene
ALK4	dodecane	AHC2	p-xylene
ALK5	tridecane	O1	caryophyllene oxide
ALK6	tetradecane	L1	γ -nonalactone
ALK7	pentadecane	L2	dihydroactinolide
M1	α-thujene	U3	Unknown 3
M2	α-pinene	U4	Unknown 4
M3	camphene	U5	Unknown 5
M4	sabinene	U6	Unknown 6
M5	β-pinene	U7	Unknown 7
M6	myrcene	U8	Unknown 8
M7	α -phellandrene	U9	Unknown 9
M8	delta-3-carene	U11	Unknown 11
M9	m-cymene	U12	Unknown 12
M10	limonene	U13	Unknown 13
M11	β-(E)-ocimene	U14	Unknown 14
M12	γ-terpinene		

4.5.3 Environmental differences between geographical location and influence on the aroma profile

In this study, differences in the volatile composition and sensory profile were observed between eight genotypes and two geographical locations. Previously, Turner et al (2021c) used the same genotypes grown in different years in the UK and identified that differences in temperatures (air and soil) played an important role in determining the overall flavour of celery. Environmental data including temperature, rainfall and relative humidity were collected at the nearest weather station to the farm of growth and provided by G's Fresh UK and Grupo G's España (Table 4.3) to compare the differences in the climate of geographical location. These environmental and geographical differences and how they influence the chemical composition of celery are only hypothesized due to the inadequate study of different growing conditions on celery. However, abiotic stresses from factors including temperature, humidity, water, and mineral availability have been commonly observed in literature to influence secondary metabolic profiles in plants (Ramakrishna & Ravishankar, 2011; Miller, Shulaev & Mittler, 2008; Arbona, Manzi, de Ollas & Gómez-Cadenas, 2013).

Table 4.3. Environmental data recorded at the nearest weather station to the farm of growth and provided by G's Fresh (UK) and Grupo G's España

	Ely, Cambridgeshire (UK)					Águilas, Murcia (Spain)				
Weeks after transplant	Air Temp (°C)	Rainfall (mm)	Relative Humidity (%)	Wind speed (m/s)	Dew point (°C)	Air Temp (°C)	Rainfall (mm)	Relative Humidity (%)	Wind speed (m/s)	Dew point (°C)
1	17.0	0.0	73.0	2.4	15.4	15.3	0.0	79.6	0.8	1.9
2	14.7	0.0	81.3	1.5	18.7	15.4	0.1	76.3	1.1	3.9
3	16.4	0.1	66.1	1.3	20.0	19.9	0.0	72.8	2.4	4.1
4	17.0	0.0	94.8	1.6	18.4	17.4	0.1	63.7	2.9	1.1
5	18.9	0.0	98.5	1.5	20.4	16.9	0.0	82.1	1.0	6.9
6	19.8	0.0	99.7	3.0	16.3	16.4	0.0	81.2	1.9	6.1
7	18.2	0.0	99.4	1.4	6.5	16.6	0.0	82.5	1.2	6.3
8	20.4	0.0	99.0	1.9	16.3	18.5	0.0	84.7	0.8	8.2
9	21.4	0.1	70.5	2.1	18.2	18.9	0.0	78.3	1.3	6.9
10	20.9	0.0	71.8	2.6	13.9	19.8	0.0	79.4	1.4	7.2
11	17.3	0.2	99.9	1.0	12.4	17.9	0.3	71.1	2.2	5.1
12	18.4	0.0	98.6	2.3	12.9	16.9	1.8	78.3	2.1	8.0
13	15.8	0.0	93.9	2.0	12.4	19.0	0.6	74.3	2.4	6.6
Average	**18.2**	**0.0**	**88.1**	**1.9**	**15.5**	**17.6**	**0.4**	**77.3**	**1.7**	**6.0**

Utilising two seasons for growing and using the same eight genotypes, Turner et al. (2021c) identified that warmer temperatures had a positive correlation with sesquiterpene and phthalide generation, whereas growing in lower temperatures led to celery with a higher monoterpene content. As similarly discussed by the authors (2021c), data from two harvests is insufficient when stating any relationships between environment and volatile composition, however, collating the data collected in this investigation, the dataset is completed with eight genotypes in a multi-site and multi-year experiment. Similarities in the chemical profile were observed in genotypes 12, 18, 22 and 25 in how they reacted to being grown in an alternative environment; suggesting that genotype predetermines the protective or coping mechanisms for the crop when exposed to abiotic and biotic stresses.

Celery grown in 2018 in the UK were subjected to temperatures much warmer than considered normal for the UK and the environmental values do not express any significant differences between geographical location (Table 4.3) apart from the dew point, whereby UK grown celery was grown in an environment where the average dew point value was 15.5 °C, substantially higher when compared to the 5.7 °C experienced by Spanish-grown celery. The observed dew point temperature indicates the temperature required for the air to cool to reach a relative humidity of 100 %. The average daily temperature of UK grown celery is 18.2 °C and much closer to the dew point value, confirming the increased humidity experienced by UK grown celery. Exposure to high dew points promotes the growth of pathogens, inhibiting crop growth and subsequently, compromising the crop to biotic stresses (Park & Park, 2011). Specific stresses such as those caused by a pathogen will cause the crop to prepare a stress response and additionally, increase the rate of plant-to-plant signalling as a form of communication, explaining the increased content of monoterpene compounds observed by UK grown crop (Table 4.1). Sampaio, Edrada-Ebel and Da Costa (2016) studied the influence of environmental factors on the secondary metabolic profile of *Tithonia diversifolia*, observing a variation within the metabolic profile in the leaves and stems, expressing a stronger association with rainfall and humidity levels than with temperature and solar radiation. The primary metabolite content of *Tithonia diversifolia* expressed a strong positive correlation with relative humidity whereas secondary metabolite content expressed a strong negative correlation with humidity. A similar reaction was observed in the present

study, whereby more secondary metabolites in the form of volatile compounds were identified in Spanish grown celery, where relative humidity was lower (Table 4.3).

Due to minimal differences in the climate data, investigating differences in agriculture including water and soil composition must be included in the discussion, as these factors will also influence the flavour outcome. A consequence of the arid and semi-arid conditions of Águilas, Spain and the increasing shortage of water for crop irrigation, desalinated seawater is often used in southern regions of Spain (Martinez-Alvarez, Maestre-Valero, González-Ortega, Gallego & Martin-Gorriz, 2019). Conversely, the crop irrigation system in place within the UK is by fresh water by a nearby reservoir, supplied by the river Little Ouse in this instance. Although rigorous pre-treatment processing and filtration steps would have been completed upon both water supplies, the mineral composition of water will be vastly diverse due to differences in the original source. This will lead to variances in the soil for uptake in minerals such as calcium, sodium, magnesium, zinc, and iron.

Growing in different geographical locations involves growing on different soil types, this will lead to differences in the soil properties including water holding capacity and mineral composition. UK celery was grown on loamy and sandy soils with naturally high groundwater, allowing for high water availability and nutrient uptake, whereas the Calcisol soils of Spain are known for their accumulation of calcium carbonate from precipitation brought about by evaporation under arid and semi-arid conditions (FAO, 2001). The presence of surplus calcium carbonate in the soil would cause a stress response by the crop. To promote healthy growth, the crop must uptake soil and waterborne micronutrients and inorganic elements which are necessary for functional growth and involved in an array of essential pathways including the synthesis of secondary metabolites such as isoprenoid through the non-mevalonate pathway; the building block for monoterpenes and sesquiterpenes. Primarily, carbon-, nitrogen-, sulphur- and phosphorous- fixation is involved in the synthesis of substrates and precursors involved in primary and secondary metabolism (Waterman & Mole, 2019). The micronutrient and element content of the soil and its permeability will influence the uptake of water and minerals from the soil to be utilised within the crop. These micronutrients can be applied by the plant for a range of uses, for example, copper has been identified to improve the flavour of fruits and vegetables along with increasing sugar and lignin content, zinc promotes the transformation and

consumption of carbohydrates in plants and iron is a prominent micronutrient involved in the synthesis of organic acids (Mousavi, Galvai & Razaeim 2021; Broadley, Brown, Cakmak, Rengel & Zhao, 2021). Applying fertilisers (organic or inorganic) will increase the soil micronutrient content leading to the desired elements being available for crop uptake. Calcium and boron deficiencies, known causes of black heart and hollow stem in celery, are both nutrient-deficient illnesses that can be avoided through the application of appropriate sprays and fertiliser (Rubatzky, Quiros & Simon, 1999). However, van Wassenhove, Dirinck, Schamp and Vulsteke (1990) identified the negative impact of using nitrogen-based fertilizer on celery and its volatile composition. Contrary to what has been discussed above, an increased application of a nitrogen fertilizer (organic and/or mineral nitrogen) led to a reduction in the aroma-determining compounds in two celery cultivars. In fact, applying no fertilizer resulted in a higher content of volatile compounds including phthalides, whereas an overall decrease was observed between 1000 and 2000 μg/kg of fresh material when a nitrogen fertilizer was applied. D'Antuono, Neri and Moretti (2002) similarly observed a decrease in volatile content as nitrogen fertilizer volume was increased, especially in compounds such as limonene, myrcene and β-selinene. However, total phthalide content along with β-caryophyllene and α-selinene were identified in high proportions when 300 kg/ha of nitrogen was used on celery. It is possible that Spanish grown celery was exposed to higher levels of nitrogen, thus leading to a lower proportion of monoterpenes, sesquiterpenes and phthalides within the aroma composition.

Factors that accompany field placement will be a less significant cause of variation but when these factors are combined, they will play a more significant role in determining the secondary metabolite content in celery. The most obvious difference between geographical location would be the altitude of each field; UK celery was grown on an east-facing field that was -1 to 1 m above sea level, whereas the field in Águilas was south-facing 390 m above sea level. Higher altitudes will result in lower temperatures and limitation on light exposure (Cui et al., 2018). Cui et al. (2018) investigated the physiological changes of *Leymus secalinus* and the effect of altitude, observing an increase in soluble sugars as elevation increased but a decrease in chlorophyll *a* and *b*, leading to a decrease in the crop's ability to absorb light. Both these reactions were noted as defence mechanisms and adaption strategies to the change in environment. These environmental differences experienced by the Spanish celery

would increase the crop's ability to synthesise ketones and aldehydes, in response to these abiotic stresses. The solar radiation would be significantly higher in the UK-grown celery due to the lower altitude along with growing in the summer months. This will increase the duration of light exposed to the crop and thus, increasing the rate of photosynthesis. Although not discussed in celery, higher exposure to UV-B in tree foliage led to an increase in flavonoids as a protective mechanism (Nissinen et al., 2017) and if a similar response occurred in celery, this would lead to an increase in terpenes to aid with plant-to-plant communication and to potentially synthesis further compounds.

Synthesising aromatic compounds is a typical response from the crop to abiotic and biotic stresses for protection and adaption to the growing environment and it is clear the celery grown in UK reacted differently to the celery grown in Spain. Turner et al (2021c) previously suggested that increased sesquiterpene and phthalide content was due to temperature stress, yet similar temperatures and other climate conditions were experienced by the Spanish crop, leading to variation in the chemical composition. Differences in soil, water and fertilizer composition used upon the UK- and Spanish-grown celery caused a change in the availability of minerals and elements available for primary and secondary metabolite production and along with the placement of the field which altered the duration of light, caused a change in the crop's defence mechanism and adaption strategy.

4.6. Conclusions

Geographical location displayed a strong influence over the aroma composition of eight celery genotypes and the influence expressed by genotype remained significant. Changes in composition caused by these factors led to differences in the aroma profile and, hence, sensory differences between genotypes and celery grown in different geographical locations were identified. Completing volatile analysis and sensory evaluation of the eight genotypes of celery demonstrated that celery genotypes grown and harvested in UK were perceived with a strong green aroma and cucumber flavour compared to the celery grown and harvested in Spain. A wider range of compound families were identified within Spanish celery samples, imparting a significantly different aroma profile which was perceived to be more closely associated with fresh fennel and coriander flavour. Identifying more compounds,

including aldehydes and ketones in Spanish-grown celery allowed for the explanation of the association to cucumber flavour.

Combining findings presented in this study and in the previous study completed by the authors, the genetic make-up of the crop regulates the synthesis of primary and secondary metabolites in response to abiotic and biotic stresses. Nonetheless, the environmental stresses experienced by the UK and Spanish crops were different and thus, a different defence mechanism was required. This was reflected by the number of compounds expressing significant differences between genotypes, the variation caused by genotype in the UK crop as well as the variation in perception between genotypes from sensory evaluation. The influence of geographical location on the aroma compositional was also evident, through the variation observed due to the location and in addition to most compounds also expressing significant differences caused by geographical location. The chemical composition was different in both locations, mostly caused by the aldehyde and ketone content that was expressed in a significantly higher proportion of the volatile composition when sampling celery grown in Spain. A similar response was observed between harvest years, whereby, significant compositional differences from the warmer temperatures' of 2018 celery were observed, ultimately leading to an increased sesquiterpene and phthalide content in the eight genotypes when grown in a considerably warmer climate in response to stress.

All eight genotypes used within these studies were observed to be influenced by both genotype and external factors including the environment (air temperatures, soil temperatures, relative humidity), geographical location (altitude and placement of field) and agronomic techniques (application of fertilisers, water availability and irrigation systems). Two genotypes (12 and 25) demonstrated consistency in their performance across harvest year and location; 12 remained a high "extreme", profiled with strong fresh coriander and fennel attributes notes which was reflected through its abundance in strong aroma compounds. On the other hand, genotype 25 was presented as a low "extreme" and was only profiled with a cucumber flavour, expressing significant correlations with related compounds; predominantly, aldehydes and ketones. This consistency makes these lines strong candidates to drive breeding programmes aimed at developing celery with distinct flavour profiles that will appeal to different consumer groups.

With apparent differences in the aroma and sensory profile, identifying which harvest year, environment, geographical location, and agronomy produced the most appealing celery is impossible to identify without carrying out consumer preference trials combined with sensory profiling. Combining the data collected from this study and experiences alike with consumer preference tests would aid in the identification of attributes that consumers find important on celery products including preference on sweet, bitter and flavour intensities. The findings from this study would be offered to celery breeders and fresh produce growers to guide celery production with aroma profile targets in mind. Furthermore, by educating breeders about the environment including location, genotype, and agronomy; a deeper understanding will be provided on the role these factors play in determining and influencing the aroma profile and therefore, the sensory perception of celery. Combining all these considerations will lead to a higher quality and better tasting product. Additionally, selecting cultivars according to the growing environment or contrariwise, rather than using the same cultivar across circumstances will allow for a more consistent product.

4.7. Relative abundance

As displayed in the previous chapter, observing the results in an alternative form, such as approximate quantities by utilising the internal standard produced results that were similar to that of percentage composition.

In the biplots observed below, A, B and C explains 75.51 %, 66.88 % and 78.17 % of the total variation observed within the data. Displayed in Figure 4.4 A, the clear division between celery grown in the UK and in Spain that was observed in Figure 4.1 stands, confirming that there is a significant difference between the UK and Spanish grown celery in all eight genotypes when considering both percentage composition and approximate abundance. The sensory associations identified using percentage composition have been confirmed using relative abundance. Overall, monoterpenes and sesquiterpenes remained most strongly associated with UK grown celery whereas Spanish grown celery were displayed a stronger association to aldehydes and ketones. Table 4.4 displays the relative abundance data collected from this trial and used to construct Figure 4.1.

Table 4.4. Relative abundance of volatile compounds identified in the headspace of eight celery genotypes using SPME GC/MS and harvested in UK and Spain

		Relative Abundance (mg/L)																P-value		
		UK								SP										
Code	Compound name	5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G	E	GxE
A1	2-methyl-1-butanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.53	1.34	0.72	1.05	1.30	1.07	1.32	0.75	***	***	***
A2	3-methyl-3-butenol	1.91	2.09	4.28	2.66	1.43	1.19	1.26	2.03	1.70	1.09	1.54	1.30	1.00	1.44	1.32	1.15	*	*	*
A3	(*E*)-2-pentenol	3.20	2.59	2.62	1.65	2.32	3.31	4.57	2.39	3.01	2.41	0.99	0.42	1.01	0.94	1.14	2.43	*	ns	*
A4	pentanol	1.00	0.83	1.54	1.14	1.57	2.27	3.11	1.55	5.93	1.82	0.54	1.08	2.17	2.42	1.86	0.70	**	**	**
A5	hexanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.54	1.10	1.30	0.88	0.97	1.08	1.21	1.42	***	***	***
A6	isoborneol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.46	0.26	0.37	0.56	0.67	0.45	0.83	***	***	***
AL1	2-methyl-2-butenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.63	1.04	1.47	1.10	1.05	1.78	1.88	1.13	***	***	***
AL2	hexanal	1.78	1.99	2.00	1.32	0.79	1.60	0.00	1.16	102.80	67.94	22.06	18.82	62.73	66.15	63.11	62.09	***	***	***
AL3	(*E*)-2-hexenal	1.27	1.86	1.39	2.41	1.40	2.46	1.69	1.36	2.21	1.63	0.49	0.65	1.52	1.56	1.43	1.36	ns	ns	ns
AL4	heptanal	0.46	0.00	0.13	0.41	0.18	0.31	0.00	0.66	1.94	1.57	0.84	1.05	1.43	1.51	1.55	1.82	**	**	**
AL5	(E)-2-heptenal	1.17	1.80	1.16	1.99	1.47	1.27	1.38	1.31	21.66	22.93	9.98	13.37	29.37	20.42	18.07	14.08	***	***	***
AL6	octanal	0.45	0.00	0.27	2.16	0.73	0.40	0.68	0.75	2.73	2.68	0.93	1.38	4.63	2.23	1.32	1.93	***	***	***
AL7	benzaldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.79	4.66	3.14	4.07	4.75	4.10	4.11	4.42	***	***	***
AL8	meta-tolualdehyde	2.91	6.19	2.25	3.21	2.18	4.02	2.48	4.28	1.62	1.12	1.22	1.99	1.79	1.67	1.42	2.27	**	**	**
AL9	nonanal	1.27	1.86	1.39	2.41	1.40	2.46	1.69	1.36	2.52	1.67	0.64	0.77	1.63	1.79	1.51	1.51	*	*	*
AL10	(*E*,*Z*)-2,6-nonadienal	1.17	1.80	1.16	1.99	1.47	1.27	1.38	1.31	1.40	1.30	0.33	0.36	1.15	1.00	1.18	0.20	**	**	**
AL11	phenylacetaldehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87	0.66	0.43	0.92	0.72	0.63	0.57	0.67	***	***	***
AL12	*(E)*-2-octenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.63	9.42	2.51	2.96	9.41	9.93	7.17	8.69	***	***	***

AL13	*(E,E)*-3,5-octadien-2-one	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.76	6.62	1.50	1.79	6.05	6.32	5.67	5.56	***	***	***
AL14	*(Z)*-2-nonenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.42	0.18	0.27	0.83	0.64	0.60	0.52	***	***	***
AL15	*(2E, 4E)*-nonadienal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.37	0.19	0.28	0.45	0.41	0.36	0.43	***	***	***
K1	2-pentanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.17	2.10	1.66	2.03	2.15	2.26	2.16	1.69	***	***	***
K2	2-heptanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.55	0.38	0.51	0.44	0.46	0.67	0.57	***	***	***
K3	2-hexanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.34	0.19	0.14	0.26	0.37	0.32	0.41	***	***	***
K4	3-heptanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.54	1.29	0.51	0.53	1.12	1.32	1.12	1.53	***	***	***
K5	2-nonanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.72	1.36	1.83	1.71	1.18	1.53	1.55	1.94	***	***	***
K6	1-octen-3-one	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.83	10.17	4.86	5.06	12.09	8.68	8.86	7.31	***	***	***
K7	3,5-octadienone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.56	2.89	1.00	1.77	3.47	2.25	3.25	1.20	***	***	***
E1	methyl butanoate	0.22	0.10	0.24	0.21	0.23	0.21	0.22	0.14	0.54	0.49	0.43	0.37	0.48	0.48	0.39	0.42	ns	ns	ns
E2	methyl pentanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	2.04	0.09	0.38	2.11	1.87	0.76	1.43	***	***	***
E3	methyl hexanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.78	0.20	0.17	0.72	1.09	0.71	0.65	***	***	***
E4	carveol acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.18	3.61	1.56	1.56	2.91	3.06	3.83	2.19	***	***	***
E5	hexy isobutanoate	1.19	1.77	0.74	0.35	1.12	1.04	0.88	1.06	0.10	2.04	0.09	0.38	2.11	1.87	0.76	1.43	***	***	***
M1	α-thujene	2.84	6.39	2.37	4.96	6.60	5.13	2.45	3.73	1.08	1.00	1.05	1.10	0.95	1.01	0.99	1.43	**	**	**
M2	α-pinene	1.00	10.52	6.31	3.91	11.48	16.07	17.73	8.15	3.01	1.34	1.78	1.78	2.18	2.00	2.42	1.03	*	*	*
M3	camphene	1.96	2.30	2.12	2.08	1.95	2.45	2.32	1.82	2.29	1.57	1.56	2.07	2.04	1.50	2.31	1.30	ns	ns	ns
M4	sabinene	13.43	38.92	4.45	42.54	25.92	15.12	4.31	28.20	1.56	1.26	1.08	1.30	1.52	1.46	1.29	0.99	**	**	**

M5	β-pinene	5.19	14.21	9.65	19.95	10.73	11.95	4.72	6.70	8.12	5.80	2.57	5.66	9.82	2.84	5.64	3.59	*	*	*
M6	myrcene	3.56	3.25	0.00	2.89	4.44	5.76	7.81	4.04	1.07	1.53	3.07	3.14	1.38	3.13	1.30	1.15	*	*	*
M7	α-phellandrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.13	10.49	7.96	7.55	6.51	11.18	7.84	7.32	***	***	***
M8	delta-3-Carene	19.66	25.91	15.23	28.91	24.42	28.18	14.45	20.47	6.01	5.50	1.11	4.30	6.23	5.36	5.29	4.78	***	***	***
M9	m-cymene	1.49	1.75	16.52	8.15	6.46	1.02	1.21	7.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M10	limonene	180.86	301.91	143.22	238.96	268.23	183.07	159.60	180.68	48.60	51.68	41.44	45.28	30.83	35.61	35.60	18.00	***	***	***
M11	β-trans-Ocimene	0.89	1.31	0.69	1.87	1.20	0.85	2.11	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M12	*γ*-terpenine	19.35	31.50	15.64	44.77	38.07	31.93	11.21	31.77	2.78	3.93	3.79	4.28	3.35	3.18	2.72	2.01	**	**	**
M13	terpinolene	1.24	1.09	0.77	0.61	1.71	0.86	0.37	0.61	1.13	0.75	0.25	0.43	0.55	0.84	0.00	0.48	ns	ns	ns
M14	*allo*-ocimene	1.20	0.68	0.92	4.37	1.77	0.80	2.71	1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M15	pentylcyclohexa-1,3-diene	2.61	4.41	1.11	1.63	1.92	1.93	0.27	2.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M16	*p*-(1,3,8)menthatriene	0.95	2.10	0.71	1.40	1.14	0.89	0.31	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M17	β-cyclocitral	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.34	0.19	0.39	0.41	0.32	0.25	0.24	***	***	***
M18	*p*-mentha-1,5,8-triene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.03	0.27	1.20	0.15	0.40	1.09	0.17	***	***	***
M19	D-carvone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.73	0.77	0.31	0.32	0.68	0.98	0.40	1.18	**	**	**
M20	L-carvone	0.68	0.88	0.28	1.03	1.56	0.63	0.77	0.48	1.07	1.03	0.76	1.15	0.83	1.46	0.45	0.68	ns	ns	ns
M21	*cis*-dihydrocarvone	0.17	0.87	0.26	0.75	0.97	0.36	0.31	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M22	*trans*-dihydrocarvone	3.56	5.27	2.61	3.03	3.98	2.35	1.75	3.11	1.21	0.78	0.51	0.56	0.69	0.86	0.00	1.11	*	*	*
MA1	(+)-*cis*-*p*-mentha-2,8-dienol	0.52	0.81	0.44	2.36	1.67	0.75	3.33	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA2	*trans*-pinocarveol	0.99	1.76	1.03	3.48	2.06	0.38	1.56	1.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA3	*trans*-carveol	0.52	0.54	0.45	0.30	0.54	0.51	0.41	0.30	1.85	1.31	1.11	0.97	0.95	1.36	0.60	1.41	*	*	*

MA4	thymol	2.40	2.64	1.80	4.52	2.02	2.10	0.84	2.74	0.38	0.34	0.25	0.35	0.34	0.49	0.23	0.31	*	*	*
MA5	carvacrol	1.11	2.09	0.76	0.72	1.09	0.55	0.80	0.73	0.08	0.31	0.29	0.44	0.31	0.43	0.15	0.19	ns	ns	ns
MA6	*cis*-pinocarveol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87	0.61	0.19	0.22	0.59	1.43	0.36	0.91	***	***	***
MA7	camphor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.46	0.38	0.38	0.52	0.60	0.38	0.70	***	***	***
MA8	*cis*-carveol	4.42	4.12	5.92	5.26	5.92	3.27	3.68	5.28	0.40	0.18	0.14	0.17	0.17	0.29	0.13	0.38	*	*	*
MA9	α-terpineol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.39	0.33	0.43	0.46	0.53	0.27	0.59	***	***	***
MA10	(E)-8-hydroxylinalool	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.43	0.22	0.41	0.15	0.39	0.00	0.46	***	***	***
MA11	caryophylladienol II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.45	0.15	0.21	0.28	0.47	0.18	0.52	***	***	***
S1	α-ylangene	4.74	6.13	2.72	0.68	1.10	2.60	3.29	4.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
S2	α-copaene	0.49	0.45	0.26	0.27	0.72	0.55	0.21	0.37	0.44	0.25	0.10	0.00	0.00	0.35	0.56	0.74	ns	ns	ns
S3	(E)-β-caryophyllene	20.07	38.08	16.68	18.43	31.05	21.53	10.76	10.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
S4	β-caryophyllene	0.78	1.52	0.58	0.40	0.95	0.76	0.32	0.54	1.53	1.72	2.47	2.24	1.25	3.83	1.28	1.71	*	*	*
S5	(+)-aromadendrene	0.80	1.59	0.77	0.70	1.05	1.13	0.37	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
S6	curcumene	1.43	3.90	1.23	3.29	3.07	1.92	0.54	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
S7	α-humulene	1.92	4.87	1.50	3.80	3.80	2.12	0.83	1.32	0.22	0.24	0.15	0.18	0.52	0.25	0.06	0.12	***	***	***
S8	β-selinene	13.70	18.09	5.88	33.78	16.18	9.71	13.98	14.96	0.84	0.86	0.49	2.89	0.45	1.24	0.88	1.22	***	***	***
S9	valencene	0.09	0.00	0.30	0.00	0.74	0.37	0.16	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
S10	α-selinene	2.75	4.14	1.79	4.70	3.90	2.31	2.93	2.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
S11	kessane	0.20	0.83	0.96	0.35	0.56	0.46	0.13	0.00	0.26	0.21	0.12	4.49	0.45	0.11	0.24	0.09	*	*	*
S12	cuparene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.10	0.03	0.00	0.00	*	*	*
S13	liguloxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.10	0.00	0.26	0.00	0.00	*	*	*

P1	3-butylhexahydrophthalide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.03	0.00	0.00	0.00	0.00	0.00	0.13	ns	ns	ns
P2	3-n-butylphthalide	0.66	1.22	1.41	1.18	1.53	0.96	1.26	1.01	13.12	10.36	9.16	18.70	14.03	15.05	12.34	10.21	***	***	***
P3	*(Z)*-3-butylidenephthalide	21.88	64.53	60.20	126.33	93.92	56.00	56.80	72.04	0.46	0.26	0.23	0.29	0.71	0.45	0.22	0.27	***	***	***
P4	sedanenolide	1.14	1.61	7.27	1.18	2.02	4.36	4.94	4.76	2.02	2.74	5.57	20.24	4.22	4.65	2.10	1.11	ns	ns	ns
P5	*trans*-neocnidilide	0.54	0.94	1.01	1.76	1.62	0.85	0.87	1.03	2.98	1.16	1.21	0.32	1.17	0.12	2.54	2.86	ns	ns	ns
P6	(E)-ligustilide	0.52	0.91	0.97	1.70	1.57	0.82	0.84	1.00	0.04	0.04	0.15	0.24	0.18	0.45	0.04	0.03	ns	ns	ns

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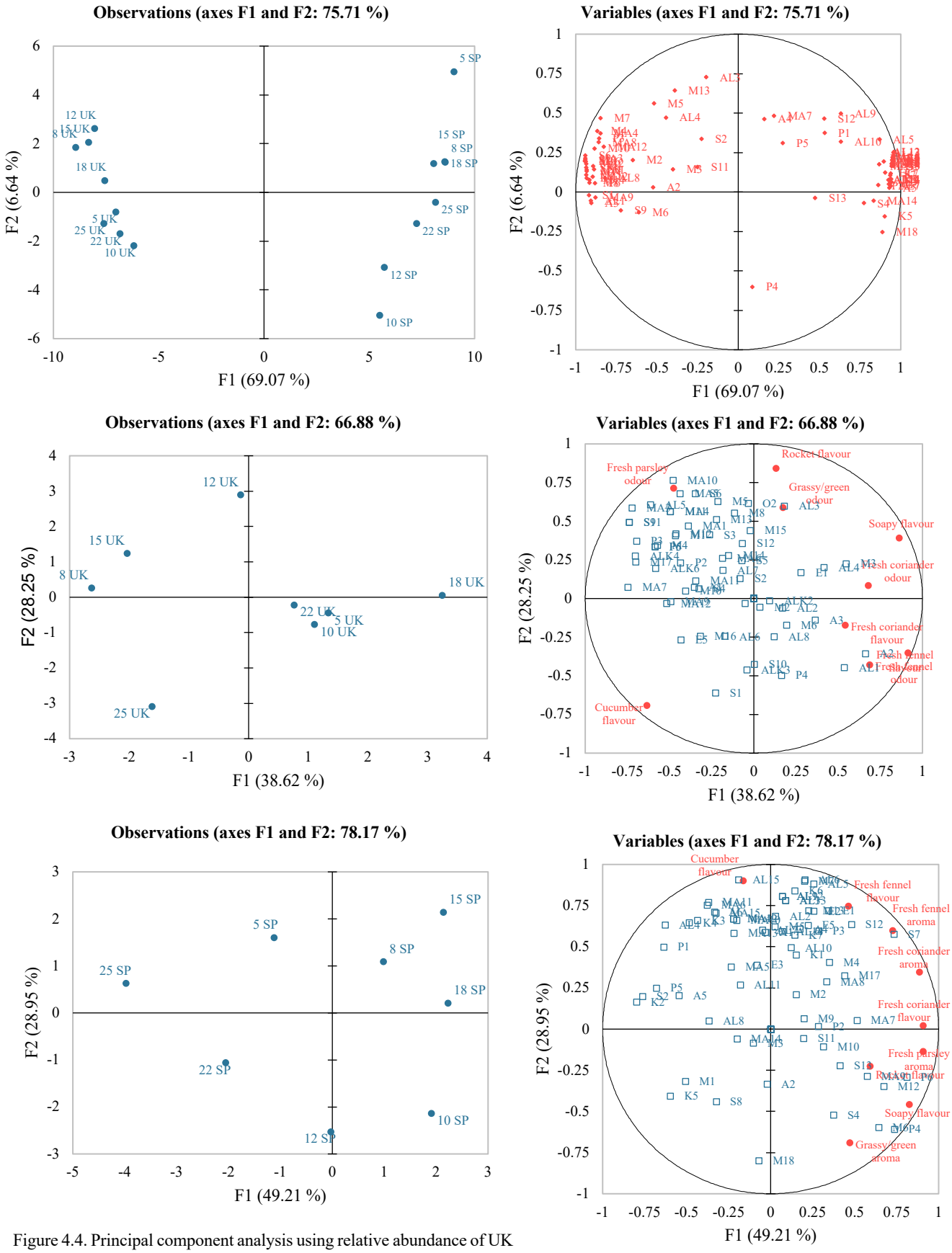
A)



3255 Figure 4.4. Principal component analysis using relative abundance of UK

3256 and Spanish grown celery (A) volatile components (B) UK celery

3257 volatile compounds with sensory attributes (C) Spanish celery volatile compounds with sensory attributes

4.8. References

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CHAPTER 5: Examining the compositional differences of eight celery genotypes grown in two different locations in Spain

5.1. Introduction to Chapter

As presented in previous chapters, significant differences in the aroma composition caused by both genotype and location and harvest year led to significant differences in the perceived sensory characteristics. Comparing celery grown in the UK in different years (chapter 3) displayed significant differences and more differences were observed when comparing to celery grown in Spain (chapter 4). Due to the connections that the project sponsors, Tozer Seeds Ltd in addition to G's Fresh, have with Spain, it was decided that growing these eight genotypes in two different locations would provide useful information. Celery is commonly grown in Spain and is supplied to the UK fresh produce marked, especially during the winter months and therefore, analysing the aroma composition of these two locations will be representative of variation within the Spanish-grown celery. The two locations chosen for this study, Cartagena and Águilas, can be found in the region of Murcia and were harvested within two weeks of each other.

By comparing the differences in variables such as the temperature, rainfall, field location and field environment, we aim to understand and identify more variables that influence the aroma composition of celery. Up until now, we have investigated climate conditions including temperature, rainfall, and relative humidity in addition to water and soil composition, all leading to differences in the secondary metabolite production within celery. Examining the differences in climate conditions and field placement, we can now investigate how factors such as angle of slope, altitude of field and the field's distance from the sea may impact the volatile composition of celery. These factors have yet to be discussed in celery and although, we only hypothesise, we aim to provide further insight to fresh produce growers on the impact of field placement upon the aroma quality of celery,

5.2. Introduction

Consumed in many cultural cuisines, celery cultivation is global, especially thriving in warm conditions between 16 °C and 21 °C with well distributed rainfall or with appropriate irrigation systems

in place allowing for good water availability. For this reason, celery is commonly grown in Europe, America, and Asia whereby the optimal conditions can be supplied. Due to the range of countries that celery can be successfully cultivated in, the number of celery cultivars available for use is vast with some cultivars even suitable for growth in warmer or subtropical conditions (Malhorta, 2012). To meet the demands of the consumer, countries such as the United Kingdom utilise the warmer winter climates of countries like Spain where celery can be grown all year round. Here, a greater range of commercial cultivars are grown in Spain than in the UK and so the variety of celery that the UK consumer eats between the months of November to March will be Spanish varieties. Using alternative cultivars introduces variation in the aroma and flavour composition of the crop, as shown in previous chapters, where significant differences between eight genotypes of celery were observed and this ultimately led to significant changes in the sensory characteristics of the celery. Additionally, growing in different geographical locations was observed to play a significant role in influencing the aroma profile of celery (Turner et al. 2021a; Chapter 4). By growing the same eight genotypes in UK and Spain, significant differences in the aroma profile and the sensory characteristics were also observed.

Spain is known as Europe's most climatically diverse country ranging from a semi-arid climate (south-east) to a warm-summer continental climate (north-east), hot-summer Mediterranean climate (coast) to an oceanic climate (north). Displaying such a variety of climates allows for a diverse range of fresh produce, including celery to be grown. Few studies have been completed that utilise a country such as Spain to investigate and compare the influence of the aroma composition in celery using different locations. Combining published data and stating the different geographical locations including the cultivar origin or harvested location, displayed clear variation in the aroma profile, partially due to geographical location but also due to cultivar (Turner, Lignou, Gawthrop & Wagstaff, 2021b). Shojaei, Ebrahimi and Salimi (2009) investigated the chemical composition of wild celery collected in three regions in Iran (Koohrang, Bazoft and Samsami) and observed differences in the percentage composition of many compounds commonly identified in celery including monoterpenes, sesquiterpenes and phthalides (van Wassenhove, Dirinck, Vulsteke & Schamp, 1990; Orav, Kailas & Jegorova, 2003). Phthalides, which are characteristic compounds of celery (Macleod & Ames, 1989; Kurobayashi, Kouno, Fujita, Morimitsu & Kubota, 2006) were observed to vary in their composition

to the aroma profile, for example Shojaei et al. observed (Z)-ligustilide, the main component of these three ecotypes of celery, to comprise 47 %, 33 % and 37 %, respectively. Furthermore, β-selinene, a compound identified by Lund, Wagner, and Bryan (1974) to possess a strong celery-like odour, was observed by Shojaei et al. to comprise 1.6 %, 4.5 % and 2.5 % of the aroma profile of each ecotype, respectively. Although assessing the differences in chemical composition due to location of growth was not the original aim of their study, clear differences were observed here and indicate the influence of location on the chemical profile of celery.

Where Shojaei et al. (2009) did not focus on the location influence, this study aims to investigate the influence of growing celery in two different locations within Spain, both of which display different climates whilst using the same eight genotypes which were transplanted and harvested within two weeks of each other. Although only 77 km apart, Cartagena displays a hot semi-arid climate whereas Águilas displays a Mediterranean climate and therefore, we aimed to identify causes of the aroma composition in celery genotypes by investigating the differences in the climate experienced during growth. Completing this study will educate growers, particularly those growing celery in similar climates, on the impact of the aroma composition and changes occurring within the crop due to climate differences.

5.3. Materials and Methods

5.3.1. Celery material and MIAPAE standard

5.3.1.1 Sample information

The eight varieties used in this experiment were chosen due to their differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each line used in this paper, the origins of these parental breeding lines and their images postharvest can be found in Appendix X. Prior to GC/MS analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis

5.3.1.2. Timing, Location and Environment

Celery seed (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd (Cobham, United Kingdom) were grown in commercial conditions in two locations in Spain and harvested in 2019. Harvest one was transplanted in Campo de Cartagena (37°39'12.6"N 0°53'33.1"W) late-November and harvested early March. The average air temperature was 16.8 °C with 0.1 mm average daily rainfall and an average relative humidity of 67.8 %. Conversely, harvest two was transplanted in Águilas (37°45'55.7"N 1°15'34.9"W) early-December and harvested mid-March. The average air temperature was 17.6 °C with 0.2 mm average daily rainfall and an average relative humidity of 77.3 %. Prior to harvest, the celery was subjected to regular in-field assessment to ensure standards for commercial quality were met, including visual and taste tests. These celeries were harvested within a close timeframe of the commercial produce also being grown in the field, acting as an indicator for commercial maturity.

5.3.1.3. Raw material collection, processing storage

The celery was grown in three randomised blocks in the centre of the field to reduce any influence from edge effects at a density of 10 plants m^{-2} and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles, sealed in labelled bags and packed into cool boxes and transported to the UK in refrigerated conditions using G's Fresh Ltd courier. Transportation took two days and samples were collected from G's Fresh (Ely, Cambridgeshire) before transportation back to the University of Reading. Samples for aroma analysis were immediately frozen at -80 °C for one week and subsequently freeze-dried for five days. Samples were then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ) and stored in an airtight container for a maximum of two weeks before analysis with gas chromatography/mass spectrometry (GC/MS).

5.3.2. Chemicals Reagents

For GC/MS analysis, calcium chloride and the alkane standard C6-C25 (100 μg/mL) in diethyl ether were obtained from Merck (Poole, UK).

5.3.3. Volatile analysis using SPME GCMS

The celery sample (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and then filled to 5 mL using HPLC-grade water in a 15 mL SPME vial fitted with a screw cap. Samples were analysed by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA).

Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m 250 µm 0.25 µm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of the injector, interface and detector was 250 °C and the sample injection mode was splitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions.

5.3.4. Statistical analysis

The percentage composition was calculated from the peak area data collected by SPME GC/MS analysis and quantitative data for each compound identified in the SPME GC/MS analysis were analysed by both two-way analysis of variance (ANOVA) and principal component analysis (PCA) using Spearman's Correlation on XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the two-way ANOVA, Tukey's Honest Significant

Difference post hoc test was applied to determine which sample means differed significantly ($P<0.05$) between the celery genotypes. This data is shown in Table 5.1. Only those compounds exhibiting significant differences between geographical location, genotype, and their interaction (geographical location x genotype) were included in the principal component analysis. To compose the PCA plots that combine both sensory and instrumental data, the volatile data was added as supplementary data on top of the flavour and aroma attributes.

5.4. Results and Discussion

5.4.1. Using SPME GCMS identified significant differences in the compositional differences in all eight genotypes

In total, 110 compounds were identified in the headspace of the eight celery genotypes in both harvests (Cartagena and Águilas) and these compounds are displayed in Table 5.1. Seventy-six compounds were identified in Cartagena-grown celery across the eight genotypes, including: 21 monoterpenes, 14 aldehydes, 11 sesquiterpenes, six phthalides and esters and four ketones, alcohols and monoterpenoid alcohols. An additional 21 compounds were identified in Águilas-grown celery including extra monoterpenes, monoterpenoid alcohols, ketones and alcohol compounds. Quantitative differences were observed between the two locations as well as the eight genotypes in this study and two-way ANOVA revealed significant differences in the aroma composition caused by both factors and their interaction. More compounds were identified not to express any significant difference than previous studies (chapters 3 and 4) due to growing in similar geographical locations and harvesting in within two weeks of each other. These included lower boiling monoterpenes as identified similarly in chapters 3 and 4 where we observed lower boiling monoterpenes (α-thujene, α-pinene, camphene and limonene) expressed no significant difference between genotype, harvest year or geographical location, highlighting the importance of these compounds to the crop and how fundamental these secondary metabolites are for the crop's defence mechanism (Turner et al. 2021a). Sesquiterpenes, alcohols, esters and phthalides

Reflected in all previous chapters as well as a plethora of literature, monoterpenes comprise the highest proportion of the aroma composition on celery (van Wassenhove et al. 1990; Orav et al. 2003;

Rożek, Nurzyńska-Wierdak, Sałata & Gumiela, 2016; Turner et al. 2021a). Cartagena produced celery that expressed an average monoterpene composition of 46.7 % and genotypes 8 and 12 displayed the highest limonene content (36 % and 32 %). Limonene was the most abundant compound in Cartagena celery, and this is reflected within literature whereby it is the most reported compound in celery and has been observed to comprise up to 80 % of the volatile composition of celery (Sowbhagya, Srinivas & Krishnamurthy, 2009). Genotype 22 displayed the lowest proportion of monoterpenes. Celery grown in Águilas displayed monoterpenes to constitute a lower proportion of the aroma composition, comprising 31 % of the total volatile content. Genotype 10 and 12 expressed the highest overall monoterpene content in the Águilas harvest and genotype 15 expressed the lowest proportion. Although differences in the monoterpene composition were observed, overall, 14 out of the 29 monoterpenes identified expressed no significant difference between genotype and location of growth further strengthening our hypothesis that these compounds are regularly synthesised regardless of genotype and environment.

Table 5.1: Percentage composition of volatile compounds identified in the headspace of eight celery parental genotypes

				Percentage Composition (%) [c]																	
				Cartagena								Águilas								P-value	
Code	Compound name	LRI [a]	ID [b]	5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G [d]	E [e]	Gx E [f]
	Alcohols																					
A1	3-methyl-3-butanol	730	A	0.56± 0.22	0.22± 0.02	0.69± 0.20	0.24± 0.10	1.1± 0.15	0.22± 0.22	0.34± 0.09	0.82± 0.43	0.60± 0.35	0.40± 0.06	0.91± 0.27	0.59± 0.13	0.36± 0.05	0.57± 0.22	0.54± 0.02	0.49± 0.13	ns	ns	ns
A2	2-methyl-1-butanol	742	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.08± 0.01 [ab]	0.07± 0.03 [ab]	0.12± 0.02 [b]	0.11± 0.01 [ab]	nd [a]	0.08± 0.04 [ab]	0.07± 0.05 [ab]	0.09± 0.02 [ab]	***	ns	***
A3	(E)-2-pentenol	758	A	0.79± 0.58	0.88± 0.37	1.0± 0.02	0.81± 0.21	2.0± 0.27	1.5± 0.06	1.2± 0.04	1.3± 0.16	0.72± 0.34	1.3± 0.25	1.1± 0.18	0.71± 0.09	0.60± 0.09	0.81± 0.31	0.87± 0.24	0.52± 0.06	ns	ns	ns
A4	pentanol	763	A	1.1± 0.90 [abc]	0.18± 0.13 [ab]	0.20± 0.10 [ab]	0.16± 0.01 [ab]	0.84± 0.14 [abc]	nd [a]	0.63± 0.34 [abc]	0.37± 0.24 [abc]	1.6± 0.27 [c]	0.50± 0.11 [abc]	0.76± 0.28 [ab]	0.49± 0.06 [abc]	1.1± 0.13 [abc]	0.87± 0.34 [abc]	1.5± 0.51 [bc]	0.88± 0.22 [abc]	**	**	**
A5	hexanol	862	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.53± 0.19 [ab]	0.44± 0.27 [ab]	0.79± 0.44 [b]	0.40± 0.21 [ab]	0.33± 0.08	0.40± 0.10 [ab]	0.48± 0.14 [ab]	0.47± 0.23 [ab]	***	***	***
A6	octanol	1072	A	0.77± 0.54 [ab]	0.56± 0.30 [ab]	1.1± 0.78 [ab]	0.74± 0.24 [ab]	0.71± 0.55 [ab]	0.79± 0.64 [ab]	0.90± 0.42 [ab]	0.94± 0.88 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
	Aldehydes																					
AL1	2-methyl-2-butenal	739	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.16± 0.07 [bc]	0.15± 0.08 [bc]	0.14± 0.06 [bc]	0.13± 0.02 [abc]	0.23± 0.03 [c]	0.19± 0.04 [bc]	0.19± 0.05 [bc]	0.09± 0.03 [ab]	***	***	***
AL2	(E)-2-pentenal	753	A	0.40± 0.14 [abc]	0.41± 0.22 [abc]	nd [a]	0.14± 0.04 [a]	0.27± 0.27 [ab]	0.33± 0.10 [ab]	0.34± 0.05 [ab]	0.52± 0.25 [abc]	0.78± 0.04 [bc]	0.13± 0.08 [ab]	0.34± 0.14 [abc]	nd [a]	0.78± 0.08 [bc]	0.80± 0.36 [c]	0.77± 0.09 [bc]	0.38± 0.11 [abc]	***	***	***
AL3	hexanal	800	A	16± 3.3	10± 2.8	5.5± 2.2	7.0± 0.34	13± 5.3	15± 8.6	23± 12	13± 0.95	25± 7.8	24± 6.2	13± 5.2	8.6± 3.6	22± 7.5	24± 4.9	25± 7.0	22± 6.3	*	*	*
AL4	(E)-2-hexenal	849	A	0.03± 0.04 [ab]	nd [a]	nd [a]	0.11± 0.01 [abc]	0.14± 0.13 [abc]	0.25± 0.04 [abcd]	0.12± 0.10 [abc]	nd [a]	0.56± 0.13 [cd]	0.57± 0.24 [cd]	0.30± 0.10 [abcd]	0.30± 0.07 [abcd]	0.55± 0.11 [cd]	0.54± 0.19 [cd]	0.57± 0.15 [cd]	0.51± 0.20 [bcd]	***	**	***
AL5	heptanal	901	A	1.6± 0.56	1.7± 0.50	0.89± 0.09	1.3± 0.31	2.3± 0.15	2.0± 0.74	2.0± 0.43	1.9±0.37	0.68± 0.18	0.58± 0.18	0.51± 0.13	0.48± 0.10	0.49± 0.35	0.57± 0.13	0.61± 0.20	0.72± 0.12	ns	ns	ns
AL6	(E)-2-heptenal	954	A	2.3± 0.46 [abcd]	1.8± 0.75 [abc]	1.9± 0.98 [abc]	1.3± 0.12 [ab]	2.2± 0.66 [abcd]	2.4± 0.99 [abcd]	2.1± 0.72 [abcd]	1.6± 0.33 [abc]	0.83± 0.75 [a]	0.49± 0.23 [a]	1.0± 0.36 [ab]	0.81± 0.64 [a]	0.77± 0.55 [a]	0.69± 0.33 [a]	1.0± 0.45 [ab]	0.75± 0.40 [a]	***	***	***
AL7	benzaldehyde	969	A	0.76± 0.08 [a]	0.61± 0.09 [a]	0.30± 0.03 [a]	0.53± 0.09 [a]	0.97± 0.01 [a]	0.75± 0.07 [a]	0.90± 0.42 [a]	0.99± 0.17 [a]	3.3± 1.8 [b]	1.7± 0.50 [ab]	1.9± 0.14 [ab]	1.9± 0.26 [ab]	1.7± 0.10 [ab]	1.6± 0.48 [ab]	1.7± 0.22 [ab]	1.9± 0.22 [ab]	***	***	***

AL 8	octanal	1007	A	0.84± 0.35	1.7± 0.56	0.71± 0.09	1.3± 0.07	2.0± 0.25	0.97± 0.01	1.1± 0.22	1.1± 0.06	0.86± 0.19	0.95± 0.22	0.56± 0.10	0.63± 0.13	1.6± 0.35	0.78± 0.21	0.54± 0.04	1.0± 0.22	ns	ns	ns
AL 9	phenylacetaldehyde	1049	A	0.27± 0.10 ab	0.15± 0.11 ab	0.34± 0.07 ab	0.22± 0.01 ab	0.26± 0.06 ab	0.15± 0.04 ab	nd a	0.29± 0.04 ab	0.31± 0.13 ab	0.24± 0.04 ab	0.26± 0.06 ab	0.42± 0.06 b	0.26± 0.02 ab	0.24± 0.06 ab	0.23± 0.98 ab	0.29± 0.05 ab	*	ns	*
AL 10	(E)-2-octenal	1057	A	0.87± 0.25 abc	1.2± 0.79 abc	0.87± 0.14 abc	0.47± 0.04 a	0.44± 0.09 a	1.2± 0.29 abc	0.68± 0.35 ab	0.79± 0.08 ab	3.3± 1.3 bc	2.2± 1.5 abc	1.5± 0.39 abc	1.4± 0.39 abc	3.4± 0.89 bc	3.5± 1.2 c	2.8± 0.96 abc	3.5± 1.0 c	***	***	** *
AL 11	m-tolualdehyde	1086	B[1]	0.70± 0.15	0.95± 0.34	0.73± 0.02	0.65± 0.13	1.5±0.14	0.85± 0.05	0.80± 0.19	1.0±0.19	0.72± 0.57	0.66± 0.26	0.71± 0.17	0.91± 0.19	0.64± 0.06	0.68± 0.32	0.57± 0.10	0.97± 0.08	ns	ns	ns
AL 12	p-tolualdehyde	1088	B[1]	0.28± 0.20	0.48± 0.43	0.15± 0.04	0.22± 0.07	0.44± 0.25	0.94± 0.63	0.90± 0.20	0.43± 0.08	nd	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
AL 13	nonanal	1105	A	0.47± 0.08	0.52± 0.22	0.65± 0.14	0.40± 0.01	0.53± 0.05	0.65± 0.05	0.68± 0.10	0.68± 0.03	0.68± 0.11	0.59± 0.18	0.39± 0.10	0.35± 0.13	0.57± 0.16	0.64± 0.35	0.61± 0.08	0.59± 0.11	ns	ns	ns
AL 14	(E,E)-2,4-octadienal	1110	A	0.15± 0.13	0.23± 0.11	0.09± 0.09	0.16± 0.02	0.24± 0.03	0.22± 0.01	nd	nd	0.15± 0.05	0.13± 0.04	0.11± 0.01	0.13± 0.03	0.16± 0.02	0.15± 0.03	0.14± 0.05	0.20± 0.02	ns	ns	ns
AL 15	(*E*,Z)-2,6-nonadienal	1162	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.08± 0.06 ab	0.15± 0.03 abc	0.11± 0.02 abc	0.12± 0.02 abc	0.29± 0.10 c	0.23± 0.02 bc	0.23± 0.16 bc	0.28± 0.05 c	***	***	** *
AL 16	(Z)-2-nonenal	1165	A	0.08± 0.07	0.20± 0.11	0.30± 0.07	0.15± 0.03	0.06± 0.06	0.28± 0.02	0.19± 0.04	nd	0.08± 0.03	0.07± 0.02	0.04± 0.03	0.14± 0.02	0.10± 0.01	0.08± 0.01	0.06± 0.05	0.12± 0.10	ns	ns	ns
AL 17	myrtenal	1207	B[2]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.19± 0.02 ab	0.14± 0.02 a	0.10± 0.03 a	0.11± 0.01 a	0.16± 0.04 ab	0.15± 0.04 ab	0.08± 0.06 a	0.37± 0.21 b	***	***	** *
AL 18	(2E, 4E)-nonadienal	1156	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.36± 0.11 ab	0.48± 0.24 b	0.20± 0.03 ab	0.16± 0.05 ab	0.41± 0.11 ab	0.35± 0.11 ab	0.46± 0.22 b	0.20± 0.17 ab	**	**	**
	Esters																					
E1	methyl butanoate	717	A	0.37± 0.15	0.21± 0.30	0.57± 0.03	0.36± 0.05	0.94± 0.13	0.15± 0.05	0.45± 0.13	0.44± 0.04	0.22± 0.14	0.18± 0.01	0.25± 0.04	0.17± 0.01	0.18± 0.04	0.18± 0.04	0.16± 0.02	0.19± 0.03	ns	ns	ns
E2	methyl pentanoate	884	A	0.14± 0.16 a	nd a	nd a	0.13± 0.13 a	nd a	nd a	nd a	nd a	0.34± 0.23 b	0.24± 0.02 ab	0.37± 0.13 b	0.40± 0.09 b	0.23± 0.07 ab	0.39± 0.18 b	0.27± 0.05 ab	0.30± 0.05 ab	***	***	** *
E3	methyl hexanoate	921	A	2.2± 1.9	0.72± 0.35	2.1± 0.66	1.2± 0.23	1.0± 0.38	0.88± 0.38	1.5± 0.98	0.82± 0.21	0.25± 0.12	0.29± 0.16	0.12± 0.01	0.08± 0.03	0.25± 0.09	0.38± 0.10	0.28± 0.10	0.24± 0.11	ns	ns	ns
E4	methyl octanoate	1122	A	0.19± 0.27	0.26± 0.11	0.12± 0.02	0.18± 0.01	0.18± 0.07	0.35± 0.07	0.37± 0.09	0.18± 0.08	nd	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
E5	lavandulyl acetate	1290	B[3]	0.86± 0.28 b	1.1± 0.55 b	1.8± 0.74 b	0.66± 0.01 b	1.6± 0.55 b	1.3± 0.21 b	1.4± 0.28 b	1.6± 0.53 b	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	*	*	*
E6	cis-pinocarvyl acetate	1343	B[4]	0.08± 0.05	0.05± 0.06	0.08± 0.08	0.19± 0.01	0.12± 0.11	0.21± 0.20	0.17± 0.05	nd	0.21± 0.05	0.14± 0.02	0.22± 0.04	0.17± 0.04	0.20± 0.04	0.27± 0.08	0.20± 0.05	0.29± 0.10	ns	ns	ns
E7	hexyl isobutanoate	1378	B[5]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.15± 0.12 ab	0.15± 0.12 ab	0.40± 0.04 b	0.22± 0.11 ab	0.18± 0.13 ab	0.11± 0.16 ab	0.36± 0.23 ab	0.13± 0.11 ab	**	**	**
	Total																					

	Ketones																				
K1	2-methyl-3-pentanone	746	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.10± 0.05 [ab]	0.08± 0.02 [ab]	0.19± 0.02 [b]	0.10± 0.01 [ab]	0.07± 0.01 [a]	0.09± 0.02 [ab]	0.09± 0.01 [ab]	0.09± 0.02 [ab]	***	***	***
K2	3-heptanone	884	A	0.05± 0.07	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.08± 0.04 [a]	nd [a]	0.14± 0.05 [b]	0.13± 0.08 [b]	0.12± 0.08 [b]	0.05± 0.02 [a]	0.09± 0.03 [a]	0.13± 0.01 [b]	0.13± 0.03 [b]	0.13± 0.04 [b]	**	*	**
K3	2-heptanone	889	A	0.39± 0.15	0.36± 0.09	0.43± 0.09	0.39± 0.05	0.57± 0.08	0.65± 0.43	0.70± 0.39	0.69± 0.13	0.49± 0.14	0.48± 0.15	0.31± 0.08	0.17± 0.12	0.39± 0.08	0.49± 0.12	0.44± 0.16	0.56± 0.18	ns	ns	ns
K4	1-octen-3-one	976	A	4.9± 0.77	3.6± 1.1	6.5± 0.91	3.7± 1.4	4.9± 0.77	4.8± 0.91	5.3± 1.1	4.2± 0.05	3.0± 0.55	3.9± 1.7	2.9± 0.17	2.3± 0.35	4.4± 0.61	3.3± 0.73	3.5± 1.3	3.9± 0.95	ns	ns	ns
K5	(E,E)-3,5-octadien-2-one	1070	B[6]	0.22± 0.16 [ab]	0.38± 0.13 [ab]	nd [a]	0.38± 0.07 [ab]	0.14± 0.14 [a]	0.29± 0.08 [ab]	0.50± 0.29 [abc]	nd [a]	0.79± 0.14 [abc]	1.1± 0.29 [bc]	0.60± 0.14 [abc]	0.81± 0.23 [ab]	1.3± 0.15 [c]	0.82± 0.19 [abc]	1.3± 0.41 [c]	0.63± 0.45 [abc]	***	***	***
K6	acetophenone	1073	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.30± 0.16 [b]	0.25± 0.16 [b]	0.27± 0.05 [b]	0.31± 0.04 [b]	0.25± 0.01 [b]	0.26± 0.07 [b]	0.28± 0.07 [b]	0.29± 0.02 [b]	***	***	***
K7	3,5-octadien-2-one	1092	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	2.2± 0.65 [b]	2.4± 1.1[b]	0.92± 0.38 [ab]	0.81± 0.32 [ab]	2.1± 0.77 [b]	2.2± 1.0 [b]	2.2± 0.81[b]	2.1± 0.91[b]	***	***	***
K8	p-methyl-acetophenone	1179	B[7]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.11± 0.04 [ab]	0.07± 0.01 [a]	0.04± 0.03 [a]	0.06± 0.04 [a]	0.08± 0.04 [ab]	nd [a]	0.07± 0.05 [a]	0.22± 0.10 [b]	***	***	***
K9	dihydrojasmone	1378	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.62± 0.33 [b]	0.69± 0.38 [b]	0.06± 0.04 [ab]	0.17± 0.13 [ab]	0.71± 0.36 [b]	0.63± 0.26 [ab]	0.30± 0.21 [ab]	0.57± 0.15 [ab]	***	***	***
	Alkanes																					
ALK1	nonane	900	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.84± 0.44 [ab]	0.62± 0.36 [ab]	0.69± 0.21 [ab]	0.27± 0.14 [a]	1.7± 0.34 [b]	0.41± 0.06 [ab]	0.36± 0.16 [ab]	0.90± 0.35 [ab]	*	**	**
ALK2	decane	1000	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	1.6± 0.18 [b]	1.7± 0.33 [bc]	1.5± 0.36 [b]	1.6± 0.05 [b]	2.2± 0.21 [c]	1.9± 0.05 [bc]	1.9± 0.18 [bc]	1.6± 0.19 [b]	***	***	***
ALK3	undecane	1100	A	0.28± 0.40	0.41± 0.31	0.58± 0.58	0.19± 0.09	0.20± 0.10	0.41± 0.05	0.46± 0.02	nd	0.60± 0.31	0.27± 0.10	0.57± 0.04	0.63± 0.02	0.55± 0.03	0.33± 0.03	0.43± 0.12	0.52± 0.05	ns	ns	ns
ALK4	dodecane	1199	A	0.34± 0.31	0.30± 0.09	0.36± 0.08	0.32± 0.05	0.37± 0.11	0.25± 0.11	0.16± 0.06	nd	0.49± 0.23	0.20± 0.03	0.37± 0.10	0.31± 0.05	0.26± 0.03	0.29± 0.03	0.27± 0.04	0.34± 0.08	ns	ns	ns
ALK5	tridecane	1299	A	0.34± 0.48	0.14± 0.20	0.12± 0.12	0.08± 0.04	nd	0.16± 0.04	nd	nd	0.16± 0.03	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
ALK6	tetradecane	1399	A	0.81± 0.93	0.41± 0.25	0.63± 0.05	0.24± 0.21	0.43± 0.27	0.38± 0.28	0.14± 0.13	0.12± 0.12	0.16± 0.12	0.02± 0.03	0.02± 0.01	0.03± 0.01	0.02± 0.01	0.04± 0.03	0.01± 0.02	0.07± 0.06	ns	ns	ns
ALK7	pentadecane	1499	A	nd	nd	nd	nd	nd	nd	nd	nd	0.15± 0.02	nd	0.03± 0.05	nd	0.18± 0.02	0.14± 0.01	0.14± 0.02	nd	ns	ns	ns
	Monoterpenes																					

M1	α-thujene	993	B[2]	1.1± 0.42	0.59± 0.19	2.4± 0.85	0.92± 0.07	1.8± 0.22	0.96± 0.10	1.0± 0.32	1.5± 0.01	0.64± 0.31	0.52± 0.19	1.1± 0.17	0.78± 0.20	0.42± 0.02	0.58± 0.14	0.64± 0.06	0.72± 0.22	ns	ns	ns
M2	α-pinene	943	A	0.68± 0.12	0.49± 0.03	0.69± 0.02	0.43± 0.03	0.84± 0.03	0.71± 0.08	0.41± 0.07	0.63± 0.02	0.83± 0.14	0.49± 0.26	1.0± 0.30	0.81± 0.16	0.77± 0.33	0.69± 0.10	1.1± 0.58	0.75± 0.46	ns	ns	ns
M3	camphene	960	A	1.2± 0.21	0.74± 0.13	1.9± 0.19	0.88± 0.14	1.8± 0.14	1.0± 0.06	1.1± 0.22	1.6± 0.08	0.73± 0.21	0.57± 0.05	0.93± 0.05	0.94± 0.13	0.73± 0.12	0.45± 0.32	0.96± 0.11	0.68± 0.14	ns	ns	ns
M4	sabinene	981	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.37± 0.25 [b]	0.29± 0.08 [b]	0.34± 0.19 [b]	0.32± 0.09 [b]	0.31± 0.08 [b]	0.38± 0.15 [b]	0.30± 0.07 [b]	0.34± 0.07 [b]	***	***	***
M5	β-pinene	989	A	3.2± 0.62	4.0± 0.66	1.6± 0.03	2.5± 0.15	3.7± 0.33	1.1± 0.05	2.7± 0.66	4.2± 0.57	2.3± 0.63	2.1± 1.1	1.5± 0.38	2.6± 0.65	3.5± 1.4	1.1± 0.18	2.5± 1.3	2.9± 1.9	ns	ns	ns
M6	myrcene	992	A	0.60± 0.18 [a]	0.34± 0.08 [a]	1.6± 0.94 [b]	1.6± 0.06 [b]	0.68± 0.01 [a]	1.2± 0.05 [ab]	0.53± 0.06 [a]	0.59± 0.03 [a]	0.51± 0.03 [a]	0.54± 0.19 [a]	1.8± 0.46 [b]	1.4± 0.06 [b]	0.48± 0.10 [a]	1.1± 0.25 [ab]	0.56± 0.18 [a]	0.51± 0.05 [a]	*	*	*
M7	p-mentha-2,8-diene	1003	B[8]	1.6± 0.78 [b]	1.0± 0.64 [b]	1.8± 0.78 [b]	1.6± 0.19 [b]	1.4± 0.54 [b]	1.4± 0.68 [b]	1.7± 0.05 [b]	1.5± 0.35 [b]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	ns	***	***
M8	α-phellandrene	1013	A	1.9± 0.11 [b]	1.9± 1.1 [b]	0.54± 0.23 [a]	1.8± 0.33 [b]	2.1± 1.4 [b]	1.8± 0.45 [b]	2.1± 0.65 [b]	2.1± 0.63 [b]	0.37± 0.16 [a]	0.31± 0.03 [a]	0.52± 0.06 [a]	0.40± 0.06 [a]	0.33± 0.04 [a]	0.39± 0.03 [a]	0.39± 0.07 [a]	0.37± 0.03 [a]	***	***	***
M9	α-terpinene	1017	A	0.48± 0.32 [b]	nd [a]	0.22± 0.20 [ab]	0.34± 0.02 [ab]	0.16± 0.06 [ab]	0.19± 0.10 [ab]	0.18± 0.17 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	*	*	*
M10	delta-3-carene	1019	A	0.21± 0.15	0.24± 0.11	0.27± 0.01	0.11± 0.10	0.13± 0.02	0.24± 0.03	0.17± 0.06	0.21± 0.03	0.72± 0.33	0.69± 0.39	0.94± 0.74	0.63± 0.44	0.54± 0.30	0.58± 0.30	0.77± 0.38	0.77± 0.46	ns	ns	ns
M11	o-cymene	1032	A	5.4± 0.91	6.5± 2.0	5.1± 0.05	6.6± 0.64	3.2± 0.28	5.4± 0.33	3.8± 0.09	5.8± 1.2	3.8± 0.94	3.7± 1.1	4.6± 1.3	3.4± 0.67	2.3± 0.94	3.9± 0.82	3.4± 1.5	3.3± 1.1	ns	ns	ns
M12	limonene	1034	A	27± 11	36± 11	27± 1.8	32± 1.8	20± 0.57	25± 1.7	19± 8.5	29± 8.6	11± 4.9	19± 1.9	24± 7.6	21± 2.1	11± 6.1	12± 5.1	15± 5.3	11± 5.3	ns	ns	ns
M13	β-(E)-ocimene	1049	B[9]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	1.3± 0.91 [ab]	0.71± 0.32 [a]	nd [a]	nd [a]	1.7± 0.29 [ab]	1.1± 0.28 [a]	nd [a]	3.1± 0.43 [b]	***	***	***
M14	γ-terpinene	1066	A	0.37± 0.03 [ab]	0.97± 0.54 [abc]	1.5± 0.31 [abc]	0.80± 0.18 [ab]	0.34± 0.03 [ab]	0.77± 0.08 [ab]	1.3± 0.54 [abc]	0.36± 0.05 [ab]	0.72± 0.12 [ab]	2.6± 1.4 [c]	2.2± 0.36 [bc]	2.0± 0.35 [abc]	1.2± 0.24 [abc]	1.1± 0.24 [abc]	1.1± 0.20 [abc]	1.1± 0.36 [abc]	**	**	**
M15	terpinolene	1097	A	0.27± 0.04 [a]	0.33± 0.31 [a]	0.11± 0.10 [a]	0.28± 0.03 [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.35± 0.08 [a]	0.25± 0.18 [a]	0.13± 0.08 [a]	0.20± 0.14 [a]	0.38± 0.14 [a]	0.34± 0.14 [a]	nd [a]	0.25± 0.18 [a]	*	*	*
M16	β-thujone	1124	B[1]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.06± 0.02 [ab]	0.01± 0.02 [a]	0.08± 0.01 [abc]	0.20± 0.04 [c]	0.05± 0.02 [ab]	0.08± 0.02 [ab]	0.17± 0.12 [b]	0.06± 0.02 [ab]	***	***	***
M17	p-mentha-1,5,8-triene	1135	B[8]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.07± 0.02 [ab]	0.02± 0.02 [a]	0.16± 0.04 [abc]	0.55± 0.15 [c]	0.07± 0.01 [ab]	0.17± 0.05 [abc]	0.50± 0.27 [ac]	0.09± 0.06 [abc]	**	**	**

M18	allo-ocimene	1132	B[10]	0.05±0.03 ab	0.01±0.01 ab	0.15±0.09 b	0.51±0.24 b	0.05±0.06 ab	0.11±0.03 ab	0.46±0.17 b	0.06±0.04 ab	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	ns	***	***
M19	camphor	1157	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.27±0.15 bc	0.17±0.04 abc	0.22±0.06 abc	0.17±0.05 abc	0.18±0.08 abc	0.23±0.06 bc	0.15±0.03 ab	0.38±0.13 c	***	***	***
M20	isoborneol	1173	A	0.11±0.16	0.19±0.18	0.77±0.22	0.29±0.06	0.42±0.10	nd	0.04±0.03	0.19±0.09	0.25±0.14	0.17±0.03	0.16±0.06	0.17±0.04	0.19±0.04	0.25±0.04	0.18±0.05	0.23±0.12	ns	ns	ns
M21	cis-dihydrocarvone	1208	A	nd	nd	nd	nd	nd	nd	nd	nd	0.35±0.03	0.28±0.02	0.30±0.05	0.25±0.06	0.23±0.12	0.20±0.14	nd	0.39±0.06	ns	*	ns
M22	safranal	1215	A	0.28±0.13	0.17±0.12	0.19±0.05	0.17±0.09	0.16±0.02	0.21±0.18	0.14±0.06	0.62±0.33	nd	nd	nd	nd	nd	nd	nd	nd	**	ns	ns
M23	pentylcyclohexa-1,3-diene	1166	B[2]	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.20±0.06 ab	0.13±0.09 ab	0.19±0.08 ab	0.20±0.02 ab	0.16±0.05 ab	0.19±0.02 ab	0.12±0.09 ab	0.30±0.14 ab	***	***	***
M24	trans-dihydrocarvone	1240	B[11]	0.42±0.18 ab	0.69±0.21 b	0.64±0.14 b	0.29±0.03 ab	0.51±0.15 ab	0.54±0.16 ab	0.31±0.08 ab	0.55±0.02 ab	0.10±0.03 ab	0.06±0.04 a	0.08±0.02 a	0.08±0.01 a	0.06±0.02 a	0.11±0.03 ab	0.06±0.04 a	0.14±0.09 ab	ns	**	*
M25	β-cyclocitral	1230	A	0.04±0.05	0.02±0.03	nd	0.12±0.01	0.24±0.06	0.06±0.06	0.08±0.06	nd	0.10±0.04	0.12±0.02	0.11±0.03	0.18±0.02	0.15±0.01	0.12±0.02	0.10±0.01	0.14±0.06	ns	ns	ns
M26	L-carvone	1248	A	0.17±0.24 a	0.11±0.10 a	nd a	0.12±0.01 a	0.08±0.08 a	0.21±0.02 a	nd a	nd a	0.38±0.22 a	0.26±0.11 a	0.18±0.06 a	0.14±0.02 a	0.23±0.08 a	0.36±0.03 a	0.17±0.08 a	0.45±0.23 a	*	*	*
M27	D-carvone	1262	A	1.8±0.62	1.0±0.24	1.7±0.02	1.7±0.48	2.2±0.75	2.2±1.1	1.8±0.70	1.8±0.11	0.33±0.13	0.27±0.06	0.60±0.13	0.36±0.17	0.30±0.10	0.48±0.11	0.52±0.11	0.47±0.18	ns	ns	ns
M28	thymol	1290	A	0.04±0.06 ab	0.05±0.06 ab	nd a	0.05±0.04 ab	nd a	nd a	nd a	nd a	0.15±0.09 ab	0.12±0.07 ab	0.15±0.01 ab	0.16±0.01 ab	0.12±0.01 ab	0.19±0.08 b	0.09±0.03 ab	0.16±0.05 ab	***	***	***
M29	carvacrol	1317	A	0.17±0.14	0.25±0.15	0.25±0.06	0.32±0.01	0.22±0.06	0.38±0.18	0.10±0.10	0.10±0.07	0.44±0.21	0.36±0.27	0.45±0.05	0.53±0.08	0.31±0.12	0.56±0.23	0.19±0.07	0.39±0.14	ns	ns	ns
	Monoterpenoid Alcohols																					
MA1	p-mentha-2,8-dien-1-ol	1122	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.15±0.03 b	0.16±0.01 b	0.15±0.03 b	0.13±0.02 ab	0.12±0.07 ab	0.13±0.02 ab	0.12±0.03 ab	0.19±0.13 b	***	***	***
MA2	dihydrolinalool	1142	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.75±0.31 abc	0.33±0.26 abc	0.93±0.08 bc	1.2±0.06 c	0.78±0.18 abc	0.64±0.30 abc	0.29±0.11 ab	0.48±0.24 abc	***	***	***
MA3	trans-pinocarveol	1147	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.29±0.09 ab	0.21±0.10 ab	0.11±0.06 ab	0.10±0.01 ab	0.20±0.10 ab	0.47±0.32 ab	0.15±0.03 ab	0.57±0.42 b	**	**	**

MA4	terpinen-4-ol	1184	A	0.19± 0.12	0.15± 0.03	0.08± 0.08	0.09± 0.04	0.10± 0.07	0.17± 0.14	0.08± 0.03	0.21± 0.16	0.10± 0.09	0.15± 0.04	0.13± 0.03	0.18± 0.02	0.06± 0.04	0.15± 0.06	nd	0.20± 0.04	ns	ns	ns
MA5	p-cymen-8-ol	1204	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.19± 0.05 [b]	0.15± 0.06 [b]	0.09± 0.04 [ab]	0.09± 0.01 [ab]	0.10± 0.02 [ab]	0.18± 0.03 [b]	0.08± 0.06 [ab]	0.18± 0.05 [b]	***	***	** *
MA6	cis-carveol	1246	B[12]	0.09± 0.04 [b]	0.05± 0.05 [b]	0.08± 0.06 [b]	0.07± 0.07 [b]	0.06± 0.04 [b]	0.11± 0.03 [b]	0.05± 0.02 [b]	0.14± 0.02 [b]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	ns	***	** *
MA7	trans-carveol	1147	B[2]	0.17± 0.15	0.43± 0.29	0.31± 0.10	0.21± 0.03	0.25± 0.25	0.54± 0.33	0.52± 0.25	0.30± 0.04	0.51± 0.07	0.45± 0.21	0.64± 0.09	0.44± 0.02	0.34± 0.07	0.51± 0.14	0.26± 0.09	0.60± 0.23	ns	ns	ns
MA9	α-terpineol	1211	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.19± 0.05	0.15± 0.06	0.09± 0.04	0.09± 0.01	0.10± 0.02	0.18± 0.03	0.08± 0.06	0.18± 0.05	ns	ns	ns
MA10	caryophylladienol II	1665	B[13]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.07± 0.05 [b]	nd [a]	0.09± 0.01 [b]	0.09± 0.02 [b]	0.08± 0.01 [b]	0.11± 0.03 [b]	0.08± 0.02 [b]	0.09± 0.03 [b]	ns	***	** *
	Oxides																					
O1	limonene oxide	1141	A	1.2± 0.35	1.5± 0.85	2.1± 0.87	0.76± 0.13	2.0± 0.86	1.4± 0.25	1.5± 0.24	2.1± 0.97	nd	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
O2	caryophyllene oxide	1610	A	0.46± 0.06	0.61± 0.48	0.66± 0.26	0.37± 0.06	0.66± 0.20	0.58± 0.29	0.61± 0.47	0.42± 0.42	0.25± 0.06	0.27± 0.08	0.28± 0.04	0.24± 0.09	0.26± 0.03	0.33± 0.11	0.22± 0.03	0.27± 0.11	ns	ns	ns
	Lactones																					
L1	γ-nonalactone	1372	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.06± 0.01 [bcde]	0.06± 0.02 [bcde]	0.04± 0.01 [abc]	0.03± 0.01 [abc]	0.06± 0.01 [bcde]	0.07± 0.01 [cde]	0.09± 0.03 [de]	0.10± 0.01 [e]	***	***	** *
L2	dihydroactinolide	1557	B[14]	0.10± 0.01	0.07± 0.10	0.09± 0.09	nd	0.14± 0.14	nd	nd	nd	0.04± 0.06	0.07± 0.05	0.07± 0.02	nd	0.16± 0.01	0.08± 0.06	0.10± 0.03	0.03± 0.02	ns	ns	ns
	Sesquiterpenes																					
S1	α-ylangene	1384	B[11]	0.17± 0.11 [b]	0.05± 0.03 [ab]	0.07± 0.04 [ab]	0.15± 0.09 [ab]	0.09± 0.05 [ab]	0.19± 0.15 [b]	0.10± 0.04	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	ns	***	** *
S2	α-copaene	1390	A	0.13± 0.11	0.11± 0.08	0.05± 0.05	nd	nd	0.07± 0.07	0.33± 0.19	0.24± 0.02	0.14± 0.04	0.09± 0.06	0.06± 0.02	nd	nd	0.12± 0.05	0.24± 0.07	0.22± 0.18	ns	ns	ns
S3	β-caryophyllene	1445	A	0.36± 0.10	0.73± 0.03	1.4±0.38	0.55± 0.31	0.43± 0.13	0.98± 0.74	0.59± 0.51	0.47± 0.03	0.67± 0.52	0.60± 0.40	1.4± 0.73	1.0± 0.15	0.46± 0.17	1.2± 0.13	0.55± 0.28	0.69± 0.28	ns	ns	ns
S4	(+)-aromadendrene	1452	A	0.03± 0.03	0.15± 0.03	0.04± 0.04	0.07± 0.04	0.14± 0.08	0.08± 0.05	0.10± 0.07	0.07± 0.02	nd	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
S5	α-humulene	1479	A	0.88± 0.01	0.58± 0.03	0.62± 0.15	0.35± 0.28	0.52± 0.10	0.39± 0.19	0.32± 0.06	0.24± 0.21	0.11± 0.02	0.10± 0.06	0.07± 0.05	0.08± 0.02	0.19± 0.04	0.07± 0.06	0.03± 0.03	0.13± 0.05	ns	ns	ns
S6	β-selinene	1508	B[15]	0.46± 0.01	0.62± 0.06	0.50± 0.18	3.6± 1.7	0.65± 0.11	0.33± 0.12	0.58± 0.34	0.90± 0.20	0.35± 0.25	0.31± 0.16	0.30± 0.17	1.3± 0.29	0.17± 0.06	0.40± 0.26	0.36± 0.15	0.50± 0.12	ns	ns	ns

S7	valencene	1514	A	0.08±0.07	0.05±0.04	0.15±0.03	3.3±0.32	0.25±0.10	0.13±0.10	0.05±0.06	0.20±0.08	nd	nd	0.03±0.02	2.1±0.16	0.01±0.02	0.01±0.01	0.02±0.02	0.36±0.05	ns	ns	ns
S8	α-selinene	1515	B[16]	0.09±0.01	0.06±0.04	0.08±0.01	1.7±1.5	0.08±0.01	0.08±0.03	0.16±0.11	0.14±0.04	0.06±0.04	0.04±0.03	0.04±0.03	0.14±0.03	0.02±0.02	0.06±0.05	0.05±0.04	0.07±0.02	ns	ns	ns
S9	cuparene[$]	1530	B[2]	nd	nd	nd	nd	nd	nd	nd	nd	0.01±0.02	nd	nd	nd	0.04±0.01	0.01±0.01	nd	0.03±0.04	ns	ns	ns
S10	(E)-nerolidol	1540	A	0.04±0.03	0.02±0.02	0.20±0.06	0.03±0.03	0.20±0.06	nd	0.08±0.03	0.08±0.02	0.03±0.02	0.02±0.02	nd	nd	0.06±0.02	0.04±0.04	0.04±0.03	0.04±0.03	ns	ns	ns
S11	kessane	1557	B[2]	nd [a]	nd [a]	nd [a]	2.1±1.2 [b]	nd [a]	nd [a]	0.15±0.06 [ab]	nd [a]	0.05±0.03 [ab]	0.01±0.01 [ab]	nd [a]	2.0±0.13 [b]	nd [a]	0.01±0.02 [ab]	nd [a]	0.36±0.05 [ab]	*	*	*
S12	liguloxide[$]	1560	B[17]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.05±0.01 [a]	nd [a]	0.04±0.05 [a]	nd [a]	0.01±0.01 [a]	*	*	*
	Phthalides																					
P1	3-propylidene phthalide	1600	A	0.02±0.07 [ab]	0.04±0.02 [ab]	nd [a]	0.24±0.11 [b]	0.08±0.04 [ab]	0.07±0.04 [ab]	0.06±0.06 [ab]	0.07±0.08 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	ns	***	***
P2	3-butyl hexahydro phthalide	1662	B[2]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.05±0.04 [abc]	0.01±0.02 [ab]	0.05±0.01 [abc]	nd [a]	0.06±0.01 [bc]	0.08±0.02 [c]	0.04±0.01 [abc]	0.06±0.01 [bc]	ns	***	***
P3	3-butylphthalide	1676	B[2]	6.2±1.6	6.5±2.7	6.7±0.87	7.3±1.9	7.7±1.3	8.2±3.1	8.3±1.7	7.5±1.9	4.2±1.1	3.6±0.81	5.6±1.1	8.5±0.86	4.9±0.93	5.6±1.4	5.2±1.3	4.6±0.87	ns	ns	ns
P4	Z-3-butylidene phthalide	1685	B[18,19]	0.32±0.23	0.36±0.38	0.40±0.37	0.30±0.01	0.93±0.25	0.35±0.23	0.37±0.24	0.36±0.11	0.22±0.20	0.09±0.04	0.13±0.01	0.13±0.01	0.25±0.06	0.17±0.06	0.09±0.01	0.14±0.04	ns	ns	ns
P5	sedanenolide	1748	B[18,19]	1.0±0.50 [a]	0.81±0.54 [a]	2.9±0.63 [a]	2.1±0.97 [a]	2.4±0.95 [a]	1.8±1.7 [a]	0.69±0.56 [a]	0.94±0.25 [a]	1.1±0.30 [a]	0.96±0.03 [a]	3.7±1.1 [a]	9.2±1.1 [b]	1.5±0.49 [a]	2.0±0.89 [a]	0.92±0.52	1.3±1.1	***	***	***
P6	trans-neocnidilide	1755	B[2]	0.45±0.27	0.41±0.37	2.7±0.62	0.11±0.03	0.43±0.16	2.3±1.3	1.0±0.05	1.3±0.04	1.4±1.1	0.45±0.24	1.2±0.24	0.14±0.01	0.37±0.15	1.7±0.55	1.0±0.23	1.1±0.19	ns	ns	ns
P7	(E)-ligustilide	1764	B[18,19]	nd	0.07±0.06	0.13±0.01	0.05±0.01	0.40±0.26	0.15±0.07	0.36±0.31	0.04±0.01	0.03±0.02	0.03±0.02	0.09±0.03	0.11±0.03	0.25±0.04	0.05±0.02	0.02±0.01	0.03±0.02	ns	ns	ns

[a]Linear retention index on a DB5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; [1]Radulovic et al., 2010; [2]Adams et al., 2005; [3]Bader et al., 2003; [4]Stashenko et al., 2003; [5] Lucero et al., 2006; [6]Beaulieu et al., 2001; [7]Lucero, Estell & Frederickson, 2003; [8]Courtois et al., 2009; [9]Sabulal et al., 2007; [10]Havlik et al., 2006; [11]Bylaite et al., 2006; [12] Chagonda and Chalchat, 2005; [13]Morteza-Semnani et al., 2007; [14]Chuang et al., 2007; [15]Yu et al., 2007; [16]Zeng et al., 2007; [17]Pripdeevech and Saansoomchair, 2013; [18]Turner et al., 2021a; [19]Turner et al., 2021c; [$] tentatively identified, spectral quality value of 70 was used for this compound. [c] Percentage composition of total peak area divided by compound peak area; means labelled with letters are significantly different ($p < 0.05$) according to the GxE interaction; means of three replicate samples; tr, trace amounts <0.10%; nd, not detected. [d] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [e] Geographical location. [f] Genotype. [g] geographical location x genotype interaction.

Across all genotypes, sesquiterpenes were observed to comprise of 3.6 % and 2.2 % of the aroma composition of celery grown in Cartagena and Águilas, respectively. Most reported sesquiterpenes, β-caryophyllene and β-selinene (Philippe, Suvarnalatha, Sankar & Suresh, 2002; Ehiabhi et al., 2006; Turner et al., 2021b) were observed to comprise on average 0.68 % and 0.95% in Cartagena and 0.82 % and 0.46 % in Águilas, respectively. Both these compounds have been observed to exhibit celery-like and herbal odour characteristics and in particular, β-selinene has been observed as one of the major components of celery volatile oil (Lund, Wagner, and Bryan, 1973; Macleod & Ames, 1989; Philippe, Suvarnalatha, Sankar & Suresh, 2002)

In contrast to sesquiterpenes, aldehydes were observed to comprise a higher proportion of the aroma profile in both locations, contrary to what has been identified in literature whereby minimal aldehydes were identified. On average, celery grown in Águilas comprised a higher proportion of aldehydes than celery grown in Cartagena (37.8 % and 22.3 %). Genotype 18 expressed the highest aldehyde content in Cartagena grown celery, primarily constituted of hexanal which comprised 22 % of the overall aroma composition whereas genotype 15 in Águilas expressed a similar proportion of hexanal in addition to (E)-2-heptanal, which contributed up to 11 % of the aroma composition. Aldehydes composed the lowest proportion of the aroma composition in genotypes 10 and 12 in both locations (Table 5.1). Genotype, location, and their interaction both exhibited a significant influence over the composition of hexanal, (E)-2-heptanal, benzaldehyde and (E)-2-octanal, compounds that have been observed to contribute green, fresh, almond, fatty and herbal odour characteristics to celery (Turner et al, 2021c). Less studied within celery, Gold and Wilson (1963) identified ten aldehydes in distilled celery juice; several of these including hexanal, heptanal and octanal were also identified in the present study.

Identified in chapter 4, ketones were further noted in Spanish-grown celery where they were not previously identified in UK grown celery (Turner et al, 2021a). In total, nine ketones were observed in both Spanish-grown celery and Águilas-grown celery, displaying the highest proportion of ketones as well as the highest number identified, comprising on average 7.6 % of the total volatile composition compared to 5.5 % in Cartagena-grown celery. 1-Octen-3-one was the ketone observed to form the highest proportion of the ketone composition in both locations and Cartagena-grown celery expressed

a higher proportion of this compound. Similar to the aldehyde content, genotype 12 expressed the lowest proportion of these compounds in both locations and genotypes 10 and 15 exhibited high percentages of ketones. It was expected that ketones and aldehydes responded in a similar manner due to their close relation in structure, both containing a carbonyl group, as well as similarities in volatility. Although not as reactive as aldehydes, ketones are readily available for chemical reactions (Brown, 2019).

Phthalides have been discussed throughout this project as the key compounds that contribute to the aroma and flavour of celery (Macleod & Ames, 1989; Kurobayashi et al., 2006; Turner et al., 2021a; 2021b; 2021c). With odour descriptors including "celery", "cooked celery" and "herbal" (Turner, Dawda, Wagstaff, Gawthrop & Lignou, 2021c), these compounds have been observed to vary significantly between genotypes as well as between geographical location and harvest year (Turner et al, 2021a). However, when comparing the average phthalide composition, location indicates to play more of a significant influence in 3-propylidene phthalide, 3-butyl hexahydro phthalide and Sedanenolide abundance (Table 5.1). Genotype 12 grown in Águilas expressed the highest proportion of phthalides (18 %) due to its particularly high 3-butylphthalide and sedanenolide content whereas genotype 10 grown in Cartagena expressed the highest proportion of trans-neocnidilide, comprising 2.7 % of the aroma profile. 3-Butylphthalide content was not significant between both locations and although this compound displays a higher odour activity value than sedanenolide, strong celery odour characteristics still remains associated with this compound (Lund, Wagner & Bryan, 1973).

Significant differences were observed in the aroma composition of eight celery genotypes grown in two regions of Spain (Cartagena and Águilas) and completing principal component analysis using only the significant compounds expressed in the two-way ANOVA according to genotype, location, and their interaction, allowed us to visualise the differences in the chemical profile between the two harvests (Figure 5.1). A clear difference in the profiles were observed through the separation of celery grown in Cartagena and Águilas along the F2 axis. Principal component one (F1) and two (F2) explained 75.06 % of the total variation within the dataset and it can be observed that the first axis separates genotypes 10 and 12 for both locations and 18 and 25 from Águilas along with 15 from Cartagena. The second axis separates celery grown in Cartagena with celery grown in Águilas.

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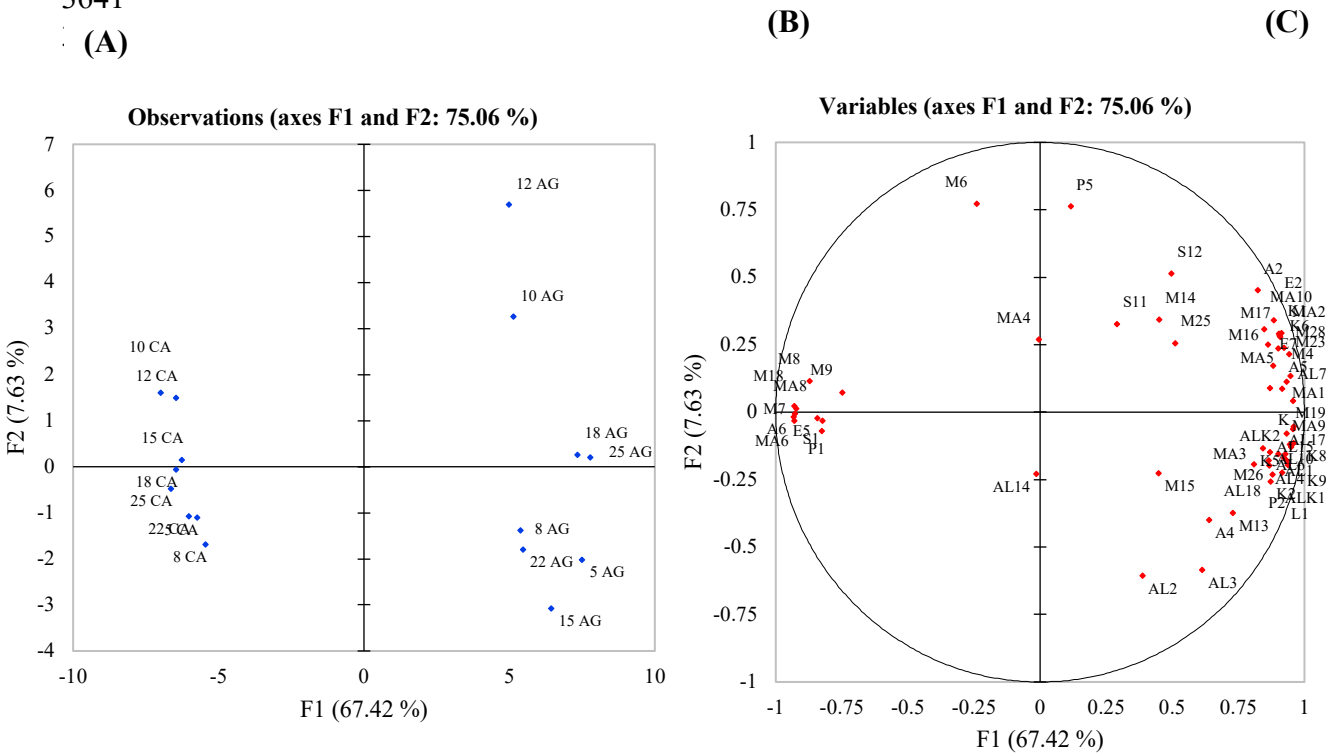

A2	2-methyl-1-butanol	M7	p-mentha-2,8-diene
A4	pentanol	M8	α-phellandrene
A5	hexanol	M9	α-terpinene
A6	octanol	M13	β-(E)-ocimene
AL1	2-methyl-2-butenal	M14	γ-terpinene
AL2	(E)-2-pentenal	M15	terpinolene
AL3	hexanal	M16	β-thujone
AL4	(E)-2-hexenal	M17	p-mentha-1,5,8-triene
AL6	(E)-2-heptenal	M18	allo-ocimene
AL7	benzaldehyde	M19	camphor
AL10	(E)-2-octenal	M23	pentylcyclohexa-1,3-diene
AL14	(E,E)-2,4-octadienal	M25	β-cyclocitral
AL15	(*E*,Z)-2,6-nonadienal	M26	L-carvone
AL17	myrtenal	M28	thymol
AL18	(2E, 4E)-nonadienal	MA1	p-mentha-2,8-dien-1-ol
E2	methyl pentanoate	MA2	dihydrolinalool
E5	lavandulyl acetate	MA3	trans-pinocarveol
E7	hexyl isobutanoate	MA4	terpinen-4-ol
K1	2-methyl-3-pentanone	MA5	p-cymen-8-ol
K2	3-heptanone	MA6	cis-carveol
K5	(E,E)-3,5-octadien-2-one	MA8	α-terpineol
K6	acetophenone	MA9	caryophylladienol II
K7	3,5-octadien-2-one	L1	γ-nonalactone
K8	p-methyl-acetophenone	S1	α-ylangene
K9	dihydrojasmone	S11	kessane
ALK1	nonane	S12	liguloxide
ALK2	decane	P1	3-propylidene phthalide
M4	sabinene	P2	3-butyl hexahydro phthalide
M6	myrcene	P5	Sedanenolide

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Figure 5.1. Principal component analysis of eight celery samples harvested in Cartagena (CA) and Águilas (AG) showing correlations with volatile compounds (A) Projection of the samples; (B) Distribution of variables; (C) Compound codes as appear in plot (B).

Variation within cultivar is observed more clearly in celery grown in Águilas as well as closer association with more volatile compounds in comparison to celery grown in Cartagena through the wider spread of genotypes across the biplot (Figure 5.1). Although similarities were observed between the chemical profiles collected from both harvests, Águilas expressed a higher content of ketones and aldehydes (Table 5.1) which is displayed in Figure 5.1 through the close association of these compounds. Celery grown in Cartagena all displayed a close association with monoterpenes (M7, M8, M9, M18), monoterpenoid alcohols (MA6, MA8) as well as 3-propylidene phthalide (P1), octanol (A6), lavandulyl acetate (E5) and α-ylangene (S1). On the other hand, more variety in the compounds associations with certain genotypes was observed in Águilas grown celery; genotypes 5, 8, 15 and 22 were most closely associated with monoterpenes (M13, M15, M26), aldehydes (AL1, AL2, AL3, AL4, AL6, AL10, AL15, AL17, AL18), ketones (K2, K5, K7, K8, K9) and 3-butyl hexahydro phthalide. Conversely, genotypes 10, 12, 18 and 25 displayed a closer relationship with monoterpenes (M4, M14, M16, M19, M23, M25, M28), monoterpenoid alcohols (MA1, MA2, MA5, MA10), sesquiterpenes (S11, S12), alcohols (A2, A5), methyl pentanoate (E2) and sedanenolide (P5). Although observed to comprise a high proportion of the aroma composition, genotypes 10 and 12 displayed a much lower association with ketones and aldehydes than genotypes 5, 8, 15, 18, 22 and 25. Where celery grown in Cartagena expressed more similarities in the chemical composition, genotypes 10 and 12 in Águilas caused a shift in the graph and take the appearance of outliers, displaying the most significant differences in the chemical composition than the other genotypes.

Genotype and growing location displayed a significant influence over the volatile content of eight celery genotypes. Growing location expressed a significant influence over some compounds whereas genotype did not, including p-mentha-2,8-diene (M7), allo-ocimene (M18), cis-carveol (MA6), α-ylangene (S1), 3-propylidene phthalide (P1) and 3-butyl hexahydro phthalide (P2). Conversely, genotype expressed significant influence over some compounds where growing location did not, including 2-methyl-1-butanol (A2), (E,E)-2,4-octadienal (AL14) and safranal (M22) (Table 5.1, Figure 5.1). In previous chapters (3 and 4), genotype and environment both displayed a significant influence over the chemical profile of these eight genotypes and this led to a change in the sensory profile. Unfortunately, sensory profiling was not completed on the Cartagena-grown celery, however,

utilising previous information gathered from comparing data collected from UK-grown celery and Spanish-grown celery, we hypothesis that these differences observed in the composition will lead to differences in the sensory characteristics. Celeries containing a large proportion of monoterpenes were perceived to be closely associated to fresh fennel and herbal flavours and celery containing more ketones and aldehyde compounds were perceived to be more closely related to fresh coriander and cucumber. Celeries expressing high abundance of compounds related to green, grass, herbal, and fresh odour descriptors such as hexanal and heptanal will increase the association to grass odours and flavours. Finally, celery expressing a high phthalide content will most likely have the typical strong distinct flavour that celery is known for as these are the characteristic compounds of celery. We would expect to observe significant differences between growing locations as well as between genotype upon completion of sensory profiling, as displayed in chapters 3 and 4. Although these sensory characteristics cannot be investigated, we can investigate the differences in growing environments which would have influenced the differences in composition between celery grown in Cartagena and Águilas.

5.4.2. Comparing differences in the growing environment may explain compositional differences observed in the aroma profile

In this study, compositional differences were observed between the eight genotypes and two locations in Spain (Cartagena and Águilas). Chapter 1 revealed the environmental influence over the chemical composition of celery, where data collected from previous studies investigated the aroma profile of celery which were grown in vastly different climates. All these studies expressed variation within the chemical profile. Chapter 3 investigated the relationship between growing environment and the chemical composition, further highlighting the increase in sesquiterpene and phthalide content in higher temperatures such as those experienced in 2018 by UK-grown celery compared to the increase in monoterpene content in lower temperatures such as those experienced in 2020 by UK-grown celery. Comparing Spanish-grown celery with UK-grown celery, there were also clear differences in the environment, however this was due to the availability of certain micronutrients in the soil and water for the crop to uptake, leading to differences in the primary and secondary metabolites synthesised, leading to alterations in the response to stress. Throughout chapters 3 and 4, the environmental data discussed

provided insight to the differences observed within the chemical composition of the eight genotypes and similarly, can be applied here (Table 5.2).

Table 5.2: Weather data displaying the average daily temperature, rainfall, and relative humidity for each week of growth from field transplantation to harvest, collected from the nearest weather station and provided by G's Grupo España.

	Cartagena			Águilas		
Week	Air Temperature (°C)	Rainfall (mm)	Relative humidity (%)	Air Temperature (°C)	Rainfall (mm)	Relative humidity (%)
1	18.0	0.1	68.1	15.3	0.0	79.6
2	19.4	0.0	65.9	15.4	0.1	76.3
3	18.9	0.0	76.3	19.9	0.0	72.8
4	17.0	0.4	65.8	17.4	0.1	63.7
5	17.1	0.0	70.8	16.9	0.0	82.1
6	15.4	1.0	78.4	16.4	0.0	81.2
7	15.7	0.0	73.2	16.6	0.0	82.5
8	14.7	0.0	58.7	18.5	0.0	84.7
9	14.5	0.1	62.6	18.9	0.0	78.3
10	18.3	0.0	51.8	19.8	0.0	79.4
11	16.7	0.0	49.2	17.9	0.3	71.1
12	17.7	0.0	70.8	16.9	1.8	78.3
13	14.5	0.0	78.0	19.0	0.6	74.3
14	17.0	0.0	70.1	17.6	0.4	77.3
15	17.7	0.0	76.7			
Average	16.8	0.1	67.8	17.6	0.2	77.3

Águilas experienced an increased overall average air temperature, rainfall, and relative humidity, particularly during the last half of growth when compared to Cartagena. The humid and temperate conditions experienced in Águilas (Table 5.2) is typical of a maritime climate and would explain the compositional differences observed in the Águilas-grown celery (Table 5.1, Figure 5.1). The warmer temperatures along with the higher rainfall and relative humidity experienced by the crop will lead to different environmental pressures. Around week 12 and leading up to harvest, Águilas-grown celery were subject to close to 3 mm of rain, a significantly higher volume than Cartagena-grown celery where it remained dry from week 10 to harvest. Investigating how rainfall, humidity and temperature influenced the volatile emission from apple trees; Vallat, Gu and Dorn (2005) identified

rainfall to significantly influence the emission of volatiles from apple trees, specifically noting an increase in aldehyde emission. This corresponds with the data presented in this study (Table 5.1) whereby Águilas expressed a higher aldehyde content. Similarly, ketones responded in a similar manner as expected due to their close relation to aldehydes.

Growing in Cartagena, the conditions experienced by the crop, lower temperatures, and drier conditions, reflect those in the 2020 UK grown celery, both expressing an increased monoterpene content when compared to the crop grown in warmer conditions. These results correspond with Vallat, Gu and Dorn (2005) who concluded that a drier climate for the growth of apple trees resulted in the formation of more secondary metabolites in response to stress. Similarly, Takabayashi, Dicke and Posthumus (1994) observed an increase in terpene compounds in response to biotic stress from herbivore pests in infested leaves. Although no data collected in this study insinuated the risk of a biotic attack but there is no way of completely removing the risk as celery is susceptible to many pests and diseases. Many celery varieties have been bred with resistance to various viruses including *fusarian* yellow and celery mosaic virus, however, many of the bacterial diseases common in celery occur due to poor environmental conditions such as blight, damping-off and soft rot due poor drainage, heavy rainfall and contaminated soil. Pesticides and herbicides were applied to the crop however, there is still a risk that a biotic attack occurred which would lead to an increase of monoterpene synthesis (Raid, R, 2004) (Table 5.1).

When observing the locations of the field, we can hypothesise that these differences account for the observed differences in the composition (Table 5.1, Figure 5.1). Águilas-grown celery was located around 35 km from the sea which is much further than the 10.7 km distance for the celery grown in Cartagena. There are local diurnal winds that are caused by the difference in the heating of land and sea, especially in the summer. During the day land heats up quicker than the sea causing air to rise and setting up low pressure and at night, the sea retains the heat and sets up a reverse convection cell and a land breeze (Pokhrel & Lee, 2011). This may explain the lower temperatures, rainfall and humidity experienced in Cartagena (Table 5.2)

Sesquiterpenes and phthalides expressed minimal significant differences between the two locations in this instance, however, chapters 3 and 4 both highlighted significant differences in the

composition of phthalides. Only sedanenolide expressed a significant difference according to genotype and this has also been observed throughout the project, concluding that sedanenolide content is determined through genotype, yet the environment still plays a significant role in the abundance of this compound. In chapter 3, it was hypothesised that sesquiterpenes and phthalides were positively correlated with warmer temperatures, this statement does not apply to the findings in this study due to similarities in the average daily air temperature which gave rise to non-significant differences in the phthalide content between Cartagena- and Águilas- grown celery.

Although not discussed within this chapter due to unavailable information, the following factors, although less significant when discussed individually, will combine to form the local microclimate which will introduce variation within the environment and will bring about differences in the crop's response to stress. Firstly, if the field of growth was south facing, on a slope, this would increase the exposure and duration of direct sunlight, leading to an increased rate of photosynthesis. Alternatively, north facing slopes are prone to cooler temperatures in comparison to south facing slopes, whilst retaining more moisture within their soils (Måren, Karki, Prajapatim Yadav & Shrestha, 2015). Secondly, the angle of the slope would be a further factor as well, although most arable crops are grown on relatively flat surfaces to prevent high run-off and promote good filtration, this may not be possible in some areas of Spain. Thirdly, the altitude of the field will apply environmental pressures to the crop, particularly through changes in the average temperature, however both Cartagena and Águilas, are located close to the coast and the land is risen above sea level. Nowak, Nowak, Nobis & Nobis (2015) observed significant changes in the composition of weed species, identifying altitude and correlated temperatures to have a major role in causing these changes. Although the altitude influence will be less obvious here, when comparing the UK celery with Spanish celery, this explains further the significant differences identified in aroma composition (Chapter 4).

In contrast to comparing UK-grown celery with Spanish-grown celery, where many significant differences were observed, the volatile content for both Spanish-grown celery trials generated fewer significant differences between genotypes for all compound groups. Responding to abiotic and biotic stresses through the synthesis of secondary metabolites for protection is a common and expected response by the crop and in previous chapters, we have been able to observe clear differences in what

causes the response. When comparing both Spanish crops, the causes of these differences become less clear due to the environmental similarities in the experiments experienced by the crops, including the temperatures, seasons, geographical locations, and maturity. However, the drier climate experienced by the Cartagena celery caused an increase in the monoterpenoid content whereas higher rainfall and relative humidity led to a celery with an increased ketone and aldehyde content.

5.5. Conclusion

Location and genotype expressed a significant influence over the aroma composition of the eight celery genotypes used in this experiment, particularly observed in the monoterpene, aldehyde, and ketone content. These compound groups are suspected to have been synthesised in response to the environmental stresses experienced by the crop. For example, growing in dry conditions such as the Cartagena celery experienced, where there was a low relative humidity and minimal rain, this would lead to the synthesis of monoterpenes, as observed by Vallet, Gu and Dorn (2005) in apple trees. Furthermore, they observed aldehydes to be positively correlated with rainfall and higher temperatures, as experienced by the Águilas celery.

Previously, the aroma composition identified in the Águilas celery was profiled using the trained sensory panel, associating many of the genotypes to be associated with fresh fennel and coriander flavours and although we were not able to carry out sensory profiling on the Cartagena celery, we expect that significant differences in the sensory profile would have been identified. However, the information provided within this chapter will educate growers on the influence of other environmental factors not discussed in previous chapters such as rain and relative humidity. It is common practice for countries such as the UK to utilise warmer countries such as Spain to grow their fresh produce during their winter months to meet consumer demand of fruit and vegetables availability all year round, however as displayed within this chapter, chapters 3 and 4; growing in various geographical locations where the climate is different has a significant effect on the chemical composition of celery and will lead to significant changes in the sensory profile. These changes have the possibility to either have a positive or detrimental effect on the quality of the crop and in an industry such as fresh produce where

quality is so vital, ensuring flavour and aroma should be considered just as important a factor as appearance and yield.

3812 **5.6. Relative abundance**

3813 **Table 5.4. Relative abundance of volatile compounds identified in the headspace of eight celery parental genotypes**

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		Relative Abundance (mg/L)																		
		AG								CA								P-value		
Code	Compound name	5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G	E	GxE
A1	2-methyl-1-butanol	1.53	1.34	0.72	1.05	1.30	1.07	1.32	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**	***	***
A2	3-methyl-3-butanol	1.70	1.09	1.54	1.30	1.00	1.44	1.32	1.15	0.47	0.17	0.52	0.28	0.70	0.20	0.26	0.37	**	***	***
A3	pentanol	5.93	1.82	0.54	1.08	2.17	2.42	1.86	0.70	0.96	0.18	0.16	0.20	0.53	0.00	0.44	0.15	***	***	***
A4	(E)-2-pentenol	3.01	2.41	0.99	0.42	1.01	0.94	1.14	2.43	0.67	0.70	0.72	0.97	1.25	1.15	0.76	0.65	***	***	***
A5	hexanol	1.54	1.10	1.30	0.88	0.97	1.08	1.21	1.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
A6	isoborneol	0.65	0.46	0.26	0.37	0.56	0.67	0.45	0.83	0.08	0.17	0.53	0.35	0.26	0.00	0.02	0.08	***	**	**
AL1	2-methyl-2-butenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.63	1.04	1.47	1.10	1.05	1.78	1.88	1.13	***	***	***
AL4	*(E)*-2-pentenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.31	0.00	0.15	0.19	0.26	0.21	0.23	***	***	***
AL5	hexanal	102.80	67.94	22.06	18.82	62.73	66.15	63.11	62.09	14.50	9.06	4.19	8.60	8.49	12.89	16.12	6.23	***	***	***
AL6	*(E)*-2-hexenal	2.21	1.63	0.49	0.65	1.52	1.56	1.43	1.36	0.03	0.00	0.00	0.14	0.09	0.23	0.10	0.00	***	***	***
AL7	heptanal	1.94	1.57	0.84	1.05	1.43	1.51	1.55	1.82	1.49	1.67	0.65	1.66	1.46	1.62	1.34	0.97	ns	ns	ns
AL8	*(E)*-2-heptenal	21.66	22.93	9.98	13.37	29.37	20.42	18.07	14.08	2.10	1.45	1.98	1.59	1.35	1.59	1.24	0.76	***	***	***
AL9	benzaldehyde	3.79	4.66	3.14	4.07	4.75	4.10	4.11	4.42	0.68	0.52	0.25	0.64	0.62	0.71	0.62	0.47	***	***	***
AL10	octanal	2.73	2.68	0.93	1.38	4.63	2.23	1.32	1.93	0.80	1.43	0.52	1.61	1.27	0.71	0.65	0.54	***	**	**
AL11	*(E)*-2-octenal	12.63	9.42	2.51	2.96	9.41	9.93	7.17	8.69	0.80	0.95	0.64	0.87	0.42	0.94	0.38	0.40	***	***	***

AL12	phenylacetaldehyde	0.87	0.66	0.43	0.92	0.72	0.63	0.57	0.67	0.26	0.16	0.25	0.27	0.17	0.14	0.00	0.14	*	*	*
AL13	*meta*-tolualdehyde	1.62	1.12	1.22	1.99	1.79	1.67	1.42	2.27	0.62	0.79	0.52	0.78	0.91	0.61	0.48	0.48	**	**	**
AL14	*para*-tolualdehyde	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.32	0.12	0.26	0.29	0.82	0.60	0.21	***	***	***
AL15	nonanal	2.52	1.67	0.64	0.77	1.63	1.79	1.51	1.51	0.43	0.42	0.45	0.49	0.34	0.47	0.42	0.33	**	**	**
AL16	*(E)*-2-nonenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.15	0.22	0.18	0.04	0.20	0.11	0.00	***	***	***
AL17	(*2E, 4E*)-nonadienal	0.46	0.37	0.19	0.28	0.45	0.41	0.36	0.43	0.12	0.19	0.07	0.19	0.15	0.16	0.00	0.00	**	**	**
AL18	*(Z)*-2-nonenal	0.35	0.42	0.18	0.27	0.83	0.64	0.60	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
AL19	*(E,E)*-3,5-Octadien-2-one	8.76	6.62	1.50	1.79	6.05	6.32	5.67	5.56	0.19	0.32	0.00	0.45	0.10	0.27	0.35	0.00	***	***	***
AL20	(*E*,*Z*)-2,6-Nonadienal	1.40	1.30	0.33	0.36	1.15	1.00	1.18	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
E1	methyl propanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.21	0.58	0.58	0.70	0.50	0.62	0.38	***	***	***
E2	methyl butanoate	0.54	0.49	0.43	0.37	0.48	0.48	0.39	0.42	0.32	0.15	0.41	0.44	0.59	0.08	0.30	0.18	***	***	***
E3	carveol acetate	2.18	3.61	1.56	1.56	2.91	3.06	3.83	2.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
E4	methyl hexanoate	0.88	0.78	0.20	0.17	0.72	1.09	0.71	0.65	2.12	0.57	1.60	1.44	0.66	0.73	1.11	0.39	**	**	**
E5	amyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.18	0.00	0.00	0.00	0.00	ns	ns	ns
E6	methyl octanoate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.21	0.10	0.22	0.12	0.28	0.25	0.07	ns	*	*
E7	cis-pinocarvyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.07	0.07	0.23	0.08	0.11	0.08	0.00	*	ns	ns
E8	butyl butanoate	0.10	2.04	0.09	0.38	2.11	1.87	0.76	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	*	*
K1	2-methyl-3-pentanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.12	0.20	0.27	0.19	0.20	0.21	0.20	*	*	*
K2	2-pentanone	2.17	2.10	1.66	2.03	2.15	2.26	2.16	1.69	0.91	0.31	1.17	0.91	1.21	0.99	0.78	0.46	**	**	**
K3	2-heptanone	0.63	0.55	0.38	0.51	0.44	0.46	0.67	0.57	0.05	0.00	0.00	0.00	0.00	0.00	0.04	0.00	**	**	**
K4	2-hexanone	0.44	0.34	0.19	0.14	0.26	0.37	0.32	0.41	0.14	0.00	0.20	0.27	0.19	0.00	0.21	0.00	***	***	***

K5	3-heptanone	1.54	1.29	0.51	0.53	1.12	1.32	1.12	1.53	0.35	0.34	0.32	0.48	0.37	0.57	0.50	0.35	**	**	**
K6	2-nonanone	1.72	1.36	1.83	1.71	1.18	1.53	1.55	1.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
K7	1-octen-3-one	9.83	10.17	4.86	5.06	12.09	8.68	8.86	7.31	4.30	3.08	4.75	4.33	3.16	3.70	3.53	2.04	***	***	***
K8	3,5-octadien-2-one	2.56	2.89	1.00	1.77	3.47	2.25	3.25	1.20	0.19	0.32	0.00	0.45	0.10	0.27	0.35	0.00	***	***	***
M1	α-pinene	1.08	1.00	1.05	1.10	0.95	1.01	0.99	1.43	0.63	0.47	0.49	0.53	0.53	0.51	0.25	0.31	***	***	***
M2	beta thujene	3.01	1.34	1.78	1.78	2.18	2.00	2.42	1.03	0.94	0.50	1.82	1.14	1.15	0.73	0.68	0.72	*	*	*
M3	camphene	2.29	1.57	1.56	2.07	2.04	1.50	2.31	1.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M4	sabinene	1.56	1.26	1.08	1.30	1.52	1.46	1.29	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M5	beta-pinene	8.12	5.80	2.57	5.66	9.82	2.84	5.64	3.59	2.95	4.00	1.16	3.06	2.31	0.83	1.59	2.09	***	***	***
M6	myrcene	1.07	1.53	3.07	3.14	1.38	3.13	1.30	1.15	0.57	0.31	1.07	1.97	0.43	0.92	0.35	0.29	ns	ns	ns
M8	delta-3-carene	6.01	5.50	1.11	4.30	6.23	5.36	5.29	4.78	0.19	0.19	0.20	0.11	0.09	0.18	0.08	0.08	***	***	***
M7	α-terpinene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.18	0.41	0.11	0.18	0.13	0.00	***	***	***
M9	p-cymene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.01	6.99	3.68	8.26	2.03	3.93	2.43	2.93	***	***	***
M9	α-phellandrene	15.13	10.49	7.96	7.55	6.51	11.18	7.84	7.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M10	limonene	48.60	51.68	41.44	45.28	30.83	35.61	35.60	18.00	24.80	39.38	19.70	40.17	12.52	17.95	10.80	14.77	***	***	***
M11	gamma terpenine	2.78	3.93	3.79	4.28	3.35	3.18	2.72	2.01	0.34	1.12	1.03	0.95	0.21	0.55	0.91	0.18	***	***	***
M12	terpinolene	1.13	0.75	0.25	0.43	0.55	0.84	0.00	0.48	0.24	0.43	0.09	0.35	0.00	0.00	0.00	0.00	**	**	**
M13	*p*-(1,3,8)-menthatriene	0.12	0.03	0.27	1.20	0.15	0.40	1.09	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
M14	beta-cyclocitral	0.29	0.34	0.19	0.39	0.41	0.32	0.25	0.24	0.04	0.03	0.00	0.15	0.15	0.03	0.04	0.00	**	**	**
M15	L-carvone	1.07	1.03	0.76	1.15	0.83	1.46	0.45	0.68	0.12	0.10	0.00	0.14	0.05	0.20	0.00	0.00	**	**	**
M16	D-carvone	1.73	0.77	0.31	0.32	0.68	0.98	0.40	1.18	1.69	0.92	1.20	2.16	1.37	1.36	1.03	0.88	ns	ns	ns
M17	*trans*-dihydrocarvone	1.21	0.78	0.51	0.56	0.69	0.86	0.00	1.11	0.36	0.58	0.45	0.36	0.33	0.43	0.18	0.27	ns	ns	ns

M18	*trans*-carveol	1.85	1.31	1.11	0.97	0.95	1.36	0.60	1.41	0.13	0.32	0.23	0.26	0.17	0.47	0.36	0.15	ns	ns	ns
MA1	*cis*-pinocarveol	0.87	0.61	0.19	0.22	0.59	1.43	0.36	0.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA2	camphor	0.68	0.46	0.38	0.38	0.52	0.60	0.38	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA3	thymol	0.38	0.34	0.25	0.35	0.34	0.49	0.23	0.31	0.04	0.03	0.00	0.05	0.00	0.00	0.00	0.00	**	**	**
MA4	pinocarvone	0.63	0.39	0.33	0.43	0.46	0.53	0.27	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA5	(E)-8-hydroxylinalool	0.21	0.43	0.22	0.41	0.15	0.39	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA6	caryophylladienol II	0.68	0.45	0.15	0.21	0.28	0.47	0.18	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA7	carvacrol	0.08	0.31	0.29	0.44	0.31	0.43	0.15	0.19	0.16	0.19	0.17	0.38	0.14	0.24	0.05	0.04	*	*	*
MA8	cis-carveol	0.40	0.18	0.14	0.17	0.17	0.29	0.13	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	***	***	***
MA9	p-cymen-8-ol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.77	0.89	0.64	0.64	0.39	0.56	0.35	0.40	***	***	***
S1	α-copaene	0.44	0.25	0.10	0.00	0.00	0.35	0.56	0.74	0.13	0.13	0.04	0.00	0.00	0.06	0.18	0.12	*	*	*
S2	(Z)-caryophyllene	1.53	1.72	2.47	2.24	1.25	3.83	1.28	1.71	0.34	0.70	0.94	0.73	0.27	0.56	0.31	0.23	***	***	***
S3	α-humulene	0.22	0.24	0.15	0.18	0.52	0.25	0.06	0.12	0.03	0.15	0.03	0.09	0.08	0.04	0.05	0.03	**	**	**
S4	Selinene ebta	0.84	0.86	0.49	2.89	0.45	1.24	0.88	1.22	0.42	0.59	0.35	4.10	0.41	0.19	0.32	0.42	***	***	***
S5	valencene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.04	0.11	4.52	0.13	0.08	0.03	0.09	***	***	***
S6	α-selinene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.06	0.06	1.88	0.05	0.05	0.09	0.07	***	***	***
S7	cuparene	0.26	0.21	0.12	4.49	0.45	0.11	0.24	0.09	0.04	0.03	0.14	0.04	0.12	0.00	0.05	0.04	*	*	*
S8	kessane	0.02	0.00	0.00	0.00	0.10	0.03	0.00	0.00	0.00	0.00	0.00	2.95	0.00	0.00	0.09	0.00	ns	ns	ns
S9	liguloxide	0.00	0.07	0.00	0.10	0.00	0.26	0.00	0.00	0.09	0.05	0.05	0.00	0.08	0.00	0.00	0.00	ns	ns	ns
P1	3-butylhexahydrophthalide	0.10	0.03	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ns	ns	ns
P2	3-n-butylphthalide	13.12	10.36	9.16	18.70	14.03	15.05	12.34	10.21	5.33	5.41	4.72	8.64	4.82	5.40	5.03	3.53	***	***	***

P3	*(Z)*-3-butylidenephthalide	0.46	0.26	0.23	0.29	0.71	0.45	0.22	0.27	0.26	0.31	0.28	0.37	0.58	0.23	0.20	0.17	ns	ns	ns
P4	sedanenolide	2.02	2.74	5.57	20.24	4.22	4.65	2.10	1.11	0.89	0.68	2.05	2.47	1.49	0.98	0.36	0.43	***	***	***
P5	*trans*-neocnidilide	2.98	1.16	1.21	0.32	1.17	0.12	2.54	2.86	0.36	0.33	1.88	0.13	0.27	1.42	0.65	0.65	*	*	*
P6	*trans*-ligustilde	0.04	0.04	0.15	0.24	0.18	0.45	0.04	0.03	0.00	0.06	0.09	0.06	0.24	0.12	0.19	0.02	*	*	*

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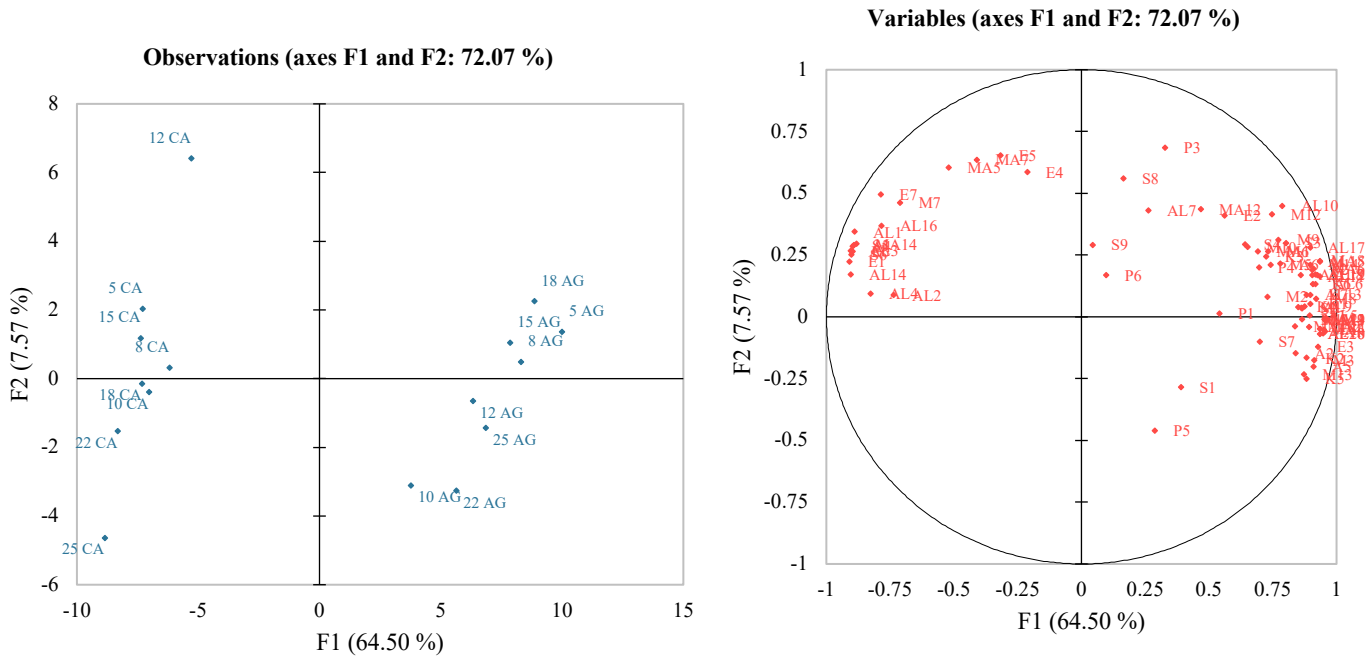

Figure 5.2. Principal component analysis using relative abundance of the volatile compounds identified in the headspace of celery grown in Cartagena (CA) and Águilas (AG)

In a similar manner to the previous chapters, the conversion of data from percentage composition to approximate quantities (mg/L) confirms the conclusions that have been made in the chapter. When observing the data collected comparing the celery grown in Cartagena with celery grown in Águilas as displayed in Table 5.4 and Figure 5.2, we can see that the strong association with most of the volatile compounds remains as well as the separation between the two different celery growing regions. Nearly all monoterpenes, sesquiterpenes and phthalides were identified at a higher relative abundance in the Águilas-grown celery whereas Cartagena-grown celery displayed a higher abundance of various aldehydes (AL1, AL2, AL4, AL14, AL16). If sensory testing was performed on this harvest, we may have witnessed the strongest association to the flavour attribute cucumber than previous harvests.

5.7. References

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5.7. Synthesis of results obtained in chapters 2, 3, 4 and 5

5.7.1. Introduction to the purpose of the chapter

Using the same eight genotypes, grown in both Spain and the UK over four years, the response to the different growing environments between the genotypes was clearly observed in chapters 3, 4 and 5. By combining all datasets collected throughout the project, we were able to examine the impact of growing in multiple geographical locations, across several years, whilst using the same eight celery genotypes.

5.7.2. Results and discussion

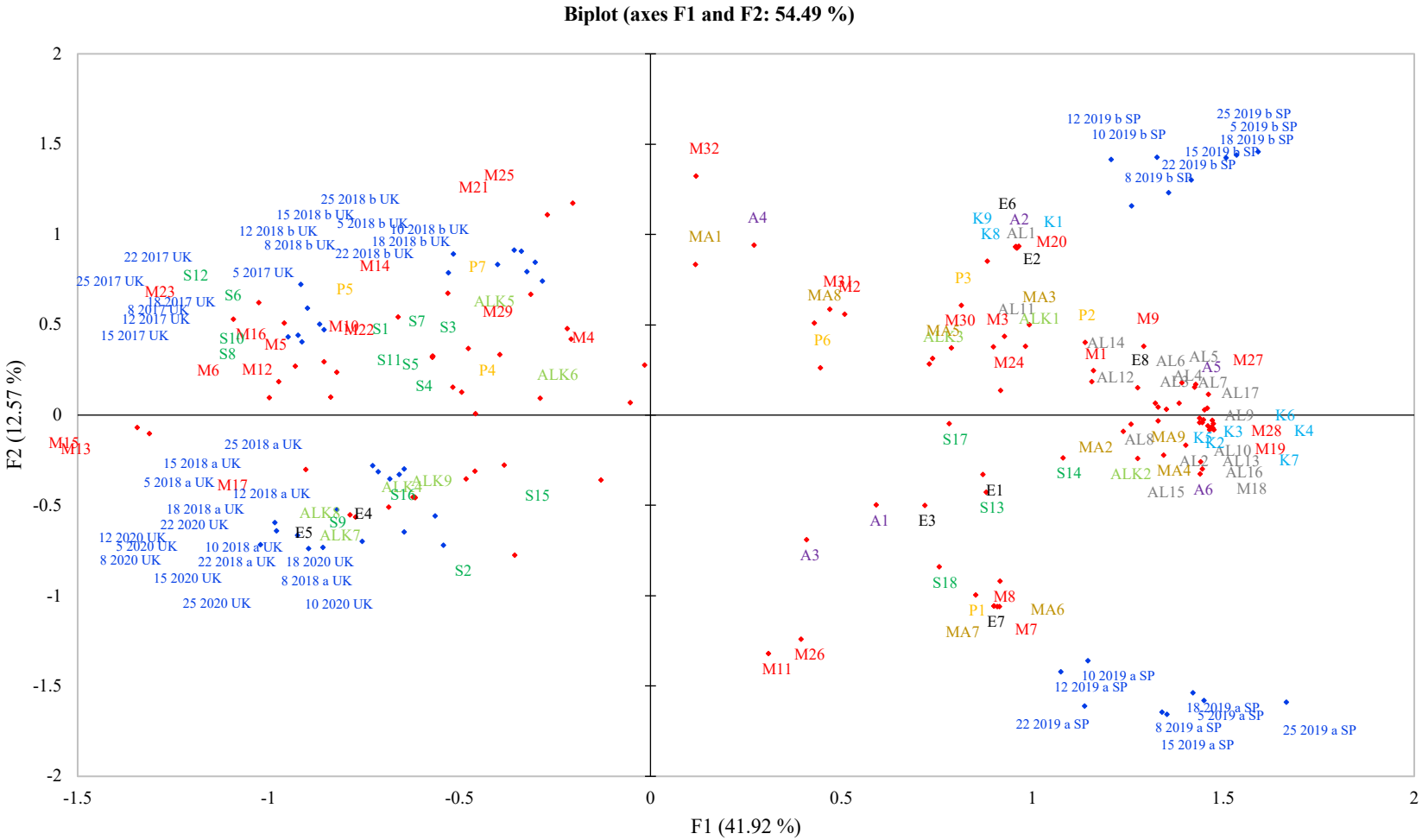
Completing a two-way ANOVA (GxE) revealed quantitative differences between all factors and their interactions and using the significantly different compounds, principal component analysis was completed (Figure 5.2). Principal component analysis was completed to visually analyse the variation in percentage composition of volatile compounds between 2017 and 2020, combining data collected from eight celery genotypes grown in both the UK and Spain. The differences in aroma composition were clearly observed through the apparent separation of all harvests (Figure 5.2). Principal component one (F1) and two (F2) explained 54.49 % of the total variation within the dataset; geographical location was separated along F2 whereas F1 separated harvest years 2017, 2018b and 2019b. Monoterpenes, monoterpenoid alcohols, aldehydes and ketones displayed a significant association with celery grown in Águilas (2019b) and celery grown in late-September, Ely, UK in 2018 (2018b) and 2017 also displayed a close association predominantly to monoterpenes, sesquiterpenes and phthalides.

Celery grown in UK 2020 and 2018 both displayed less of an association with monoterpene and sesquiterpene compounds. If we were able to compare the sensory profile of the UK grown celery, we would expect that the 2017 and 2018b celeries would be scored higher by the panel in aroma and flavour attributes due to their significantly higher abundance in these compounds (Figure 5.2). As observed in chapters 3 and 4, the aroma profile in Spanish celery was significantly different to UK-grown celery. Águilas-grown celery (2019b) expressed sesquiterpenes and phthalides at a lower abundance than UK grown celery (Chapter 3). Between regions, the volatile composition expressed

significant differences however, this was to a lesser extent than when we compared Spanish celery to UK-grown celery. Águilas celery expressed a significantly higher proportion of ketones and aldehydes compared to both Cartagena and UK grown celery. Less variation was observed within celeries grown in the UK (2017, 2018, 2020), particularly between 2018a and 2020 grown celery. Spanish-grown celery displayed the most obvious separation, not only expressing a large separation from the UK but also between regions.

Figure 5.2. Principal component analysis of eight celery genotypes harvested between the years of 2017 and 2020, grown in UK and Spain, correlations between volatile compounds (A). Corresponding codes used in PCA (B).

(A)



A1	3-methyl-3-buten-1-ol	**M11**	α -terpinene
A2	2-methyl-1-butanol	M12	m-cymene
A3	(E)-2-penten-1-ol	M13	limonene
A4	1-pentanol	M14	β-(E)-ocimene
A5	hexanol	M15	γ-terpinene
A6	octanol	M16	terpinolene
AL1	2-methyl-2-butenal	M17	allo-ocimene
AL2	(E)-2-pentenal	M18	camphor
AL3	hexanal	M19	isoborneol
AL4	2-E-hexenal	M20	β-thujone
AL5	heptanal	M21	*p*-mentha-1,5,8-triene
AL6	2-E-heptenal	M22	neo-allo-ocimene
AL7	benzaldehyde	M23	pentylcyclohexa-1,3-diene
AL8	n-octanal	M24	cis-dihydrocarvone
AL9	phenylacetaldehyde	M25	trans-dihydrocarvone
AL10	2-E-octen-1-al	M26	safranal
AL11	m-tolualdehyde	M27	trans carveol
AL12	nonanal	M28	β-cyclocitral
AL13	(E,E)-2,4-octadienal	M29	L-carvone
AL14	(*E*,*E*)-2,6-nonadienal	M30	D-carvone
AL15	2-Z-nonen-1-al	M31	thymol
AL16	(2E, 4E)-nonadienal	M32	carvacrol
AL17	undecanal	MA1	p-mentha-2,8-dien-1-ol
E1	methyl butanoate	MA2	dihydrolinalool
E2	methyl pentanoate	MA3	trans-pinocarveol
E3	methyl hexanoate	MA4	β-terpineol
E4	1-octen-3-yl-acetate	MA5	terpinen-4-ol
E5	(E)-pinocarvyl acetate	MA6	p-cymen-8-ol
E6	carveol acetate	MA7	cis-carveol
E7	lavandulyl acetate	MA8	carveol
E8	hexyl isobutanoate	MA9	(E)-8-hydroxylinalool
K1	2-methyl-3-pentanone	MA10	caryophylladienol II
K2	3-heptanone	OX1	trans-limonene oxide
K3	2-heptanone	OX2	caryophyllene oxide
K4	1-octen-3-one	S1	cyclosativene
K5	(E,E)-3,5-octadien-2-one	S2	α-ylangene
K6	acetophenone	S3	α-copaene
K7	3,5-octadien-2-one	S4	(E)-β-caryophyllene

(B)

K8	p-methyl-acetophenone	S5	β-caryophyllene
K9	dihydrojasmone	S6	(+)-aromadendrene
ALK1	nonane	S7	curcumene
ALK2	decane	S8	α-humulene
ALK3	dodecane	S9	α-gurjunene
ALK4	tridecane	S10	β-selinene
ALK5	tetradecane	S11	valencene
ALK6	pentadecane	S12	α-selinene
ALK7	hexadecane	S13	cuparene
ALK8	heptadecane	S14	(E)-nerolidol
ALK9	octadecane	S15	kessane
M1	α-thujene	S16	β-gurjuene
M2	α-pinene	S17	liguloxide
M3	camphene	S18	rosifoliol
M4	sabinene	P1	3-propylidene phthalide
M5	β-pinene	P2	3-butyl hexahydro phthalide
M6	myrcene	P3	3-butylphthalide
M7	p-mentha-2,8-diene	P4	3Z-butylidene phthalide
M8	α -phellandrene	P5	sedanenolide
M9	delta-3-carene	P6	*trans*-neocnidilide
M10	delta-2-carene	P7	(E)-ligustilide

Colour coding observed in PCA plot can be seen as follows: alcohols (A), aldehydes (AL), alkanes (ALK), esters (E), ketones (K), monoterpenes (M), monoterpenoid alcohols (MA), sesquiterpenes (S) and phthalides (P).

To visualise the effect of genotype on the aroma composition of celery grown in various conditions, a bar chart was compiled using the total aroma composition (%) of volatile groups including monoterpenes, sesquiterpenes and phthalides (Figure 5.3). By doing this, patterns in how genotypes behave becomes clearer. Monoterpenes comprise the majority of the aroma profile for all genotypes in all harvest conditions and most predominantly in UK-grown celery in 2020. On the other hand, celery grown in Águilas in 2019 displayed a much lower monoterpene composition but a significantly increased alcohol, aldehyde, ester and ketone composition. As mentioned previously, in chapter 1, due to the simple hydrocarbon structure of monoterpenes, they are readily available to undergo reactions which produce a range of alcohols, aldehydes and ketones. These changes observed in monoterpenes and alcohols, aldehydes and ketones, in Águilas 2019 celery, was observed in all genotypes but not observed in any other harvest condition apart from genotype 22 in the Cartagena crop.

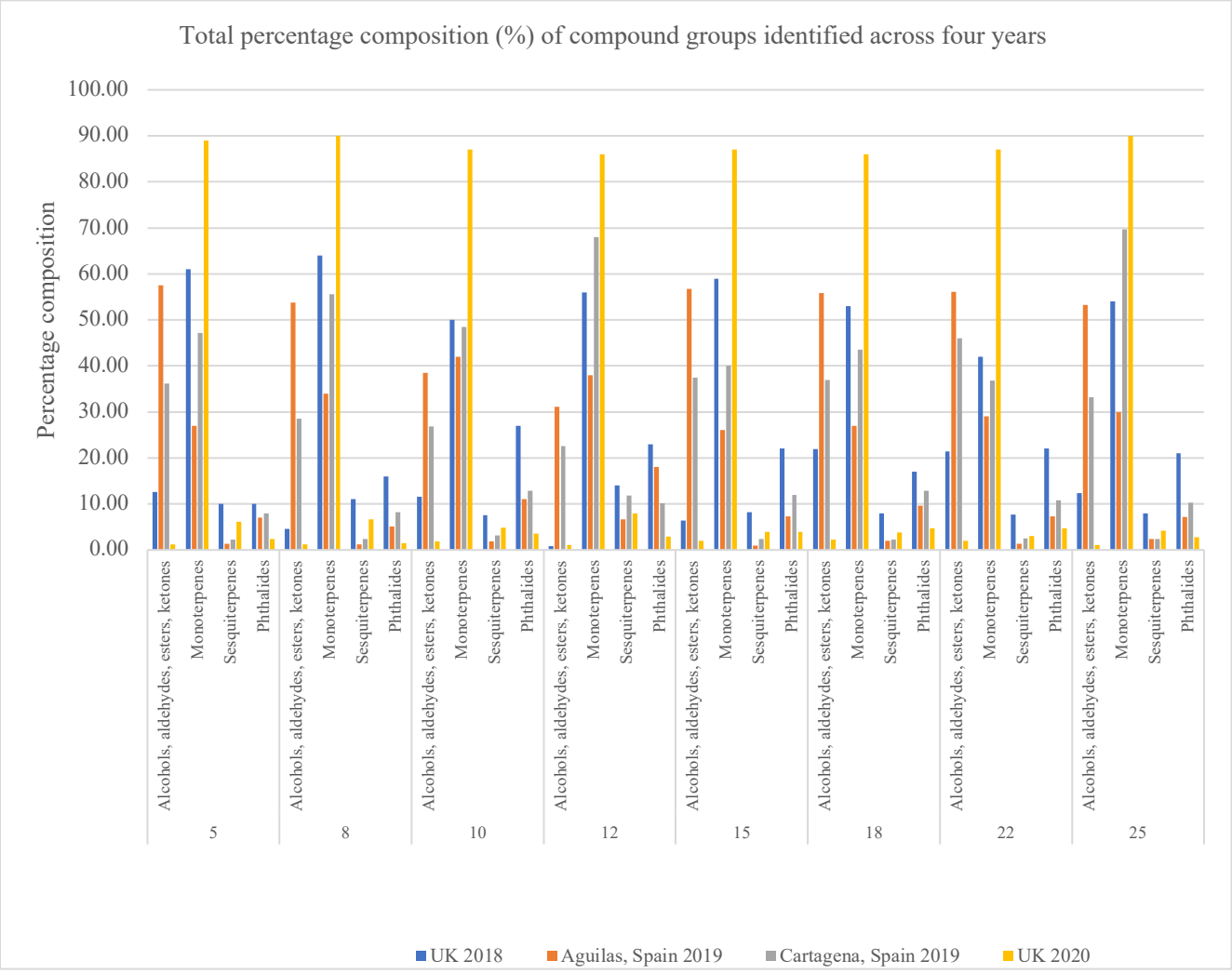


Figure 5.3. Bar chart displaying the total aroma composition (%) volatile groups of eight celery genotypes harvested between the years of 2017 and 2020, grown in UK and Spain.

Phthalides, the key aroma compounds in celery and contribute strong celery odours, comprised the highest proportion of the aroma profile of genotype 10 when grown in the UK in 2018 and genotype 12, when grown in Águilas 2019. Phthalides comprised the lowest proportion of the composition in genotypes 5 and 18 and overall, growing in UK 2020 produced celery with the lowest proportion of phthalides. In chapter 1, the synthesis of phthalides was discussed and their origins remain unknown. However, a pattern between sesquiterpenes and phthalides can be observed in all genotypes (Figure 3.2) whereby a low sesquiterpene composition is faced with an increased phthalide composition. In a similar process in which alcohol, aldehydes and ketones are formed from monoterpenes, terpenes may be involved in the synthesis of phthalides. Identified in chapter 6, phthalides were observed at lower relative abundance at pre-maturity and increase across time whereas the opposite was observed for monoterpenes. Unfortunately, no investigation has been completed and therefore, there is no evidence to support this but we hypothesise that phthalides are synthesised as the plant matures or faces various stresses from terpene breakdown.

By collating all datasets together, we identified patterns in genotypes and how they behave according to environmental conditions. When grown in the UK, the proportion of terpenes that comprise the aroma composition of genotype 5 was much higher than when it was grown in Spain, where the alcohol, aldehyde and ketone content formed an increased proportion. Phthalide content comprised a similar proportion of the composition in both Spain and UK 2018 but were much lower in UK 2020. Genotypes 8, 10, 12 and 15 all follow a similar pattern whereby growing in Spain led to a large increase in alcohols, aldehydes and ketones and growing in the UK increased the proportion of terpenes but a much lower phthalide content in UK 2020. Genotypes 18, 22 and 25 expressed a consistently higher proportion of alcohol, aldehydes and ketones across all harvests apart from in 2020 and their terpene content (both monoterpenes and sesquiterpenes) remained consistent also. Comparing locations, Spain produced a consistent crop in all genotypes and UK produced crops that were most different; this is most noticeable in the phthalide and alcohol, aldehyde and ketone content.

The influence of the variables studied in this project was observed clearly. Variation caused by genotype remained significant throughout and we hypothesise that genotype is the original cause of variation within celery, predetermining the aroma composition of the crop. Introducing differences in the environment including temperature and location, had a significant impact upon the secondary metabolite profile within the crop by stimulating either a protective or adaptive response, ultimately leading to significant differences in the sensory characteristics.

CHAPTER 6: Influence of harvest maturity on the aroma quality of two celery (*Apium graveolens*) genotypes

6.1 Introduction to Paper (as published in Food Chemistry, volume 365, 130515)

Based on the results presented in the previous chapters, external factors including geographical location, climate and agriculture all play a significant role in influencing the aroma composition of celery, furthermore, significant differences caused by genotype were also observed. This in turn led to significant differences in the sensory characteristics including appearance, aroma, flavour, and mouthfeel. As a natural response to external stresses that the crops were subject to, secondary metabolites were synthesised and deviations between genotype and environment was observed, for example, celery grown in Spain displayed a high abundance of ketones and aldehydes whereas celery grown in the UK did not express ketones and aldehydes were observed in a significantly lower abundance. Due to differences in dew point, field placement, water availability and soil composition, we hypothesised that these environmental and agronomic variables led to the differential synthesis of the compounds. Genotypes 12 and 22 were observed to perform consistently regardless of the location and environment in which they were grown in and additionally, these genotypes were observed as opposites of one and other with genotype 12 expressing high abundance of volatile compounds with strong associations with sensory attributes including fresh coriander, bitter taste and stringy mouthfeel whereas genotype 22 expressed a lower abundance of volatile compounds and a strong association with sweet taste, fresh fennel flavour and a moist mouthfeel.

As these genotypes were expressed as the most significantly different to each other, it was decided that further investigation into these genotypes was required to gain a better understanding into how their aroma develops and whether they consistently remain different to one another. Therefore, these genotypes were harvested at three different time-points during the growing cycle (pre-mature, commercial mature, post-mature) and were subject to GC/MS analysis to identify the compounds present and their abundance within each genotype and maturity followed by GC/O analysis, where we detect the most abundant compounds present and the odours that are associated with them, ultimately identifying the compounds that contribute most significantly to the celery aroma. Combining the

compounds identified through GC/MS with the compounds detected through GC/O, we examine any shifts in the aroma of celery and which compounds contribute the most to either the immature, mature or post-mature celery aroma. If these data were combined further with consumer preference data, we would be able to identify what the preferred flavour strength would be. For example, if a less celery-like and more fresh and green flavour was preferred this would direct growers towards earlier harvesting or if stronger, floral flavour, flavours that are most likely to occur in more mature celery were preferred then this would direct growers towards a later harvest date.

Sections 6.2 – 6.7 were published in Food Chemistry, 2021. (See Appendix XI for the pdf version of the manuscript)

6.2 Abstract

Celery is a fibrous horticultural vegetable grown globally and widely consumed due to its health benefits, distinct flavours, and culinary versatility. Currently, few datasets examine its aroma development across maturity which would help guide growers towards optimising harvest times whilst identifying potential consequences of harvesting outside commercial maturity. Freeze-dried celery of two genotypes, selected for biochemical and sensory differences, were harvested at three time-points and investigated using solid-phase microextraction gas chromatography/mass spectrometry (SPME GC/MS) and gas chromatography/olfactometry (GC/O). Both maturity and genotype showed significant ($P<0.05$) interactions between compounds, and harvest stage exhibited greater impact upon aroma quality than plant genotype. Thus, indicating that agronomic practice is key in determining crop quality. Monoterpenes, sesquiterpenes and phthalides begun to decrease once commercial maturity was reached, whereas alcohols were more prominent in post-mature celery. GC/O results confirmed the importance of phthalides to mature celery aroma and aroma differences caused by genotype.

6.3. Introduction

Apium graveolens, is a popular biennial crop that is grown and consumed globally; in salads as a raw ingredient or in cooking, whereby it forms the base of many soups, stocks and sauces (Rozėk,

2007). Celery has a distinct flavour profile that has been investigated extensively, with studies looking at the aroma profile of various cultivars in a variety of forms, such as fresh, dried or as an essential oil. Regardless of the material under investigation, a wide range of compounds that contribute to its strong flavour, including alcohols, aldehydes, monoterpenes, sesquiterpenes and phthalides have been identified (Gold & Wilson, 1963; van Wassenhove, Dirinck, Vulsteke & Schamp, 1990). The latter are seen as characteristic compounds. Phthalides are mainly found in members of the Apiaceae family, predominantly *Ligusticum* and *Angelica* (Karmakar, Pahari, & Mal, 2014). Phthalides including 3-*n*-butylphthalide, sedanenolide and *cis* and *trans*- ligustilide have been identified in celery, possessing odour descriptors such as "celery", "herbal" and "green" (Macleod & Ames, 1989; Kurobayashi, Kouno, Fujita, Morimitsu & Kubota, 2006).

Sellami, Bettaieb, Bourgou, Dahmani, Limam & Marzouk (2012) identified more than 25 volatile compounds in the roots, petioles and leaves of celery in the form of essential oil. Although more compounds were identified in the roots, the leaves exhibited a high concentration of aroma compounds, including phthalides. Similarly, Kurobayashi et al. (2006) utilised a combination of analytical techniques including gas chromatography olfactometry (GC/O) to analyse the odorants that characterise the aroma in raw and boiled celery and identified a much higher proportion of phthalides in the leaves rather than the petioles. Using GC/O, Kurobayashi et al. (2006) stated that sedanenolide, 3-*n*-butylphthalide and *cis*- and *trans*-sedanolide were the most distinguishing components of the celery aroma and through aroma extract dilution analysis (AEDA) quantified these compounds (3,200, 140 and 78 µg/kg respectively) to be the most abundant odour active compounds in raw celery petioles. Through sensory analysis and GC/O, these compounds were found to contribute odour characteristics such as "fragrant", "green" and "spicy" to celery.

Being such a widely consumed horticultural crop, research into the development across maturity of these key odour active compounds with celery is unexpectedly low. Yommi, Di Gerónimo, Carrozzi, Quillehauquy, Goñi & Roura (2013) monitored the quality changes (structural and textural) of self-blanching celery every seven days from day 80 (after transplanting) until day 129. It was concluded that the optimum yield and quality balance of the cultivar was attained at 122 days after transplanting, noting that a later harvest was strongly associated with lower quality due to textural

changes. Overall, there has been inadequate focus on the internal quality aspects of celery during maturity and although Yommi et al (2013) completed sensory analysis of matured petioles, the flavour attribute was labelled as 'characteristic flavour'. This is not an appropriate descriptor as the flavour profile is more complex than this. Furthermore, an analytical method such as solid-phase extraction (SPE) or solvent-assisted flavour extraction (SAFE) that generates quantitative results would monitor changes in the volatile content across maturity accurately.

While quality standards are usually based on visual evaluation (petiole shape, appearance, health) (Raffo, Sinesio, Moneta, Nardo, Peparaio & Paoletti, 2006), it can be argued that aroma and, therefore, flavour are attributes that should be considered when determining quality, as these also play an important role in consumer product acceptance. The purpose of this study was to investigate the development of aroma over maturity by utilising two different genotypes of *A. graveolens*, harvested at three different time points during plant development. The relationship between genotype and odour as well as maturity and odour were investigated using SPME and gas chromatography/mass spectrometry (GC/MS) and GC/O. From this, time points during maturation when key families of compounds were at their most abundant, such as monoterpenes that contribute fresh and citrus notes or phthalides that give the strong, characteristic herbal and celery odour were recognised. Eventually, this would help guide the fresh produce industry to introduce more flavour variation for celery and other vegetable products.

6.4. Materials & Methods

6.4.1. Celery material and Minimum Information About a Plant Aroma Experiment (MIAPAE) standard

6.4.1.1. Sample information

The two varieties used in this experiment were chosen due to their vast differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each line in this paper, the sensory properties of these genotypes can be revealed as these (along with others) were evaluated by the trained panel at the Sensory Science Centre (n = 12) (University of

Reading, UK) using Quantitative Descriptive Analysis (QDA^{TM}). Prior to GC/MS and GC/O analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis.

The first genotype, coded as line 12, has United Kingdom origins. Green and pink in colour with long, narrow petioles and ribs that appear compact and very prominent (Appendix XII). This genotype is characterised by a fibrous physiology, revealing strings of vascular tissue when a petiole is snapped, and bitter tasting.

The second genotype, coded as line 22, has North American origins with light green, compact petioles (Appendix XII). This genotype had a more typical celery appearance and is less bitter than the line above. It is not stringy, and the petiole breaks cleanly in half when snapped.

6.3.1.2. Timing, Location, and Environment

Celery seed (*Apium graveolens*) of two parental lines supplied by Tozer Seeds Ltd (Pyports, United Kingdom) were grown in commercial conditions and harvested in Cambridgeshire, United Kingdom by G's Fresh Ltd (Barway, United Kingdom) (52°21'12.9"N 0°17'15.6"E) during spring/summer 2018. Celery was grown in a field with commercial celery products and treated to the same agronomic techniques and conditions as commercial celery.

Plants were transplanted after 26 days of growing in the nursery. The first harvest occurred on day 63 after transplanting, in late July 2018 (premature, M1), the second harvest occurred on day 76 after transplanting, in mid-August 2018 (mature, M2) and the final harvest occurred on day 89 after transplanting, in late August 2018 (post-mature, M3). Average climate conditions from day one of transplanting to day 89 after transplanting were as follows: air temperature was 18 °C, average soil temperature was 22 °C and average rainfall was 0.04 mm. 20 to 25 mm of overheard irrigation was used and standard commercial fertiliser, pest and disease control regimes were applied.

6.4.1.3. Raw material collection, processing, and storage

Within the field, the celery was grown in three randomised blocks (10 plants m^{-2}) and were harvested using a celery knife. M1 celery were cut to 10 cm from the base, M2 and M3 were cut to 13 cm from the base, ensuring that no knuckles or leaves were included in the petiole cuttings. Three

biological replicates were harvested from each block. Once cut, the petioles were sealed in labelled bags for immediate transportation to the University of Reading (United Kingdom). Celery for aroma analysis was frozen at -80 °C and freeze-dried for five days. Celery was then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ) and stored in an airtight container out of sunlight exposure at room temperature for a maximum of 2 weeks before instrumental analysis.

6.3.2. Chemical reagents

For GC/MS analysis, calcium chloride solution was prepared with HPLC-grade water and added to the sample with 100 mg/L propyl propanoate in methanol, as the internal standard. For GC/O analysis, HPLC-grade water was used to rehydrate the samples and dry ice obtained from the University of Reading. The alkane standards C_6-C_{25} in diethyl ether was used for both GC/MS and GC/O analysis. All reagents were purchased from Sigma Aldrich (Gillingham, United Kingdom).

6.4.3. Solid-phase microextraction followed by GC/MS to identify changes in the aroma profile of different celery maturities and genotypes

Celery (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and filled to 5 mL using HPLC-grade water with 50 µL of 100 mg/L propyl propanoate (internal standard) in a 15 mL SPME vial fitted with a screw cap. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA). The SPME fibre stationary phase was composed of 75 µm divinylbenzene/Carboxen™ on polydimethylsiloxane; Supelco, (Bellefonte, PA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column DB5 (30 m x 250 µm x 0.25 µm thickness) (Agilent, Santa Clara, CA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C, and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of injector, interface and detector was 250 °C and the

sample injection mode was splitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 *m/z*, and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading), or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C_6–C_{25} *n*-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions. The approximate quantification (mg/L) of volatiles collected from the headspace were calculated from GC peak areas, by comparison with the peak area of the propyl propanoate standard.

6.4.4. Odour analysis using GC/O to identify changes in the perception of aroma compounds as celery matures

Celery (0.5 g) and 4.5 mL of HPLC grade water was placed in a SPME vial of 15 mL fitted with a screw cap with 50 µl of 100 mg/L propyl propanoate (internal standard). After equilibration at 37 °C for 10 min, the SPME device (divinylbenzene/Carboxen™ on polydimethylsiloxane) was exposed to the headspace above the sample for 30 minutes. After extraction, the SPME device was inserted into the injection port of an Agilent 7890B Series ODO 2 (SGE) GC/O (Agilent, Santa Clara, CA) system equipped with a HP-5MS column (30 m × 0.25 mm × 0.25 µm). The outlet was split between a flame ionisation detector and a humified sniffing port (1:1). The fibre contents were desorbed for 2 min onto five small loops of the column in a coil, which were cooled in solid carbon dioxide, contained within a 250 mL beaker. The injector and detector temperatures were maintained at 280 °C and 250 °C respectively. The oven was held at 40 °C during desorption. After desorption, the solid carbon dioxide was removed from the oven. The temperature program used was: 40 °C for 2 min isothermal, an increase of 4 °C/min to 200 °C, and an increase at 8 °C/min to 300 °C. Helium was the carrier gas with a flow rate of 2.0 mL/min. A standard of C_6–C_{25} *n*-alkanes was used to collect linear retention index (LRI) values.

Three assessors were used for the detection and verbal description of the aroma compounds. All assessors were subjected to multiple training sessions with different materials on the GC/O prior to scoring using celery material, accounting to seven hours in training. Two assessors were already considered to be well trained on the GC/O. Further training, including odour identification using 12 flavour compounds, threshold and discrimination tests using Sniffin' Sticks (Burghardt®, Wedel, Germany) were also completed prior to assessment. Assessors smelt each sample in duplicate and documented the odour description, time and odour intensity (OI) using a seven-point scale (2-8) where 3 = weak, 5 = medium and 7 = strong. Each session lasted 40 min and assessors were advised to refrain from drinking coffee and eating at least 30 min before the scoring session.

6.4.5. Statistical analysis and data pre-treatment

Raw data collected from the SPME GCMS was calculated into relative abundance according to the internal standard. The semi-quantitative data was then analysed by both one- and two-way analysis of variance (ANOVA) and principal component analysis (PCA) following Spearman's correlation, using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($P<0.05$) between harvest maturities and the celery parental lines. Only those compounds exhibiting significant differences between maturity, genotype and their interaction (maturity x genotype) were included in the principal component analysis plots.

6.5. Results and Discussion

6.5.1. Biochemical profile is more influenced by maturity than genotype

In total, 94 compounds were determined in the headspace across two celery parental lines (Table 6.1) and 91 of these were identified. Ninety-three compounds were shown to be significantly influenced by plant maturity whereas 71 compounds by plant genotype. Identified compounds include 20 monoterpenes, 13 monoterpenoid alcohols, 11 sesquiterpenes, nine alcohols and nine aldehydes, six phthalides and a range of other compounds counting esters and ketones. Monoterpenes, followed by

phthalides and sesquiterpenes, comprise most of the total volatiles collected from the headspace of the two genotypes and three maturities (Table 6.1) and are at their highest total volatile content at M1 for line 12 and M2 for line 22. Alcohols displayed an increase as the crop developed and became most abundant at M3; similar trend also observed for the aldehyde content in line 22. Sesquiterpenes and phthalides were at their highest total volatile content at M2.

GC/MS analysis identified groups of compounds that fluctuate throughout maturity and between genotype (Table 6.1). All compounds apart from *p*-cymen-8-ol, were influenced by maturity and fewer significantly influenced by genotype. Similar patterns can be observed between genotypes as the crop develops, but certain compounds prevent these patterns from occurring consistently between genotypes. For example, hexanal and propyl 3-methylbutanoate dramatically increased in line 22 at M2, causing the total aldehyde and ester content to increase accordingly.

4241 **Table 6.1** – Approximate quantities of volatile compounds identified in the headspace of celery using SPME GCMS harvested at three different maturity stages.
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				Mean relative abundance (mg/L) [f]								
				Line 12			Line 22			P-value [g]		
Code	**Compound**	**LRI expt[a]**	**ID[b]**	**M1[c]**	**M2[d]**	**M3[e]**	**M1**	**M2**	**M3**	**M[h]**	**L[i]**	**MxL[j]**
	Alcohols											
A1	3-methyl-3-buten-1-ol	730	A	nd ^{C}	4.6±1.3 ^{A}	8.6±0.91 ^{A}	nd ^{C}	3.7±0.40 ^{B}	4.3±0.76 ^{B}	***	***	***
A2	1-pentanol	763	A	0.19±0.03 ^{E}	3.7±0.53 ^{BC}	2.5±0.24 ^{CD}	0.5±0.12 ^{E}	5.7±0.85 ^{AB}	7.9±1.7 ^{A}	***	***	***
A3	1-hepten-3-ol	893	A	nd ^{C}	nd ^{C}	1.7±0.10 ^{B}	nd ^{C}	nd ^{C}	5.2±0.45 ^{A}	***	***	***
A4	*(E)*-2-hexen-1-ol	867	A	0.37±0.02 ^{C}	nd ^{C}	4.5±0.50 ^{B}	0.68±0.12 ^{C}	nd ^{C}	8.1±0.88 ^{A}	***	***	***
A5	(*E)*-2-octen-1-ol	1069	A	nd	nd	1.8±1.8	nd	nd	1.7±1.2	***	ns	ns
A6	1-octanol	1073	A	1.5±0.30	nd	nd	1.8±0.27	nd	nd	***	*	ns
A7	1-nonanol	1176	A	6.0±1.7 ^{A}	4.1±0.59 ^{AB}	5.1±0.57 ^{AB}	2.1±0.57 ^{AB}	1.4±0.17 ^{B}	3.7±1.0 ^{AB}	***	***	**
A8	1-decanol	1272	A	nd ^{C}	2.9±0.64 ^{A}	nd ^{C}	nd ^{C}	1.6±0.39 ^{B}	nd ^{C}	***	*	*
A9	1-dodecanol	1469	A	1.1±0.16 ^{A}	nd ^{C}	0.63±0.16 ^{B}	0.65±0.10 ^{B}	nd ^{C}	0.83±0.18 ^{AB}	***	ns	**
	Total			**9.2**	**15.3**	**24.8**	**5.7**	**12.4**	**31.7**			
	Aldehydes											
AH1	*(E)*-2-pentenal	754	A	4.7±0.57 ^{C}	4.1±0.99 ^{C}	7.6±1.4 ^{BC}	6.5±2.4 ^{BC}	13.6±3.2 ^{A}	11.3±1.9 ^{AB}	*	***	*
AH2	hexanal	802	A	3.1±0.32 ^{B}	14.3±3.3 ^{B}	7.1±1.1 ^{B}	5.7 ±0.60 ^{B}	134±32.3 ^{A}	153±2.2 ^{A}	***	***	***
AH3	*(Z)*-2-hexenal	855	A	1.3±0.05 ^{B}	1.7±0.10 ^{BC}	nd ^{D}	0.39±0.07 ^{CD}	2.5±0.45 ^{A}	nd ^{D}	***	**	***
AH4	*(Z)*-4-heptenal	902	A	nd	4.1±0.61	nd	nd	3.7±0.91	nd	***	ns	ns
AH5	*n*-octanal	1007	A	8.9±0.47 ^{A}	5.1±1.1 ^{B}	4.9±0.96 ^{B}	4.0±0.72 ^{B}	5.6±1.2 ^{B}	4.3±0.54 ^{B}	*	**	***
AH6	phenylacetaldehyde	1049	A	6.9±0.92 ^{BC}	4.4±0.57 ^{C}	4.5±0.25 ^{C}	15.8±2.4 ^{A}	8.4±1.9 ^{B}	3.8±0.33 ^{C}	***	***	***
AH7	2-hydroxybenzaldehyde	1056	A	nd ^{B}	nd ^{B}	4.8±0.05 ^{B}	nd ^{B}	nd ^{B}	34.6±6.3 ^{A}	***	***	***

AH8	*(E,Z)*-2,6-nonadienal	1156	A	2.1±0.38 ^{A}	nd ^{C}	nd ^{C}	1.0±0.23 ^{B}	nd ^{C}	nd ^{C}	***	***	***
AH9	*(E,E)*-2,4-nonadienal	1221	A	3.0±0.41 ^{A}	1.1±0.09 ^{C}	nd ^{D}	1.2±0.27 ^{BC}	0.44±0.28 ^{B}	nd ^{D}	***	**	*
	Total			**30**	**34.8**	**28.9**	**34.6**	**168.2**	**207**			
	Ketones											
K1	3-hexanone	779	A	nd ^{C}	nd ^{C}	1.3±0.12 ^{B}	nd ^{C}	nd ^{C}	2.1±0.45 ^{A}	***	***	**
K2	1-octen-3-one	978	A	nd ^{C}	nd ^{C}	6.7±1.3 ^{B}	nd ^{C}	nd ^{C}	4.7±1.0 ^{A}	***	ns	*
K3	2-nonanone	1090	A	2.4±0.14	nd	nd	1.6±0.51	nd	nd	***	ns	ns
	Total			**2.4**	**nd**	**28.6**	**1.6**	**nd**	**6.8**			
	Esters											
E1	methyl butanoate	720	A	nd ^{C}	0.53±0.05 ^{B}	nd ^{C}	nd ^{C}	2.3±0.09 ^{A}	nd ^{C}	***	***	***
E2	propyl 3-methylbutanoate	947	A	1.5±0.26 ^{C}	9.8±0.69 ^{C}	8.8±1.2 ^{C}	1.5±0.45 ^{C}	52.5±10.8 ^{A}	23.1±0.31 ^{B}	***	***	***
E3	bornyl acetate	1297	A	0.71±0.15 ^{B}	nd ^{B}	nd ^{B}	0.41±0.03 ^{B}	nd ^{B}	2.4±0.67 ^{A}	***	***	***
E4	(*E*)-pinocarvyl acetate	1304	B[1]	8.3±1.1 ^{A}	nd ^{C}	7.9±0.95 ^{A}	4.8±1.2 ^{B}	nd ^{C}	7.3±1.7 ^{AB}	***	*	*
E5	carveol acetate	1339	B[1]	8.7±0.54 ^{A}	nd ^{C}	10.5±0.47 ^{B}	4.2±1.1 ^{B}	nd ^{C}	5.2±1.5 ^{B}	***	***	***
E6	hexyl hexanoate	1385	A	0.36±0.07 ^{CD}	1.5±0.12 ^{B}	nd ^{D}	0.92±0.36 ^{BC}	2.6±0.69 ^{A}	nd ^{D}	***	**	*
E7	hexyl octanoate	1584	A	0.67±0.15	nd	nd	0.57±0.12	nd	nd	***	ns	ns
	Total			**20.2**	**11.8**	**27.2**	**12.4**	**57.4**	**38**			
	Monoterpenes											
M1	α-thujene	932	B[2]	12.5±1.5 ^{A}	4.6±0.34 ^{B}	1.3±0.10 ^{D}	3.4±0.32 ^{BC}	4.3±0.54 ^{B}	1.6±0.36 ^{CD}	***	***	***
M2	α-pinene	939	A	15.8±3.7 ^{A}	8.8±0.86 ^{BC}	11.4±1.3 ^{AB}	5.9±0.60 ^{C}	6.7±1.4 ^{BC}	5.0±0.40 ^{C}	*	***	**
M3	camphene	958	A	3.7±0.64 ^{C}	4.9±1.3 ^{BC}	6.8±0.97 ^{AB}	2.2±0.40 ^{C}	8.0±1.7 ^{A}	7.8±0.76 ^{A}	***	ns	**
M4	dehydrosabinene	960	A	nd ^{B}	nd ^{B}	nd ^{B}	nd ^{B}	nd ^{B}	0.5±0.14 ^{A}	***	***	***
M5	sabinene	976	A	13.3±2.5 ^{A}	5.5±1.0 ^{B}	4.6±0.17 ^{B}	3.7±0.45 ^{B}	6.7±1.2 ^{B}	3.5± 0.73 ^{B}	***	***	***
M6	β-pinene	980	A	190±37.9 ^{A}	86.9±10.8 ^{B}	14.9±2.4 ^{C}	39.3±5.6 ^{C}	16.9±2.7 ^{C}	17.4±3.2 ^{C}	***	***	***

M7	myrcene	991	A	122±25.7 ^{A}	49.6±11.8 ^{B}	15.1±2.4 ^{C}	20.3± 5.7 ^{BC}	12.3± 2.8 ^{C}	6.9± 2.3 ^{C}	***	**	***
M8	α-terpinene	1018	A	7.2±1.9 ^{A}	4.8±1.0 ^{AB}	0.84±0.02 ^{C}	3.3±0.77 ^{BC}	3.9±0.43 ^{B}	2.5±0.29 ^{BC}	***	*	**
M9	m-cymene	1027	A	185±32.7 ^{A}	71.5±10.6 ^{B}	40.8±9.2 ^{B}	59.1±26.3 ^{B}	59.2±8.0 ^{B}	25.8±0.68 ^{B}	***	***	***
M10	limonene	1034	A	1068±207 ^{A}	598±41.8 ^{B}	264±61.8 ^{C}	581±93.7 ^{B}	605±88.8 ^{B}	264±7.4 ^{C}	***	**	**
M11	γ-terpinene	1063	A	256±34.4 ^{A}	112±20.3 ^{B}	21.7±2.5 ^{C}	63.7±34.6 ^{BC}	54.0±12.9 ^{BC}	42.3±12.8 ^{C}	***	***	***
M12	terpinolene	1093	A	9.6±0.15 ^{B}	8.0±0.89 ^{BC}	15.1±2.0 ^{A}	4.4±0.74 ^{D}	7.3±1.0 ^{BCD}	6.4±1.0 ^{CD}	***	***	***
M13	p-cymene	1099	A	nd ^{C}	nd ^{C}	3.7±0.35 ^{A}	nd ^{C}	nd ^{C}	2.9±0.27 ^{B}	***	**	**
M14	β-thujone	1119	B[3]	1.6±0.50	4.2±0.82	0.96±0.20	0.77±0.18	3.0±0.45	0.86±0.13	***	**	ns
M15	*p*-mentha-1,5,8-triene	1113	B	nd ^{C}	1.3±0.26 ^{B}	1.9±0.35 ^{A}	nd ^{C}	1.4±0.16 ^{B}	1.4±0.05 ^{B}	***	ns	*
M16	citronellal	1159	A	25.4±4.2 ^{A}	9.3±2.4 ^{B}	2.8±0.12 ^{C}	4.2±0.83 ^{BC}	6.5±1.4 ^{BC}	1.2±0.06 ^{C}	***	***	***
M17	trans-dihydrocarvone	1195	A	nd	nd	2.9±0.64	nd	nd	2.8±0.18	***	ns	ns
M18	β-cyclocitral	1232	A	1.2±0.27	1.9±0.42	1.8±0.10	0.88±0.28	1.9±0.21	1.1±0.15	***	*	ns
M19	carvone	1246	A	9.2±1.7 ^{B}	18.1±3.3 ^{A}	2.1±0.41 ^{C}	7.0±1.5 ^{BC}	10.2±1.7 ^{B}	4.1±1.2 ^{C}	***	*	*
M20	L-carvone	1257	A	nd ^{C}	3.6±0.74 ^{B}	4.9±0.93 ^{B}	nd ^{C}	4.4±0.80 ^{B}	7.1±0.84 ^{A}	***	**	**
	Total			**1921**	**993**	**418**	**799**	**812**	**405**			
	Monoterpenoid alcohols											
MA1	linalool	1103	A	1.3±0.23 ^{CD}	1.6±0.34 ^{CD}	1.7±0.36 ^{C}	0.84±0.13 ^{D}	3.7±0.35 ^{A}	2.8±0.19 ^{B}	***	***	***
MA2	*p*-mentha-2,8-dien-1-ol	1122	A	nd	1.2±0.15	0.8±0.15	nd	1.1±0.20	1.1±0.29	***	ns	ns
MA3	fenchol	1127	A	16.9±1.5 ^{A}	5.6±1.0 ^{B}	1.8±0.27 ^{B}	22.5±5.5 ^{A}	1.9±0.27 ^{B}	3.9±0.86 ^{B}	***	ns	*
MA4	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	1129	A	6.8±1.6^{AB}	9.7±1.9 ^{AB}	1.8±0.35 ^{B}	7.5±1.6 ^{A}	9.3±1.1 ^{B}	1.7±0.13 ^{B}	***	ns	ns
MA5	dihydrolinalool	1136	A	nd ^{B}	nd ^{B}	6.3±1.0 ^{AB}	nd ^{B}	nd ^{B}	5.0±1.7 ^{A}	***	ns	ns
MA6	pinocarveol	1152	A	3.1±0.68 ^{B}	4.0±0.84 ^{AB}	4.2±0.22 ^{AB}	1.2±0.35 ^{C}	1.1±0.05 ^{C}	5.4±0.43 ^{A}	***	***	***
MA7	terpinen-4-ol	1184	A	nd ^{C}	1.7±0.30 ^{B}	2.9±0.68 ^{A}	nd ^{C}	nd ^{C}	2.7±0.61 ^{AB}	***	***	**
MA8	p-cymen-8-ol	1202	A	4.1±0.79	3.8±0.03	4.2±0.91	2.0±0.63	2.8±0.29	2.7±0.78	ns	***	ns

MA9	γ-terpineol	1210	A	2.6±0.71 ^{A}	nd ^{C}	1.8±0.40 ^{AB}	1.2±0.44 ^{A}	2.0±0.19 ^{AB}	2.5±0.42 ^{A}	***	ns	***
MA10	*(Z)*-carveol	1220	B[3]	nd	7.5±1.5	5.8±0.92	nd	4.9±1.0	4.2±1.1	***	**	ns
MA11	thymol	1290	A	0.87±0.15 ^{BC}	2.8±0.30 ^{A}	3.2±0.74 ^{A}	0.31±0.07 ^{C}	nd^{C}	1.4±0.37 ^{B}	***	***	**
MA12	carvacrol	1311	A	2.8±0.60 ^{B}	11.2±1.7 ^{A}	13.1±0.78 ^{A}	0.80±0.09 ^{B}	2.8±0.30 ^{B}	2.2±0.38 ^{B}	***	***	***
MA13	*(E)*-8-hydroxylinalool	1342	B[3]	0.90±0.26 ^{A}	nd ^{C}	nd ^{C}	0.38±0.05 ^{B}	nd ^{C}	nd ^{C}	***	**	**
	Total			**39.4**	**49.1**	**47.6**	**36.7**	**29.6**	**35.6**			
	Sesquiterpenes											
S1	(+)-cyclosativene	1378	A	nd ^{C}	1.1±0.12 ^{B}	nd ^{C}	nd ^{C}	3.8±0.75 ^{A}	nd ^{C}	***	***	***
S2	α-copaene	1389	A	0.36±0.10 ^{B}	1.6±0.43 ^{B}	nd ^{B}	2.1±0.30 ^{B}	10.5±1.9 ^{A}	nd ^{B}	***	***	***
S3	β-caryophyllene	1440	A	35.9±12.1 ^{A}	46.5±11.4 ^{AB}	12.8±3.3 ^{B}	15.9±3.8 ^{B}	25.6±1.1 ^{B}	6.6±2.1 ^{B}	***	***	ns
S4	α-humulene	1475	A	9.8±2.3 ^{A}	8.5±1.1 ^{BC}	5.2±1.6 ^{B}	2.2±0.29 ^{BCD}	2.0±0.41 ^{D}	1.3±0.17^{CD}	**	***	ns
S5	(+)-aromadendrene	1447	A	1.1±0.18 ^{ABC}	1.5±0.16 ^{A}	0.60±0.10 ^{C}	0.66±0.11 ^{C}	1.3±0.33 ^{AB}	0.97±0.18 ^{BC}	***	ns	**
S6	curcumene	1486	B[4]	2.0±0.21 ^{A}	nd ^{C}	nd ^{C}	1.0±0.11 ^{B}	nd ^{C}	nd ^{C}	***	***	***
S7	β-selinene	1505	B[5]	57.0±13.3	79.2± 14.6	26.4±4.5	21.6±4.2	50.5±11.5	15.0±2.0	***	***	ns
S8	valencene	1516	A	nd ^{B}	54.5±9.7 ^{A}	nd ^{B}	nd ^{B}	nd ^{B}	nd ^{B}	***	***	***
S9	α-selinene	1518	B[6]	8.3±1.6	14.2±2.4	4.0±0.72	3.5±0.12	9.3±2.1	3.3±0.84	***	***	ns
S10	(*Z*)-β-nerolidol	1535	A	nd	nd	3.2±0.34	nd	nd	3.4±0.56	***	ns	ns
S11	kessane	1554	B[3]	60.3±7.8 ^{A}	nd ^{B}	nd ^{B}	0.64±0.23 ^{B}	nd ^{B}	nd ^{B}	***	***	***
	Total			**175**	**207**	**52.2**	**47.5**	**103**	**30.6**			
	Phthalides											
P1	3-propylidene phthalide	1600	A	1.4±0.23	2.1±0.29	1.3±0.36	0.4±0.03	1.4±0.32	0.17±0.03	***	***	ns
P2	3-*n*-butylphthalide	1658	B[7]	37.2±4.5 ^{C}	124±20.2 ^{A}	103±5.5 ^{AB}	26.8±6.7 ^{C}	148±27.3 ^{A}	68.0±22.9 ^{BC}	***	ns	*
P3	*(Z)*-butylidenephthalide	1685	B[7]	nd ^{C}	2.9±0.60 ^{B}	1.5±0.28 ^{C}	nd ^{C}	4.3±0.84 ^{A}	0.84±0.07 ^{CD}	***	ns	**
P4	sedanenolide	1730	A	102±16.1 ^{C}	279±21.3 ^{A}	221±42.2 ^{AB}	56.8±12.3 ^{CD}	202±27.1 ^{B}	18.1±4.0 ^{D}	***	***	***

P5	neocnidilide	1753	B[7]	1.1±0.13 ^{C}	2.9±0.53 ^{BC}	3.2±0.63 ^{BC}	3.0±0.62 ^{BC}	10.0±1.8 ^{A}	3.8±0.52 ^{B}	***	***	***
P6	(*E*)-ligustilide	1758	B[7]	1.4±0.25 ^{B}	3.8±0.61 ^{A}	3.0±0.55 ^{A}	0.89±0.20 ^{B}	2.9±0.56 ^{A}	0.42±0.07 ^{B}	***	***	**
	Total			**143**	**415**	**333**	**87.9**	**369**	**91.3**			
	Alkanes											
ALK1	nonane	900	A	5.9±1.2 ^{AB}	9.7±2.0 ^{A}	6.8±1.1 ^{AB}	5.5±1.9 ^{AB}	nd ^{C}	9.3±1.2 ^{AB}	**	**	***
ALK2	decane	1000	A	nd ^{D}	6.4±1.2 ^{BC}	5.1±0.74 ^{CD}	nd ^{D}	22.5±4.2 ^{A}	11.1±1.6 ^{B}	***	***	***
ALK3	undecane	1100	A	2.4±1.5	2.3±0.17	nd	1.7±0.21	3.2±0.76	nd	***	ns	ns
ALK4	dodecane	1200	A	0.56±0.08 ^{D}	6.2±1.6 ^{A}	5.5±0.79 ^{A}	1.7±0.21 ^{CD}	4.6±1.0 ^{AB}	3.0±0.60 ^{BC}	***	*	*
ALK5	tridecane	1300	A	nd ^{B}	nd ^{B}	3.1±0.57 ^{A}	nd ^{B}	nd ^{B}	nd ^{B}	***	***	***
ALK6	tetradecane	1400	A	0.51±0.13 ^{C}	0.99±0.21 ^{B}	nd ^{D}	0.39±0.04 ^{C}	2.0±0.14 ^{A}	nd ^{D}	***	***	***
	Total			**9.4**	**25.6**	**20.5**	**9.3**	**32.3**	**23.4**			
	Ether											
ET1	dill ether	1184	A	nd ^{C}	nd ^{C}	3.5±1.4 ^{A}	nd ^{C}	nd ^{C}	1.6±0.36 ^{B}	***	ns	*
	Oxide											
O1	*(Z)*-limonene oxide	1145	A	12.8±3.4	nd	nd	10.8±0.53	nd	nd ^{B}	***	ns	ns
	Phenol											
PH1	eugenol	1363	A	nd	1.8±0.22	2.7±0.23	nd	2.3±0.29	2.7±0.42	***	ns	ns
	Unknowns											
U1	unknown	935		3.9±0.58 ^{A}	nd ^{D}	1.1±0.21 ^{C}	2.1±0.18 ^{B}	nd ^{D}	1.6±0.16 ^{C}	***	***	***
U2	unknown	1009		nd ^{C}	nd ^{C}	13.6±1.2 ^{A}	nd ^{C}	nd ^{C}	10.9±1.1 ^{B}	***	*	**
U3	unknown	1133		nd ^{B}	nd ^{B}	0.72±0.14 ^{B}	nd ^{B}	nd ^{B}	2.0±0.71 ^{A}	***	*	**
U4	unknown	1239		nd ^{B}	nd ^{B}	2.1±0.18 ^{B}	nd ^{B}	nd ^{B}	22.2±4.38 ^{A}	***	***	***
U5	unknown	1277		nd ^{B}	1.4±0.34 ^{B}	4.6±2.0 ^{A}	nd ^{B}	1.7±0.25 ^{B}	2.1±0.56 ^{B}	***	ns	*
U6	unknown	1466		nd ^{C}	2.6±0.57 ^{A}	nd ^{C}	nd ^{C}	1.5±0.05 ^{B}	nd ^{C}	***	**	***
U7	unknown	1698		nd ^{B}	51.8±7.7 ^{A}	nd ^{B}	nd ^{B}	nd ^{B}	nd ^{B}	***	***	***

	Total			**64.2**	**55.8**	**22.1**	**2.7**	**3.2**	**38.8**			

[a] Linear retention index on a DB-5 column. [b] A – Experimental LRI, identification of compound whereby the mass spectrum and LRI agree with those of authentic compound (A) Identification, mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectra database or (B) LRI agree with those in the literature; [1] Stashenko et al. (2003); [2] Adams et al. (2005); [3] Andriamaharavo, (2014); [4] Cao et al. (2011); [5] Yu et al. (2007); [6] Zeng et al. (2007); [7] Turner et al. (2021b); [c] Premature time-point. [d] Commercial maturity time-point. [e] Post-maturity time-point. [f] Estimated quantities (mg) collected in the headspace of celery samples containing 0.5 mL of saturated calcium chloride and filled up to 5 mL with HPLC-grade water, calculated by comparison with of 100 mg/L propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [h] Maturity. [i] Line. [j] Maturity and line interaction. Tukey's HSD - means not labelled with letters are not significantly different ($p < 0.05$) according maturity/line interaction.

Monoterpene content in line 12 was the highest at M1, with limonene, the most abundant compound, identified across both lines and maturities. Limonene's content decreased as celery developed. Most monoterpenes followed this pattern including γ-terpinene, m-cymene and β-pinene and is most noticeable in line 12. These compounds remained the most abundant monoterpenes in line 22, however, there is less of a noticeable change between M1 and M2. These compounds are known to have odour descriptors that include citrus, pine and sweet. Throughout literature, monoterpenes have been shown to be the most abundant compounds reported in various celery genotypes as shown previously by Turner, Lignou, Gawthrop & Wagstaff (2021). Orav, Kailas & Jegorova (2003) analysed the composition of Estonian grown celery essential oil and similarly, identified monoterpenes to comprise most of the flavour profile (85.3 %). Likewise, MacLeod & Ames (1989) identified 18 monoterpenes, representing around 46 % of the aroma profile of fresh supermarket bought celery and identified limonene as the major component in the celery isolate, similar to this study.

Additional monoterpenes such as p-mentha-1,5,8-triene and L-carvone in M2 and *trans*-dihydrocarvone and *p*-cymene were identified in both genotypes as maturity developed whereas dehydrosabinene only appeared in line 22 at M3. These compounds signal the deterioration of the crop through the development of the aroma from fresh and green, to woody and pine. Similarly, further monoterpenoid alcohols such as p-mentha-2,8-dien-1-ol, dihydrolinalool, terpinen-4-ol and (Z)-carveol were identified as maturity developed. Linalool, pinocarveol, thymol and carvacrol exhibited their highest abundance at M3. These compounds are responsible for floral, herbal, pine odours. For both genotypes, fenchol was the most abundant monoterpenoid alcohol with odour descriptors such as minty, medicinal and camphoreous. Compared to M1, fenchol's content at M3 was significantly lower. Monoterpenoid alcohols presented to be least influenced by genotype compared to other compound groups.

Sesquiterpenes, while fewer were identified and with lower relative abundances, contribute woody, herbal, and floral notes to celery aroma. Maturity showed to have a significant influence for all sesquiterpenes. Lund, Wagner, & Bryan (1973) and MacLeod & Ames (1989) both identified β-selinene to be an important compound to the celery aroma, although not a characteristic compound. β-Selinene and β-caryophyllene were identified as non-phthalide compounds with the highest concentrations in

celery essential oil, however, β-selinene was characterised with a celery-like odour. Using odour evaluation, β-selinene was shown to have a threshold of 1 mg/L which is low compared to 3-n-butylphthalide with an odour threshold of 10 mg/L (Lund, Wagner & Bryan, 1973). Furthermore, Ehiabhi et al. (2006) reported both β-selinene and β-caryophyllene to be major constituents of Nigerian grown *A. graveolens* and were reported to make up as much as 16.3 and 10.5 % respectively, of the aroma profile.

Findings in the present study agree with Ehiabhi et al. (2006), β-selinene and β-caryophyllene expressed their highest relative abundance at M2 and decreased once commercial maturity reached (Table 6.1). A similar pattern was observed for other sesquiterpenes including α-selinene and α-copaene and monoterpenes in line 22. α-Humulene was most abundant at M1 with curcumene and kessane only detected at M1. Kessane was also identified by Philippe, Suvarnalatha, Sankar & Suresh (2002) in the essential oil of Indian celery seed. During M3, the abundance of sesquiterpenes remained relatively low compared to monoterpenes and phthalides, however, (Z)-β-nerolidol was only identified at M3 for both genotypes. Kessane, curcumene and (Z)-β-nerolidol were all determined by Nurzyńska-Wierdak, Gruszecki and Kosior (2018) in varying amounts of celery essential oil of two varieties grown in Poland. These had been preserved through various drying techniques and harvested in July and October. Only the July harvest showed the presence of these compounds.

Phthalides exhibited a similar pattern to sesquiterpenes, showing their highest level of abundance at M2. Abundance variation within the phthalides identified were observed between maturities, with line 12 showing a much higher phthalide content than line 22. As shown by both Kurobayashi et al. (2006) and Sellami et al. (2012), phthalide compounds are important contributors to the typical *A. graveolens* aroma and therefore, having a lower abundance of these compounds at a later maturity may mean that the odour these genotypes exhibit is a much less typical celery odour. Focussing further on the phthalide compounds, a significant difference between the maturities for most of these compounds can be observed, with sedanenolide showing the most significant increase from M1 to M2 and then decreasing at M3. Apart from neocnidilide in line 22, all phthalides were at the highest abundance at this time point. 3-*n*-Butylphthalide and (Z)-butylidene phthalide showed no significant difference between genotype, only maturity, and (Z)-butylidene phthalide was not identified at M1.

The relative abundance of alcohols increased as the crop developed for both genotypes. At M3 more alcohols were identified and in most cases at a higher abundance. Compounds 1-nonanol and 1-dodecanol for line 12 were shown to be of lower abundance at M3 when compared to M1 and 1-octanol and 1-decanol were not identified in either genotype at M3. For monoterpenes, sesquiterpenes and phthalides, line 12 has been shown to have the highest abundance of these compounds when compared to line 22. However, for alcohols, aldehydes and esters, line 22 has a significantly higher abundance of these and exhibited a different pattern to line 12. At M1, line 22 expressed a similar aldehyde and ester content to line 12 at M2 and at M3, a much higher abundance of these compounds is observed. The biggest cause of this difference in esters was attributed to the large increase of propyl 3-methylbutanoate, known for its fruity, apple odour. Seven aldehydes were identified at both M1 and M2 compared to the five identified at M3. Compounds contributing to green, fresh odours such as *(Z)*-2-hexenal, (*Z*)-4-heptenal, *(E,Z)*-2,6- and *(E,E)*-2,4-nonadienal were not found in M3. Conversely, 2-hydroxybenzaldehyde was only identified at M3 and at much higher abundance in line 22, again this indicates aroma deterioration. Line 22 exhibited a higher abundance in compounds such as hexanal at all maturities, particularly at M3 where hexanal increased in relative abundance, whereas in line 12 this began to decrease after M2.

As these lines were transplanted in the same field at the same time and were grown under the same environmental conditions, minimal significant differences caused by environmental factors were expected. Therefore, any differences observed should be attributed to differences in the genotype and maturity. From the results so far, it seems that maturity has a higher impact on aroma profile differences than genotype however, the difference between genotypes in terms of patterns for different compounds across maturities is apparent. This was expected due to the differences identified by Yommi et al. (2013) and Fellman, Miller and Mattinson (2000). They observed the influence of genetics and harvest maturity on volatile compounds in different apple varieties, stating that the nature and amount of aroma compounds present in apples were cultivar dependent.

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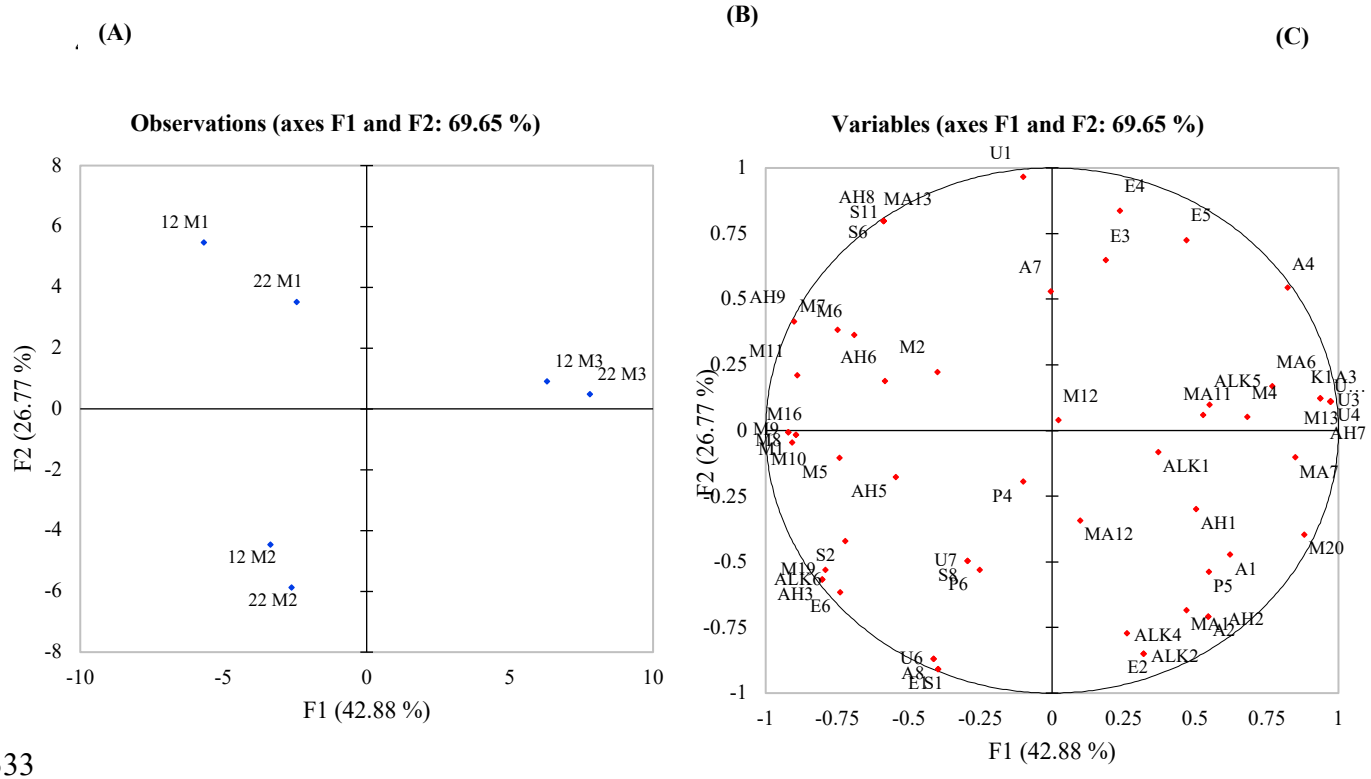

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A1	3-methyl-3-buten-1-ol	M12	terpinolene
A2	1-pentanol	M13	p-cymenene
A3	1-hepten-3-ol	M16	citronellal
A4	*(E)*-2-hexen-1-ol	M19	carvone
A7	1-nonanol	M20	L-carvone
A8	1-decanol	MA1	linalool
AH1	*(E)*-2-pentenal	MA6	pinocarveol
AH2	hexanal	MA7	terpinen-4-ol
AH3	*(Z)*-2-hexenal	MA11	carvacrol
AH5	*n*-octanal	MA12	*(E)*-8-hydroxylinalool
AH6	phenylacetaldehyde	MA13	(+)-cyclosativene
AH7	2-hydroxybenzaldehyde	S1	α-copaene
AH8	*(E,Z)*-2,6-nonadienal	S2	β-caryophyllene
AH9	*(E,E)*-2,4-nonadienal	S6	β-selinene
K1	3-hexanone	S8	α-selinene
E1	methyl butanoate	S11	kessane
E2	propyl 3-methylbutanoate	P4	sedanenolide
E3	bornyl acetate	P5	neocnidilide
E4	*(E)*-pinocarvyl acetate	P6	*(E)*-ligustilide
E5	carveol acetate	ALK1	nonane
E6	hexyl hexanoate	ALK2	decane
M1	α-thujene	ALK4	dodecane
M2	α-pinene	ALK5	tridecane
M4	dehydrosabinene	ALK6	tetradecane
M5	sabinene	U1	unknown
M6	β-pinene	U2	unknown
M7	myrcene	U3	unknown
M8	α-terpinene	U4	unknown
M9	m-cymene	U5	unknown
M10	limonene	U6	unknown
M11	γ-terpinene	U7	unknown

4342 **Figure 6.1**. Principal component analysis of two different celery genotypes at three different maturities showing
4343 correlations with volatile compounds that are significant according to factors of maturity, genotype and their interaction of
4344 maturity x genotype: (A) Projection of samples, (B) Distribution of volatile compounds, (C) Key of compounds used to
4345 construct the PCA.

Principal component analysis was used to visualise graphically the differences in the volatile compounds in the three maturity stages and the two genotypes and to examine any correlations occurring between maturity, genotype and chemical compounds (Figure 6.1). Using only the significant compounds for maturity, genotype and their interaction, a clear separation between the maturities and the chemical compounds associated can be observed. Principal component one (F1) and two (F2) explained 69.95 % of the total variation present within the data and it can be observed that the first axis discriminates M3 from M1 and M2, whereas M2 is discriminated from M1 and M3 by the second axis. Predominantly, monoterpene content expresses a strong association with F1 (42.88 %) whereas other compound groups including aldehydes, esters and phthalides are measured through F2 and explaining a lower proportion of the variation present within the data (26.77 %).

Genotype shows a stronger influence upon M1 where a larger separation can be seen between the two genotypes and a stronger association with the volatile compounds associated with line 12 M1. M1 displays a strong positive association with the majority of monoterpenes, such as α-pinene (M2), sabinene (M5), β-pinene (M6), myrcene (M7) and (M11) γ-terpinene, and aldehydes such as 1-octanol (AH5) phenylacetaldehyde (AH6), (*E,Z*)-2,6-nonadienal (AH8) and *(E,E)*-2,4-nonadienal (AH9). These are compounds are known to exhibit fresh, waxy, green notes, similar to cucumber odour. The highest number of esters were identified at M1 (Table 6.1) and these compounds contribute fruity and fresh notes however, these are at low relative abundance compared to the other maturities as seen in Table 6.1, explaining the low association of these compounds in all PCA plots. Nurzyńska-Wierdak, Gruszecki & Kosior (2018) observed both increases and decreases in the ester content of celery essential oil when comparing freeze-dried with convection drying, however these were not significant differences. Phthalides show no association with M1 in Figure 6.1 and only sesquiterpenes β-selinene (S6) and kessane (S11) show association with M1.

Developing into M2, the aroma profile shifted, with strong associations with phthalides such as sedanenolide (P4) and *(E)*-ligustilide (P6), and sesquiterpenes such as α-copaene (S1), β-caryophyllene (S2) and α-selinene (S8). The presence of these compounds allows stronger odours that are woodier, herbal and celery-like to seem more apparent, descriptors that are more common when

describing *A. graveolens* aroma. At this stage, the highest number of sesquiterpenes and phthalides were observed for both genotypes (Table 6.1).

Once M3 is reached, the spread of compounds within the quadrant (Figure 6.1) is much less compared to other maturities, with the compounds more localised. Furthermore, where more obvious groupings of compounds by M1 and M2 can be seen clearly, this is less apparent for M3. Compounds including 2-hydroxybenzaldehyde (AH7), dehydrosabinene (M4), *p*-cymene (M13) and terpinolene (M12) are strongly associated with M3 as well as the monoterpenoid alcohols; pinocarveol (MA6), terpinen-4-ol (MA7), carvacrol (MA11) and (E)-8-hydroxylinalool (MA12). M3 displaying stronger associations with these compounds and weaker associations with monoterpenes, alcohols and phthalides (fresh, green and fruit odours) suggests that the odour of these genotypes are no longer of the same quality as M2 and therefore, deterioration of the crop is beginning. The presence of certain compounds (A3, K1, M4, M13) could act as an indicator of quality decline in celery. Within the same quadrant as M3, esters bornyl acetate (E3), (E)-pinocarvyl acetate (E4), carveol acetate (E5) express a closer association than previous maturities.

Furthermore, line 22 shows significantly higher abundances in certain compounds at M3 including AH2, M4 and AH7 whereas line 12, show higher abundances in other compounds at M3 including K2, M13 and MA5 (Table 6.1). Possibly due to genetic differences or because line 22 may have progressed through developmental stages differently compared to than line 12, where the floral transition had commenced, and the plants were preparing to bolt. At the beginning of maturity, line 12 appears to be most aromatic (Figure 6.1, Table 6.1) however, as maturity occurs line 22 M2 and M3 progresses into a more aromatic line, showing these two time points to be most significantly different when combined with genotype. Line 12 M1 and line 22 M2 celery share the most similarities in terms of aroma profile and independent of genotype, M1 and M2 appear to be the most similar.

Compounds including hexanal and *(E)*-2-hexen-1-ol are known as green leaf volatiles (GLVs); these are released in the early stages of maturity and increase as the plants develop, similar to monoterpenes. Over time, the bolting process begins and the crop invests more resources into reproduction and protecting the developing floral meristem from predatory attack, as shown by Rapparini, Baraldi & Facini, (2001). This is where the concentration of terpenes was highest (Table 6.1,

M1) following flowering and in subsequent reproductive stages. As the plant develops, plant-plant and plant-insect interactions become more important, involving the synthesis of GLVs and other volatile compounds (Spinelli, Cellini, Marchetti, Mudigere & Piovene, 2011). This relationship explains the increase of monoterpenes from M1 to M2 before the crop focuses on the synthesis of alcohols and aldehydes as maturity develops.

Overall, comparing the odours between the two genotypes and three maturities, line 12 has the highest abundance of volatile compounds and is expressed as the more aromatic variety. Harvesting at any time point will result in a crop with a significantly different aroma profile. Harvesting at an earlier, similar to M1 would result in low in phthalide and high monoterpene content, resulting in a more citrus-like profile. Over commercial maturity, phthalide content remains high, maintaining strong celery notes. In order to identify whether there has been aroma quality decline and whether compounds identified in M3 contribute to off-odours, sensory profiling using a trained panel can be completed. The differences support the hypothesis that the time point of harvest does have a significant influence over the aroma of celery as well as the genotype and that genotype will influence the synthesis of odours during deterioration. This relationship is discussed further when considering the GC/O data in section 6.5.2.

6.5.2. Human olfactory analysis using GC-O shows that genotype influences development of off-flavours

In total, 103 different odours were detected in the headspace of the two celery genotypes across three different maturities using GC/O. Out of these, 65 compounds were identified using a combination of GC/MS analysis, LRI comparison to authentic standards and using the aromas they were described with (Table 6.2). Similarly to the chemistry described by GC/MS (Table 6.1), differences between genotype as the crop developed is evident in Table 6.2, with the absence/presence of compounds within genotypes contributing different odours to the overall aroma profile and thus indicating that genotype plays a role in the synthesis of odours that may indicate quality decline.

Table 6.2 – Odour description and intensity of the volatile compounds detected by GC-O in the headspace of two celery genotypes harvested at three different maturity stages.

					Average Odour Intensity [c]					
					Line 12			Line 22		
Odour Description	LRIexp[a]	Compound	ID[b]	Code[d]	M1[e]	M2[f]	M3[g]	M1	M2	M3
Alcohols										
Burnt, baked, dairy	660	1-butanol	B		-	-	4	3	4	-
Green/chemical	670	1-penten-3-ol	B		4	-	-	-	-	-
Green, plastic, fruity	706	3-pentanol	B		-	3	4	-	-	-
Soapy, green, sharp	733	3-methyl-3-buten-1-ol	A	A1	5	-	5	3	-	-
Fresh, green, fruity	859	(*Z*)-3-hexen-1-ol	B		5	-	4	-	-	-
Musty, moss	867	*(E)*-2-hexen-1-ol	A	A4	-	5	3	-	4	-
Earthy, mushroom, grass	889	1-hepten-3-ol	A	A3	8	-	4	-	5	-
Mushroom	907	2-heptanol	B, C		6	5	-	-	-	3
Mushroom, soil	978	1-octen-3-ol	B, C		7	5	6	4	7	5
Fresh, citrus, waxy	1001	3-octanol	B		7	-	5	5	6	-
Metallic, sweaty	1174	1-nonanol	A	A7	7	-	6	-	4	4
Tomato, herbal, fatty	1274	1-decanol	A	A8	-	-	5	-	5	-
Aldehydes										
Floral, green, waxy	760	(*E*)-2-pentenal	A	AH1	-	4	5	3	3	-
Fresh, green, apple	801	hexanal	A	AH2	5	5	3	6	6	4
Garbage, damp	855	(*E*)-2-hexenal	A	AH3	-	5	-	5	-	-
Biscuit, bread	901	(*Z*)-4-heptenal	A	AH4	5	-	5	4	-	-
Floral, rose, citrus	1005	n-octanal	A	AH5	-	7	-	6	-	3
Rose, honey, floral	1045	phenylacetaldehyde	A	AH6	7	5	4	5	5	4
Baked, honey, make-up powder	1057	2-hydroxybenzaldehyde	A	AH7	6	-	5	4	4	5

Floral, smoky, cherry	1071	*p*-tolualdehyde	B		-	-	5	3	-	-
Woody, moss, cucumber	1155	(*E,E*)-2,6-nonadienal	B, C		6	5	6	7	5	5
Green, cucumber, parsley	1159	(*E,Z*)-2,6-nonadienal	A	AH8	6	5	-	7	7	5
Floral, woody	1224	(*E,E*)-2,4-nonadienal	A	AH9	-	5	-	-	-	-
Ketones										
Vanilla, creamy, butter	677	1-penten-3-one	B		-	3	-	-	-	-
Bread, floral, grass	687	2-pentanone	B		-	-	4	5	6	3
Green	693	3-pentanone	B		7	-	4	5	-	-
Waxy, green, plastic	776	3-hexanone	A	K1	6	-	-	5	-	-
Green, cut grass, apple	793	2-hexanone	B		7	3	4	4	-	-
Metallic, musty	978	1,5-octadien-3-one?	A	K2	-	-	-	4	4	-
Rose, honey, floral	1041	3-octen-2-one	B		7	-	5	-	-	-
Herbal, soil, spicy	1083	2-nonanone	A	K3	-	3	5	-	5	-
Make-up powder, floral, creamy	1146	3-nonen-2-one	B		-	-	6	6	5	-
Esters										
Make-up powder, floral	947	propyl 3-methylbutanoate	A	E2	3	-	6	-	-	-
Woody, pencil shavings, liquorice	1247	linalyl acetate	B		6	-	6	-	5	-
Herbal, woody	1305	bornyl acetate	A	E3	-	-	4	-	-	4
Plastic, green, herbal	1332	carveol acetate	A	E5	-	-	4	7	-	-
Metallic, damp, musty	1381	hexyl hexanoate	A	E6	-	-	4	-	6	4
Monoterpenes										
Pine, minty, floral	931	α-thujene	A	M1	5	-	4	4	4	-
Herbal, citrus, waxy	959	camphene	A	M3	6	4	5	5	5	3
Earthy, mushroom, green	981	sabinene	A	M5	8	-	6	7	7	-

Herbal, earthy, woody	987	β-pinene	A	M6	8	7	4	7	5	5
Lemon, green, waxy	997	β-myrcene	A	M7	-	3	4	6	-	-
Musty, camphoreous	1025	α-terpinene	A	M8	6	-	4	-	-	-
Floral, fresh, mint	1031	limonene	A	M10	6	-	4	4	4	-
Waxy, woody, makeup powder	1062	γ -terpinene	A	M11	6	-	-	-	-	-
Make-up powder, floral, citrus	1094	terpinolene	A	M12	5	3	4	-	5	4
Floral, herbal, violet	1098	*p*-cymene	A	M13	6	-	3	-	-	-
Caramel, honey, floral	1109	*p*-mentha-1,5,8-triene	A	M15	5	-	6	-	-	4
Tomato, spicy	1112	β-thujone	A	M14	-	-	-	5	5	-
Floral, musty, green	1166	citronellal	A	M16	-	7	4	5	6	-
Make-up powder, herbal, floral	1195	trans-dihydrocarvone	A	M17	6	-	4	4	6	5
Floral	1231	β -cyclocitral	A	M18	-	-	6	-	-	-
Spearmint	1245	carvone	A	M19	-	-	6	5	-	3
Herbal, pine, minty	1253	L-carvone	A	M20	-	7	6	6	4	6
Oily, woody	1259	D-carvone	B, C		5	-	5	-	-	-
Monoterpenoid alcohols										
Woody, red fruit	1103	linalool	A	MA1	3	-	-	4	-	-
Herbal, cooked	1116	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	A	MA2	-	-	4	4	-	-
Cucumber, floral, woody	1150	pinocarveol	A	MA6	-	-	6	7	-	4
Mushroom, earthy, metallic	1180	terpinen-4-ol	A	MA7	-	7	3	3	-	-
Herbal	1207	γ -terpineol	A	MA9	-	-	-	4	-	-
Bread, creamy	1214	(*Z*)-carveol	A	MA10	-	-	5	5	4	-
Pine, spicy	1292	thymol	A	MA11	-	3	4	-	-	-
Herbal, starchy	1314	carvacrol	A	MA12	-	-	5	-	-	-
Herbal	1346	*(E)*-8-hydroxylinalool	A	MA13	-	3	-	-	-	-

Sesquiterpenes										
Cucumber skin, fatty	1366	(+)-cyclosativene	A	S1	-	-	3	-	3	-
Damp, bread, woody	1390	α-copaene	A	S2	-	-	4	5	6	4
Sweet, earthy	1443	β-caryophyllene	A	S3	-	-	4	-	-	3
Floral, vegetative, woody	1478	α-humulene	A	S4	-	-	4	-	4	-
Floral, rose, woody	1495	β-selinene	A	S7	-	5	4	5	5	-
Creamy	1513	α-selinene	A	S9	-	3	-	-	-	-
Vegetative	1555	kessane	A	S11	-	-	3	-	-	-
Phthalides										
Celery, vegetables	1603	3-propylidene phthalide	A	PH1	-	3	-	-	-	-
Dried celery, parsley	1660	3-*n*-butylphthalide	A	PH2	-	5	5	-	-	-
Dried celery	1676	(*Z*-butylidenephthalide	A	PH3	-	-	-	4	-	-
Dried celery	1698	*cis*-ligustilide	B, C		5	-	6	4	5	5
Fresh celery	1709	*(E)*-butylidenephthalide	B, C		7	5	6	-	-	3
Cooked celery	1715	sedanolide	B, C		6	6	6	4	5	5
Celery	1731	sedanenolide	A	PH4	6	7	6	5	5	5
Dried celery	1742	neocnidilide	A	PH5	6	7	5	-	-	-
Celery	1752	(*E*)-ligustilide	A	PH6	-	-	4	7	3	-
Furans										
Caramel, rose, strawberry	1081	furaneol	B, C		7	5	5	6	5	5
Unknowns										
Floral, fruity	608	unknown			-	-	3	-	-	-
Floral	625	unknown			-	-	3	-	-	-
Buttery, dairy	632	unknown			-	-	4	4	4	3
Plastic, green, musty	768	unknown			-	-	4	-	5	3

Fresh lime, citrus	808	unknown			4	-	-	-	-	-
Floral, fruity, green	817	unknown			-	-	4	-	6	3
Pungent, cheese	842	unknown			-	-	5	-	4	-
Lemon, soil	913	unknown			-	-	-	-	5	-
Bread	918	unknown			-	-	-	-	-	3
Mushroom, soil	971	unknown			-	-	6	-	-	-
Smokey	1130	unknown	A	UN3	-	-	-	5	-	-
Woody, floral	1284	unknown	A	UN5	-	-	-	5	6	-
Smoked tomato, musty	1324	unknown			-	5	-	-	-	-
Make-up powder, baked	1401	unknown			-	5	4	-	-	-
Vegetative, woody	1631	unknown			-	5	4	-	-	-
Dried celery	1649	unknown			-	-	5	-	-	-
Fresh celery	1722	unknown			-	6	6	-	5	-
Rotten celery	1765	unknown			-	4	4	-	-	-
Celery	1780	unknown			6	-	4	6	3	-
Celery	1800	unknown			-	-	-	5	3	-
Cooked celery	1816	unknown			5	3	-	-	-	-
Celery	1855	unknown			5	-	-	-	-	-
Total compounds					43	39	77	51	48	31

[a] Linear retention index (LRI) on DB-5 column, calculated from a linear equation between each pair of straight chain n-alkanes C_6-C_{25}. [b] Means of identifying compound (A- Mass Spectrometry B- LRI C- Aroma note recognitions only). [c] Average odour intensity recorded by three assessors recording each maturity in duplicate except line 22 where only one was completed. (scoring scale: weak = 3, medium = 5, strong = 7), - = not detected. [d] Code corresponds to compounds identified in Table 1. [e] Prematurity time-point. [f] Commercial maturity time-point. [g] Post-maturity time-point. An average odour intensity was taken by collecting the average scores from the duplicates of each assessor and dividing by the number of GC/O runs completed for the genotype and maturity. The value of average odour intensity was rounded up/down to the nearest whole number.

Within the samples, 18 monoterpenes, 12 alcohols, 11 aldehydes, ten ketones, nine monoterpenoid alcohols and phthalides and other compounds including esters (acetates and non-acetates) and sesquiterpenes were identified respectively. Out of the 103 odours that were identified, only nine of these compounds appeared in both genotypes and across the three maturities (Table 2). Across these compounds, it can be observed that line 12 had the highest recorded intensity for all of these compounds apart from hexanal and *(E,E)*-2,6-nonadienal. In most the cases, the compounds were at their highest intensity at M1 and started to decrease thereafter, with a subset then showing an increase between M2 and M3. Comparison of LRIs and odour descriptors detected in this experiment against those of authentic standards and what has been reported previously can be observed in Table 6.3.

In M1, 43 and 51 compounds were identified in the two genotypes respectively, with the majority of these compounds being monoterpenes (sabinene, β-pinene, limonene and γ -terpinene) and alcohols (1-hepten-3-ol, 1-octen-3-ol and 1-nonanol), all averaging intensity scores of around five and six (Table 6.2). No sesquiterpenes were not detected in M1 line 12, however, α-copaene and β-selinene were both detected within M1 line 22 at an intensity of five. β-selinene was identified as having a high abundance in GC/MS (Table 6.1) for both line 12 and 22 across all maturities. The absence of these compounds is with agreement with the PCA plots, whereby monoterpenes show a high association with M1 with low sesquiterpene association. Aldehydes (phenylacetaldehyde, (E,E)-2,6- and (E,Z)-2,6-nonadienal), ketones (3-pentanone, 2-hexanone and 3-octen-2-one) were detected to have a high average odour intensity in line 12, contributing cucumber, herbal and green odour notes however, only 2-pentanone was detected in line 22.

Among some of the compounds that were identified with a high average odour intensity, compounds with ‘mushroom’ and ‘earthy’ odours were very much apparent. These included 2- and 3-heptanol, 1-octen-3-ol, sabinene and β-pinene. These mushroom smelling compounds are displayed as key contributors to a M1 celery odour. Out of these compounds, sabinene and β-pinene were identified by the GC/MS and exhibited high abundance at M1. In terms of phthalides, *(E)*-3-butylidenephthalide had an odour intensity of seven at M1 line 12 and yet *(E)*-3-butylidenephthalide was not identified in line 22. Sedanenolide and sedanolide were identified throughout maturity and at a high average odour intensity for both genotypes, reflected in Table 6.1 also.

A study completed by Macleod and Ames (1989) identified *(E)*-3-butylidenephthalide, sedanolide and sedanenolide in supermarket purchased celery using GC/MS and GC/O. *(E)*-3-Butylidenephthalide was identified to have an odour of 'cooked celery', *(E)*-sedanolide and sedanenolide were both identified to have an odour of 'celery' as well as being 'pungent'. Although not identified in line 12, *(E)*-ligustilide appeared to be an important compound for line 22, showing a high average odour intensity at M1 with a gradual decrease to not being detected in M3. Neocnidilide exhibited a consistently high odour intensity across the different maturities in line 12, reaching and average odour intensity of seven at M2 before decreasing to five in M3.

At M2, 39 and 48 compounds were identified in line 12 and 22 respectively. A wide variety of compounds were observed at this time point, including a mixture of monoterpenes, alcohols, aldehydes and phthalides. Key odour descriptors for commercial mature celery include fresh, green, herbal, and earthy. These odours are achieved by compounds such as hexanal, β-pinene and phthalides such as neocnidilide and sedanenolide, all scoring at an intensity five and above (Table 6.2). According to Table 2, the aroma profile of line 22 appeared to be more complex, with more compounds being identified at M2 than line 12 including more alcohols, ketones, esters, and monoterpenes. However, more phthalides were detected in line 12 and at a higher average odour intensity. Therefore, although fewer compounds were identified in line 12 M2, it can be hypothesised that this genotype at commercial maturity had a strong celery aroma due to its high phthalide content, whereas line 22 had more odours that are green, grass-like and earthy. Sedanenolide was detected at its highest average odour intensity here and similar to the results reported in Table 6.1, line 12 reports the highest relative abundance for phthalides when compared to line 22 and is at its highest at M2. Likewise, Kurobayashi et al. (2006) reported sedanenolide, 3-n-butylphthalide, *(E)*- and *(Z)*-sedanolides as having the highest flavour dilution factor upon completion of AEDA. Further stating that odour descriptors of these compounds are similar to the expected celery odour and are more significant contributors to its odour.

Progressing onto M3, line 12 had the highest number of compounds detected here with 77, conversely line 22 had only 31 compounds detected, the lowest number out of all samples analysed. Here, genotypic differences are very apparent, contradicting Figure 6.1 whereby M3 showed to have the fewest differences caused by genotype, whereas Table 6.2 supports the hypothesis that genotype

determines how the crop matures. Correspondingly shown in Table 6.1, the highest number of monoterpenes were identified here and monoterpenoid alcohols such as terpinen-4-ol and (Z)-carveol for line 12. Conversely, these compounds were detected earlier on in maturity in line 22 and not detected at M3, potentially indicating that line 22 was further along maturity that line 12. No odour with an intensity above six was detected for both lines, showing an obvious decline in aroma quality and intensity. L-Carvone was the compound with the highest intensity in M3 line 12 and 22, with herbal, minty and pine odour descriptors.

Only four phthalides were identified with a relatively low odour intensity and compounds such as 3-n-butylphthalide, neocnidilide and *(E)*- ligustilide were not detected at all in line 22 at M3. The absence of these odour active compounds with odour descriptors such as "celery, fresh celery, dried celery" implying that M3 line 22 did not have the mature celery odour that line 12 may have. On the other hand, line 12 M3 shows an abundance of these phthalides as well as unknown compounds that express a range of celery odour descriptors from cooked, dried, and rotten celery.

Within M3, there were compounds present that were not previously detected by the assessors; these include bornyl acetate, β-caryophyllene and carvacrol (line 12). The odour descriptors that were used to describe the compounds present were 'bread', 'woody', 'sweet' and 'starchy'. The sesquiterpene, α-copaene was identified across all maturities for line 22, yet was only detected in line 12 at M3, with odour descriptors including damp, bread, and woody, indicating deterioration in line 22 as an 'off-odour'. On the other hand, these compounds have been reported in previous investigations (Pino, Rosado & Fuentes, 1997; Marongui et al., 2013) and identified in GC/MS (Table 6.1). Compounds with 'starchy' and 'bread' odours imparted a negative odour on the celery and are synthesised at a higher quantity as the vegetable matures. Due to the nature of GC/O, it is not possible to conclude that these compounds were responsible for off-odours within celery. Using sensory analysis to profile these celery maturities alongside this will help give a better indication of flavour defects within the crop.

4516 **Table 6.3** - Odour compounds, LRI values and odour descriptors identified through GC/O and GC/MS
4517

Code[a]	Compound	LRI[b]	LRI[c]	Detected odour[d]	Reported odour[e]	Code	Compound	LRI	LRI	Detected odour	Reported odour
A1	3-methyl-2-buten-1-ol	733	730	Soapy, green, sharp	Fruity, green, lavender	**M13**	*p*-cymene	1098	1099	Floral, herbal, violet	Fresh, citrus, woody
A4	(E)-2-hexenol	867	867	Musty, moss	Green, vegetative, fatty	**M14**	β-thujone	1112	1119	Tomato, spicy	Cedar, spicy, woody
A3	1-hepten-3-ol	889	893	Earthy, mushroom, grass	Oily, green, metallic	**M15**	*p*-mentha-1,5,8-triene	1109	1113	Caramel, honey, floral	Roasted
A7	nonanol	1174	1176	Metallic, sweaty	Fatty, rose, wet	**M16**	citronellal	1166	1159	Floral, musty, green	Sweet, dry, floral
A8	decanol	1274	1272	Tomato, herbal, fatty	Fatty, waxy, floral	**M17**	trans-dihydrocarvone	1195	1195	Make-up powder, herbal, floral	Warm, herbal
AH1	(*E*)-2-pentenal	760	754	Floral, green, waxy	Pungent, green, fruity	**M18**	β-cyclocitral	1231	1232	Floral	Herbal, saffron, rose
AH2	hexanal	801	802	Fresh, green, apple	Fresh, green, fatty	**M19**	L-carvone	1245	1246	Spearmint	Sweet, spearmint, herbal
AH3	(*E*)-2-hexenal	855	855	Garbage, damp	Green, aldhydic, fatty	**M20**	D-carvone	1253	1257	Herbal, pine, minty	Spice, mint, caraway
AH4	(*Z*)-4-heptenal	901	902	Biscuit, bread	Fatty, dairy, milky	**MA1**	linalool	1103	1103	Woody, red fruit	Floral, bois de rois, woody
AH5	n-octanal	1005	1007	Floral, rose, citrus	Waxy, citrus, orange	**MA2**	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	1116	1122	Herbal, cooked	Fresh, minty

AH6	phenylacetaldehyde	1045	1049	Rose, honey, floral	Floral, honey, powdery	**MA6**	pinocarveol	1150	1152	Cucumber, floral, woody	Camphoreous, woody, pine
AH7	2-hydroxybenzaldehyde	1057	1056	Baked, honey, make-up powder	Medicinal, spicy, cinnamon	**MA7**	terpinen-4-ol	1180	1182	Mushroom, earthy, metallic	Woody, earth, musty
AH8	(*E,Z*)-2,6-nonadienal	1159	1156	Woody, moss, cucumber	Green, fatty, cucumber	**MA9**	γ-terpineol	1207	1210	Herbal	Pine, floral, lilac
AH9	(*E,E*)-2,4-nonadienal	1224	1221	Floral, woody	Green, fatty, melon	**MA10**	(*Z*)-carveol	1214	1220	Bread, creamy	Caraway, spicy
K1	3-hexanone	776	779	Waxy, green, plastic	Sweet, fruity, waxy	**MA11**	thymol	1292	1290	Pine, spicy	Herbal, thyme, phenolic
K2	1-octen-3-one	978	978	Metallic, musty	Herbal, mushroom, earthy	**MA12**	carvacrol	1314	1311	Herbal, starchy	Spice, woody, camphor
K3	2-nonanone	1083	1090	Herbal, soil, spicy	Green, weedy, herbal	**MA13**	*(E)*-8-hydroxylinalool	1346	1342	Herbal	-
E2	propyl 3-methylbutanoate	947	947	Make-up powder, floral	Sweet, apple, fruity	**S1**	(+)-cyclosativene	1366	1378	Cucumber skin, fatty	-
E3	bornyl acetate	1305	1297	Herbal, woody	Woody, pine, herbal	**S2**	α-copaene	1390	1389	Damp, bread, woody	Woody, spicy, honey
E5	carveol acetate	1332	1339	Plastic, green, herbal	Green, spearmint, herbal	**S3**	β-caryophyllene	1443	1440	Sweet, earthy	Sweet, woody, spice
E6	hexyl hexanoate	1381	1385	Metallic, damp, musty	Herbal, vegetable, cut-grass	**S4**	α-humulene	1478	1475	Floral, vegetative, woody	Woody
M1	α-thujene	931	932	Pine, minty, floral	Woody, green, herb	**S7**	β-selinene	1495	1505	Floral, rose, woody	Herbal

M3	camphene	959	958	Herbal, citrus, waxy	Woody, herbal, pine	**S9**	α-selinene	1513	1518	Creamy	Amber
M5	sabinene	981	976	Earthy, mushroom, green	Woody, terpene, citrus	**S11**	kessane	1555	1554	Vegetative	-
M6	β-pinene	987	980	Herbal, earthy, woody	Woody, resinous, pine	**PH1**	3-propylidene phthalide	1603	1600	Celery, vegetables	Celery, herbal, lovage
M7	β-myrcene	997	991	Lemon, green, waxy	Terpy, herbaceous, woody	**PH2**	3-*n*-butylphthalide	1660	1658	Dried celery, parsley	Herbal, phenolic, celery
M8	α-terpinene	1025	1018	Musty, camphoreous	Woody, terpene, camphoraceous	**PH3**	(Z)-butylidenephthalide	1676	1685	Dried celery	Herbal, lovage, celery
M10	limonene	1031	1034	Floral, fresh, mint	Citrus, orange, fresh	**PH4**	sedanenolide	1731	1730	Celery	Herbal, celery
M11	γ-terpinene	1062	1063	Waxy, woody, makeup powder	Oily, woody, terpene	**PH5**	neocnidilide	1742	1753	Dried celery	Herbal, celery
M12	terpinolene	1094	1093	Make-up powder, floral, citrus	fresh, woody, sweet	**PH6**	(*E*)-ligustilide	1752	1758	Celery	-

[a]Code refers to compound code from Table 1. [b]LRI of compound detected through GC/O and confirmed through GC/MS analysis, Table 6.2. [c]LRI of compound identified through GC/MS analysis, confirmed through authentic standards, Table 6.1. [d] Aroma descriptors detected by panellists. [e] Aroma descriptors according to GoodScents.com and by assessing authentic compounds.

An indication of degradation of the sample can be observed most clearly in M3, genotype 12 whereby products of terpene oxidation are abundant (Table 6.2). As discussed in chapter 1, the structure of terpenes allows for easy modifications through the addition of functional groups, resulting in new compounds to be generated. Limonene, the most abundant compound within celery was detected at an average odour intensity of 6 at M1 and decreased to 4 once M3 approached and this was also reflected within Table 6.1, whereby the relative abundance decreased from 1068 mg/L to 264 mg/L in genotype 12 and from 581 mg/L to 264 mg/L in genotype 22. Conversely, compounds such as L-Carvone, dihydrocarvone, carveol acetate and carvacrol, examples of oxidative derivatives from limonene increased by M3. Due to the simple hydrocarbon structure of terpenes, they can easily decompose to isoprene and become the precursor for new compounds to form (Bicas, Dionísio & Pastore, 2009). Furthermore, there is a significant increase in the number of alcohols, aldehydes and ketone compounds identified in the post-mature celery (Tables 6.1 and 6.2), this is most likely due to lipid oxidation and degradation. Fatty acids such as linoleic and linolenic acid are susceptible to oxidation, this will cause the commencement of the lipoxygenase pathway, synthesising compounds such as hexanal and (E)-2-hexanal. This process explains the decrease in monoterpenes observed over time (Tables 6.1 and 6.2) but the increase of oxidative derivatives as well as ketones, aldehydes and alcohols.

Overall, comparing the odours between the three maturity stages and the two genotypes, it was observed that the most odours were identified in line 12 at M3, and a high average odour intensity compared to line 22 and other maturity stages. Despite M2 line 12 expressing a lower number of odours in comparison to M3 line 22, the average odour intensities of these compounds were much higher, particularly for phthalide compounds. From the results displayed, M2 line 12 had a much more distinct odour profile than line 22 and as line 12 matured, it remained aromatic, therefore, having a better field holding capacity and exhibiting a slow bolting trait.

In terms of aroma development, M1 exhibited a high proportion of monoterpenes and alcohols contributing to a fresh, fruity and citrus odour and low intensities of phthalides. The intensity of phthalides increased to M2, whereby a more typical celery odour was observed. Together with monoterpenes, aldehydes, sesquiterpenes and phthalides, the celery odour was present along with subtle floral, woody, and herbal notes, whilst remaining fresh and green. As the crop developed beyond

commercial maturity these fresh, green notes were at their minimum or not detected. At this stage, the aroma profile was much more herbal and woodier.

Together with 3-*n*-butylphthalide and sedanenolide, neocnidilide can be considered an important compound to the aroma in celery. Although identified in Table 6.1 at a lower relative abundance, neocnidilide scored a high average odour intensity scored across line 12 in all maturities (Table 6.2). This is supported by Marongiu et al., (2013), who identified neocnidilide at high abundance across four celery extracts using two varieties grown in Portugal and Spain, extracted using supercritical carbon dioxide extraction as well as hydrodistillation. Despite the two different extraction methods yielding different results, neocnidilide comprised most of the aroma profile of both varieties and extraction methods. Furthermore, Shojaei Ebrahimi & Salimi (2011) identified *(E)*-3-butylidenephthalide and *(Z)*-ligustilide as key phthalides in wild celery, as reflected correspondingly by the GC/O data, whereby these two compounds were scored at a high intensity for line 12 across all maturities. Ligustilide was only identified in M3 for line 12 but more apparent in line 22 (Table 6.2).

Interestingly, the compound phenylacetaldehyde, with a characteristic odour of honey, floral and rose, was found at high abundance in M1 line 22 on the GC/MS data and remained high across maturity. A similar observation was made with line 12, albeit at a lower abundance. Conversely on the GC/O, phenylacetaldehyde was detected in both genotypes across three maturities, with M1 line 12 exhibiting a stronger average odour intensity. Though not commonly identified in *A. graveolens*, Shojaei et al. (2011) identified phenylacetaldehyde in three ecotypes of wild celery grown in three regions of Iran (0.13 %, 0.03 % and 0.08 % respectively) using GC/MS on essential oil.

As there have been limited studies investigating the development of celery aroma over maturity and that combine both GC/MS and GC/O analytical techniques to investigate celery aroma, comparison with other datasets is difficult. Therefore, studies that have used GC/O or GC/MS separately have been utilised. Although commonly used, SPME may not be able to extract all the compounds present in the isolate due to the low concentrations of some flavour compounds (Lui, Su & Song, 2018). SAFE, as used by Kurobayashi et al. (2006), combined with GC/O, AEDA and sensory profiling would give a more representative aroma profile. Using a method such as AEDA allows for the detection of further compounds that were identified in GC/MS. Due to the abundance of limonene within celery (Table 6.1)

and the multiple terpene compounds that co-elute with limonene (Table 6.2), the likelihood of assessors missing or not detecting these compounds are high during GC/O. Although multiple training sessions were completed prior to GC/O, the ability for the assessor to separate and determine these compounds presents difficulties and therefore, only compounds with the lowest odour thresholds are detected. Carrying out various dilutions through AEDA will lead to the detection of compounds with higher odour thresholds that would have been otherwise masked by limonene, building a broadened profile of celery aroma. Furthermore, harvesting vegetable crops at more time points leading up to and after commercial maturity will help to assess the changes in the volatiles profile further. Exploiting different seasons, geographical locations with diverse climates and using different cultivars would help build a better understanding on how celery aroma develops and how is influenced by the various factors.

6.6. Conclusion

Out of the two genotypes that were used in this experiment, line 12 exhibited a higher abundance for most volatile compounds as well as more odours present when observing the GC/O data. The abundance of these compounds indicated that this genotype may have a more distinctive and complex aroma profile with green, herbal, and floral notes along with strong celery notes, contributed from the high abundance of phthalides detected. In contrast, line 22 indicated a more subtle aroma, more similar to cucumber during maturity, but as the crop developed, there was a bigger change in aroma than seen in line 12, with odours developing that suggested a decline in quality. The stability of line 12 in this study shows that genotype influences field holding capacity.

Monoterpenes contributed to the fresh, piney, and earthy notes and were more abundant at prematurity and commercial maturity. The woodier and herbal notes developed as the crop matured and compounds such as sesquiterpenes, monoterpenoid alcohols and most importantly, phthalides were the main contributors to this aroma. Phthalides have been shown in this study, as well as in a plethora of other experiments, to be significant contributors to celery aroma with high relative abundances identified by GC/MS and high average odour intensities from the GC/O; with odour descriptors including ‘celery’ and ‘herbal’.

According to the data presented, the development of the aroma profile of *A. graveolens* changed over time; it commenced as fresh and fruity, progressed to herbal, woody, and celery at commercial maturity, and shifted completely away from fresh and fruity towards woody, floral and damp odours at post-maturity. In order to confirm this, the addition of sensory profiling and more sensitive methods of chemical analysis are required. As shown in this study, developmental maturity has a bigger influence over aroma than genotype. However, genotype determined the way in which the flavour profile developed either through driving the synthesis of new compounds, reducing the synthesis of existing compounds, or driving the degradation of existing compounds.

These insights, especially when combined with future consumer preference studies, will provide celery growers with desirable aroma profile targets that will ensure that the crop is harvested at the optimum developmental stage. Growers should avoid taking a late harvest, even though this may improve yield, since the organoleptic profile of the crop will be compromised as overmature celery exhibit odours of lower intensity and compounds that may distort the flavour profile. This information will be useful to guide breeders to develop varieties that maintain an optimal aroma profile over a longer growing period. Furthermore, celery breeders now have access to biochemical information to assist breeding programmes and develop genotypes with improved field holding capacity which retain desirable aroma profiles.

6.7. References

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CHAPTER 7: Consumer acceptability and sensory profile of three new celery (*Apium graveolens*) hybrids and their parental genotypes

7.1 Introduction to Paper: (Published to International Journal of Molecular Sciences special issue: Breeding Next Generation Vegetables: Improving Flavour and Functional Quality

Previous chapters identified factors such as temperature, dew point, maturity, geographical location, field placement and genotype to significantly influence the aroma composition in eight celery genotypes and their sensory profiles. Combining all the data collected throughout this project, a multisite and multiyear experiment is formed where we have investigated the aroma profile of UK grown celery across three years (2017, 2018 and 2020) and compared with Spanish grown celery (2019) where we also investigated growing in two different locations in Spain (Cartagena and Águilas). Deviations in the aroma composition was observed across all variables which led to significant differences in the perceived sensory profile however, it has been confirmed that monoterpenes, sesquiterpenes and phthalides comprise most of the aroma composition of celery and these compound groups were identified throughout the project, regardless of genotype, location, and harvest year, in addition to confirming the importance of these compounds to the celery aroma.

The celery used throughout this project were grown in commercial conditions with celery destined for consumption, they were subject to the same conditions including agronomic applications (fertilizers, irrigation) and transportation methods from Spain to UK. As the eight genotypes used in this project all displayed significant differences in these different environments, the commercial celery grown in these environments will also express significant differences, therefore, the consumer will be purchasing a product that is inconsistent in quality. We identified three genotypes that displayed consistencies in how they performed, regardless of location and environment of growth, genotypes 12, 22 and 25. Genotype 12 remained a “high extreme” throughout the project, expressing a high abundance of volatile compounds and scored as the most bitter tasting with a strong rocket, green aroma, and flavour. This genotype also displayed prominent ribs that was accompanied with the stringiest mouthfeel in addition to being dark green and pink in colour. On the other hand, genotype 25 remained a “low extreme” throughout the project, expressing a low abundance of volatile compounds and scored

as the most sweet tasting with high scores for fresh cucumber flavour. The mouthfeel of this genotype was moist and crunchy and was white in colour. The relative abundance of volatile compounds in genotype 22 always remained below genotype 12 and above genotype 25 and was scored to be closely associated to fresh fennel aroma and flavour. The mouthfeel for this genotype was scored in a similar manner to genotype 25, high scoring in crunchy and moist but also green in colour and less prominent ribs compared to genotype 12. These genotypes, along with the five other genotypes used throughout this study were genetically crossed with all other genotypes twice, to ensure we had each genotype as the mother and father, in summer 2018. These were then "selfed" to produce an F1 generation in 2019 and then again in 2020 to produce an F2 population which was then sown in late-Autumn in Spain 2020 for harvest in Easter 2021.

By presenting these hybrids and their parents to a trained sensory panel as well as completing GC/MS analysis, we can observe any maternal or paternal inheritance occurring which will provide a better understand on celery inheritance in general. Furthermore, using the same genotypes and hybrids in a consumer acceptance and preference trial, we can identify whether we have been successful in developing new celery hybrid lines that meet consumer demands. This experiment is the first of its kind as no consumer trial has been completed on celery and from this we can identify (1) what the consumer wants in their celery (2) the inheritance patterns in celery and (3) whether we can use instrumental analysis linked with sensory profiling to develop new celery hybrid lines according to their secondary metabolite profile.

Sections 7.2 – 7.7 have been published in International Journal of Molecular Sciences. (See Appendix XIV for submitted manuscript)

7.2 Abstract

Celery is a stalky green vegetable that is grown and consumed globally and used in many cuisines for its distinctive taste and flavour. Previous investigations identified the aroma composition of celery and profiled its sensory characteristics using a trained panel; however, evaluation of the sensory characteristics of celery combined with a consumer panel, where consumer preferences and

acceptability are determined, is novel. In this study, three parental genotypes (12, 22 and 25) and three new hybrids (12x22, 22x12 and 25x12) were presented to a trained sensory panel (n = 12) for profiling and a consumer panel (n = 118), where liking and preference were assessed. Celery samples were analysed by SPME GC–MS and significant differences in aroma composition between all samples were identified, causing significant differences in the sensory profile. Furthermore, significant differences in attributes assessed for liking (appearance, aroma, texture and overall) were identified. Consumer segmentation identified three groups of consumers exhibiting differences in the hedonic reaction to the samples. Sweet and bitter taste along with overall flavour were identified as drivers of liking. Hybrid 25x12 was found to be the hybrid that exhibited high intensities for most of the attributes assessed.

7.3. Introduction

Celery is an aromatic vegetable that is grown and consumed globally in a range of salads, with condiments; in cooking, where it can be boiled, fried, roasted as well as forming the base of many soups, stocks, and sauces (Rożek, 2007; Malhotra, 2021; Turner, Lignou, Gawthrop & Wagstaff, 2021a). Within cuisines, celery is known to form part of the holy trinity and soffrito (Turner et al., 2021a), starring alongside carrots and onions or onions and bell peppers depending on the cuisine. Celery owes its culinary diversity to the distinct aroma and flavour profile it possesses, with a range of compounds including terpenes (monoterpenes and sesquiterpenes), alcohols, aldehydes and phthalides contributing to the overall flavour quality of celery (Turner et al., 2021a; Uhlig, Chang & Jen, 1987; Orav, Kailas & Jegorova, 2003; Sellami, Bettaieb, Bourgou, Dahmani, Limam & Marzouk, 2012; Macleod & Ames, 1989). The phthalide compounds have been established as the characteristic odorants of celery, with odour descriptors such as ‘celery’, ‘cooked celery’ and ‘herbal’. Without the presence of these compounds, celery aroma would not be so distinctive (Macleod & Ames, 1989; Turner, Dawda, Wagstaff, Lignou, 2021b).

Being such a commonly grown and consumed vegetable, the research investigating the perception of celery flavour is surprisingly small, with only few sources investigating the sensory properties of celery (Turner et al. 2021b; Yommi et al. 2013; Raffo et al. 2006; Turner, Lignou, Gawthrop & Wagstaff, 2021c; Turner, Lignou, Gawthrop & Wagstaff, 2021d). Furthermore, there has

been no research conducted that explores the sensory characteristics of celery combined with consumers' perceptions and preferences. Previous research has identified that external characteristics such as product appearance are primary influencers of initial consumer purchase, whilst internal characteristics that follow consumption (aroma, taste, flavour, texture) influence acceptability and repurchase (Francis et al., 2012; Caracciolo et al., 2020; Cavallo, Caracciolo, Cicia & Del Giudice, 2018). Without completing sensory and consumer evaluation, the acceptability of celery and the sensory characteristics that consumers find desirable within celery remain unknown and crop breeding programmes are missing key information that should direct their selection processes.

The authors have previously carried out several experiments, where they identified the aroma profile of various celery genotypes and investigated how factors such as genotype, maturity, geographical location, climate, and agronomy influence the aroma profile and the sensory characteristics using a trained panel (Turner et al., 2021b; Turner et al. 2021c). Combining data from instrumental and sensory analysis with multi-site and multi-year investigations that use the same eight genotypes has led to the discovery of three genotypes that consistently performed regardless of influencing environmental or developmental factors; genotypes 12, 22 and 25. Genotype 12 was consistently high in the abundance of volatile compounds with a high percentage of phthalides comprising the aroma profile of celery with a strong, typical celery odour. The trained panel strongly associated this genotype with a grass odour and herbal flavour, including fennel, parsley, and coriander (Turner et al., 2021b; Turner et al. 2021c). On the other hand, genotype 25 exhibited low abundance of phthalides and a high abundance of aldehydes, with the trained panel describing this genotype as having a cucumber flavour. Genotype 22 had similar aroma profile to genotype 12 but with lower abundance and was scored lower by the trained panel for aroma and flavour attributes such as fresh parsley, coriander, and fennel. In terms of mouthfeel, genotype 22 was consistently scored high for a moist and crunchy petiole and low for stringy mouthfeel, opposing genotype 12. Genotype 12 was ribbed, stringy and bitter, genotypes 22 and 25 remained crunchy, moist with minimal stringiness (Turner et al., 2021c).

Providing celery growers and breeders with the information gathered from this investigation will aid in the development of new celery hybrids that have been tailor-made according to consumer preference. The aim of this study was to evaluate the sensory characteristics of celery parental genotypes

(12, 22 and 25) and their hybrids (12x22, 25x12, 22x12) using a trained sensory panel and to assess the aroma profile of the same samples using solid phase microextraction gas chromatography/mass spectrometry (SPME GC/MS) to identify differences and similarities within the aroma profile. Consumer evaluation was also conducted to understand the acceptability, liking and preference of these genotypes and hybrids and to associate sensory and biochemical composition with these desirable characteristics.

7.4. Materials and Methods

7.4.1. Celery material and MIAPAE standard

7.4.1.1. Sample Information

The three parental genotypes used in this experiment were chosen due to their differences in physical and chemical attributes and the original genetic crosses of the hybrid were carried out in 2018 at Tozer Seeds Ltd (Pyports, United Kingdom). Although commercial confidentiality precludes revealing the exact genetic identity of each genotype used in this paper, the origins of the parental breeding lines and their image postharvest can be found in Figure 7.1.

7.4.1.2. Timing, Location and Environment

Celery seed (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd (Cobham, United Kingdom) were grown in commercial conditions and harvested in El Albujon, Murcia, Spain 2021 (37°43'05.5"N 1°03'24.3"W). Plugs were transplanted after 56 days growing in a nursery and then harvested 113 days later. Plants were lifted, packed, and despatched on the same day. Average daily air temperature was 17.7 °C with 1.0 mm average daily rainfall, average relative humidity was 81.5 % with an average daily speed of 6.3 m/s.

4830 **Figure 7.1:** Images of the petioles of the six celery samples used in the experiment

4831

	Samples					
Genotype	12	22	25	12x22	22x12	25x12
Origin	UK	USA	EU	-	-	-
Appearance						

7.4.1.3. Raw material collection, processing, and storage

The celery was grown in three randomised blocks in the centre of the field to reduce any influence from edge effects at a density of 10 plants m^{-2} and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles and sealed in labelled freezer bags with freezer blocks for transportation to the UK. A courier to the Netherlands, followed by air freight to London, Heathrow (United Kingdom) allowed celery to be at the University of Reading two days post-harvest. Celery samples used for sensory evaluation and the consumer trial were refrigerated for two days and were kept in refrigerated conditions prior to panel and volunteer collection. Samples for aroma analysis were refrigerated for two days before analysis. Panel and consumer tasting occurred on the same day as aroma analysis (P + 4).

7.4.2. Chemical Reagents

For GC/MS analysis, calcium chloride and the alkane standard C6-C25 (100 µg/mL) in diethyl ether were obtained from Merck (Poole, UK).

7.4.3. Volatile analysis using SPME GCMS

The fresh celery sample (2 g) was combined with 0.5 mL of saturated calcium chloride solution in a 15 mL SPME vial fitted with a screw cap lid and 50 µl of propyl propanoate (100 mg/L). Samples were analysed by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA) with a DB5 column (30 m x 0.25 mm x 0.25 µm) from Agilent (Palo Alto, CA, US). The SPME fibre stationary phase was composed of 75 µm divinylbenzene/Carboxen™ on polydimethylsiloxane; Supelco, (Bellefonte, PA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column DB5 (30 m x 250 µm x 0.25 µm thickness) (Agilent, Santa Clara, CA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C, and 6 min at 250 °C isothermal. Helium was used as

the carrier gas at a flow rate of 1.2 mL/min. The temperature of injector, interface and detector was 250 °C and the sample injection mode was splitless. Mass spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 *m/z*, and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading), or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C_6–C_{25} *n*-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions. The approximate quantification (mg/L) of volatiles collected from the headspace were calculated from GC peak areas, by comparison with the peak area of the propyl propanoate standard.

7.4.4. Sensory profiling

Sensory evaluation was carried out using quantitative descriptive analysis (QDA^{TM}) to determine the sensory characteristics of the celery samples and the characteristics were estimated quantitatively according to Turner et al (2021b;2021c)

7.4.5. Consumer evaluation

One hundred and eighteen volunteers were recruited across the University of Reading (male and female, aged 18 years and above, non-smokers and without allergies or intolerances to wheat, gluten and/or celery). The study was performed as an at home study due to ongoing COVID-19 restrictions, complying with social distancing and COVID-19 guidelines, as well as risk assessments in place. The study was fully explained to the volunteers and their informed written consent was obtained prior to participation. Participants collected their samples from the Sensory Science Centre (University of Reading) along with palate cleanser (crackers) and other information regarding how to access the study online. Participants were asked to complete the study within 24 hours and keep the samples refrigerated until ready to begin the test. Participants were asked, after observing the samples, to rate their liking

(appearance, aroma, taste, texture and overall) on a 9-point hedonic scale (where 1: dislike extremely, 5: neither like nor dislike, 9: like extremely) for all samples. They also indicated the appropriateness of attribute level on a 5-point Just-About-Right (JAR) scale for the following attributes: aroma intensity, bitterness, sweetness, flavour intensity and stringiness (where 1: much too low, 3: JAR and 5: much too strong). Participants were asked to indicate their preference for the hybrid genotypes only (25x12, 22x12 and 12x22) and rank various celery characteristics such as smooth exterior, moist texture, crunchy texture, sweet taste, bitter taste, and strong aroma (from most important to least important). Finally, participants were asked a series of demographic questions, purchase intent and celery consumption and were given the opportunity to leave additional comments after evaluating each sample if they wanted to. In total, six samples were evaluated (three parental genotypes and three celery hybrids in one session). Samples were presented to participants in a monadic balanced order using Williams design, with sample sets randomly assigned to consumers. Data was collected using Compusense Cloud Software. The School of Chemistry, Food and Pharmacy Research Ethics Committee (SREC) provided a favourable opinion for conduct (SREC 11/2021) and the study was conducted in March 2021.

7.4.6. Statistical analysis

Raw data collected from the SPME GCMS was calculated into relative abundance according to the internal standard. The semi-quantitative data was then analysed by one-way analysis of variance (ANOVA) and principal component analysis (PCA) using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($P<0.05$) between the celery genotypes. Only those compounds exhibiting significant differences between genotype were included in the principal component analysis. To compose the PCA plots that combine both sensory and instrumental data, the volatile data was added as supplementary data on top of the flavour and aroma attributes.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out ANOVA of sensory panel data where the main effects (sample and assessor) were tested against the sample by assessor interaction with sample as a fixed effect and assessor as a random effect. The means from sensory data were taken

over assessors and correlated with the relative abundance means from the instrumental data via PCA using XLSTAT. Internal preference mapping was used to relate sensory characteristics of celery samples to consumer liking data XLSTAT was used to carry out the following analyses: (i) PCA of the volatile and sensory panel data, (ii) one-way ANOVA for the aroma analysis and consumer liking (ii) analysis of the preference (ranking) data using Friedman's test; (iii) agglomerative hierarchical clustering (AHC) for overall liking, (iv) penalty analysis of the JAR data and (v) internal preference mapping. In more detail, for the AHC, dissimilarity of responses was determined by Euclidean distance, and agglomeration using Ward's method (set to automatic truncation). For the penalty analysis, the influence of consumer perception of appropriateness of attribute level rating (JAR) on consumer liking was evaluated by calculating the mean drop in liking rating (scale 1–9) compared with mean liking of consumers that rated the attribute as JAR (JAR 3 on a 1–5 scale), determining whether this drop in liking score was significant.

7.5. Results and Discussion

7.5.1. Volatile composition

In total, 100 compounds were identified in the headspace of the six celery samples (Table 1) including 28 monoterpenes, 16 sesquiterpenes, 12 alcohols (five of which are classified as monoterpenoid alcohols), nine aldehydes and five phthalides. Quantitative differences were observed between the genotypes used in this study and one-way ANOVA revealed significant differences in the relative abundance of aroma compounds between the genotypes in most compounds. Compounds such as (E)-2-penten-1-ol, (Z)-3-hexenol, lavandulyl acetate, δ-3-carene, β-thujone, p-1,3,8-menthatriene, fenchol and β-eudesmol expressed no significant difference between genotypes accompanied by several alkanes and unknown compounds.

A large proportion of the aroma profile was comprised of monoterpenes and sesquiterpenes with limonene, β-pinene, myrcene, γ-terpinene and β-caryophyllene exhibiting the highest relative abundance within their compound groups. These compounds are commonly present in celery and have been reported to contribute to odour notes such as woody, herbal, green, waxy, and earthy (Turner et al. 2021a; Turner et al. 2021b). Monoterpenes have been shown to have the highest proportion of the

aroma composition in various studies (Turner et al. 2021a; Orav et al. 2003; Sellami et al. 2012). Genotype 12 exhibited the highest abundance of monoterpenes, sesquiterpenes and phthalides, followed by the hybrid 22x12 and 12x22, while genotype 25 and 25x12 had a much lower abundance of these compounds. However, as reported by the authors, these terpenes are not the characteristic compounds in celery (Uhlig et al, 1987).

Sesquiterpenes, whilst at a lower relative abundance to monoterpenes are more typical to the mature celery aroma. Previously reported by the authors (Turner et al. 2021b), during maturation, the celery aroma developed significantly, starting as a fresh, citrus, green aroma due to the high proportion of monoterpenes and lack of sesquiterpene and phthalide compounds. As the celery matured, the abundance of sesquiterpenes and phthalides became much more apparent and thus, a change in the perceived aroma was identified (Turner et al. 2021b). β-Caryophyllene and β-selinene (Table 7.1) exhibited the highest relative abundance within all genotypes, and this was most obviously observed in genotype 12 and hybrid 22x12. Ehiabhi et al. (2006) reported β-caryophyllene and β-selinene to be major constituents of Nigerian grown celery and Lund, Wagner, and Bryan (1973) identified β-selinene to impart a strong celery aroma. Although less abundant in other genotypes, genotype 12 had a high abundance of kessane. Kessane was identified by Philippe, Suvarnalatha, Sankar & Suresh (2002) in the essential oil of Indian-grown celery seed, comprising between 2.2-7.6 % of the volatile profile.

4961 **Table 7.1.** Relative abundance of aroma compounds identified in the headspace of fresh celery samples

				Relative abundance (mg/L)[c]						
Code	Compound name	LRI[a]	ID[b]	12	22	25	25x12	12x22	22x12	p-value
	Alcohols									
A1	(*E*)-2-penten-1-ol	758	A	nd	0.53±0.74	0.43±0.05	nd	nd	0.83±0.09	ns
A2	pentanol	762	A	nd^{b}	nd^{b}	nd^{b}	0.48±0.14^{ab}	0.68±0.33^{a}	0.15±0.21^{ab}	**
A3	(Z)-3-hexenol	849	B[1]	4.1±2.5^{a}	4.1±1.7	nd	2.0±0.47	4.3±1.1	1.2±0.18	ns
A4	(E)-3-hexenol	852	A	6.2±2.9^{a}	3.5±1.8^{ab}	1.3±0.26^{b}	nd^{b}	3.7±0.53^{ab}	0.69±0.49^{b}	*
A5	hexanol	862	A	nd^{b}	nd^{b}	0.53±0.03^{b}	0.65±0.04^{b}	3.0±0.98^{a}	3.6±1.1^{a}	***
A6	octanol	1072	A	4.9±0.70^{ab}	5.3±0.61^{a}	1.3±0.13^{cd}	nd^{d}	2.9±1.2^{bc}	3.8±0.36^{ab}	***
A7	(Z)-3-nonenol	1153	B[2]	5.6±2.9	6.1±2.6	1.8±0.81	1.3±0.16	6.9±1.7	5.9±0.98	*
	Aldehydes									
AL1	hexanal	800	A	9.23±0.33^{ab}	0.43±0.06^{b}	0.15±0.12^{b}	0.30±0.05^{b}	0.46±0.31^{b}	91±18^{a}	***
AL2	benzaldehyde	964	A	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.24±0.04^{a}	nd^{b}	***
AL3	octanal	1008	A	7.6±1.4^{ab}	9.5±2.4^{a}	3.6±0.62^{bc}	2.4±0.58^{c}	5.3±1.3^{abc}	9.4±1.1^{a}	**
AL4	phenylacetaldehyde	1058	A	6.4±1.3^{a}	6.5±2.4^{a}	1.9±0.25^{bc}	0.96±0.43^{c}	3.7±1.6^{abc}	5.2±0.60^{ab}	**
AL5	m-tolualdehyde	1083	B[3]	nd^{b}	19±2.4^{a}	nd^{b}	nd^{b}	nd^{b}	16±1.2^{a}	***
AL6	(*E*,*E*)-2,4-octadienal	1116	A	2.0±1.1^{b}	nd^{b}	nd^{b}	nd^{b}	1.6±0.57^{b}	4.2±0.72^{a}	***
AL7	(*E*,*E*)-2,6-nonadienal	1155	A	2.3±1.6	nd	nd	0.39±0.55	nd	nd	*
AL8	(E)-2-nonenal	1171	A	3.2±0.44^{a}	2.7±0.46^{a}	0.69±0.09^{b}	0.89±0.14b	0.69±0.97^{b}	1.8±0.07^{ab}	***
AL9	undecanal	1306		nd^{c}	nd^{c}	0.93±0.28^{bc}	1.4±0.35^{bc}	1.6±0.44^{b}	3.8±0.79^{a}	***
	Esters									
E1	allyl hexanoate	1080	A	3.9±0.62^{ab}	nd^{c}	2.0±0.43^{bc}	1.2±0.92^{bc}	3.1±0.96^{ab}	6.0±1.5^{a}	***

E2	(E,Z)-3,6 nonadienol acetate	1174	B[4]	4.4±0.45^{a}	2.2±0.49^{bc}	1.0±0.12^{c}	1.5±0.15^{c}	2.2±0.41^{bc}	3.3±0.48^{ab}	***
E3	(Z)-3-hexenyl butanoate	1185	A	2.5±0.23^{b}	2.6±0.10^{b}	nd^{d}	nd^{d}	1.3±0.45^{c}	4.5±0.54^{a}	***
E4	lavandulyl acetate	1285	B[5]	0.34±0.48	0.72±0.20	0.15±0.22	0.64±0.14	0.15±0.22	1.1±0.79	ns
	Ketones									
K1	acetophenone	1077	A	8.4±1.1^{a}	nd^{b}	1.8±0.26^{b}	0.68±0.35^{b}	8.2±0.86^{a}	14±1.5^{a}	***
K2	(Z)-jasmone	1405	A	2.3±0.38^{a}	0.24±0.33^{c}	0.48±0.04^{bc}	0.10±0.15^{c}	nd^{c}	0.99±0.05^{b}	***
	Alkanes									
AK1	nonane	897	A	17±2.8^{b}	46±1.9^{a}	8.4±1.5^{b}	19±1.1^{b}	21±1.6^{b}	52±11^{a}	***
AK2	decane	998	A	nd^{c}	10±3.5^{ab}	4.9±0.93^{bc}	5.0±0.93^{bc}	6.3±3.2^{bc}	14±1.3^{a}	***
AK3	undecane	1097	A	27±9.6	23±11.2	10±2.1	9.3±1.9	12±4.1	22±5.1	ns
AK4	dodecane	1197	A	14±9.6	6.3±3.6	1.5±0.65	2.9±0.85	4.5±1.2	6.8±0.60	ns
AK5	tridecane	1297	A	18±1.2	4.0±3.8	1.1±0.20	1.1±0.92	1.7±1.3	1.9±1.2	ns
AK6	tetradecane	1397	A	40±1.5	9.5±7.9	3.2±1.8	2.7±2.0	4.6±3.5	5.5±2.8	ns
AK7	pentadecane	1498	A	35±9.1	9.3±6.1	3.3±0.84	3.3±1.9	6.0±3.9	3.2±2.3	ns
AK8	hexadecane	1599	A	17±11	4.6±2.2	1.7±0.71	1.8±0.84	3.4±1.8	4.0±1.3	ns
AK9	heptadecane	1699	A	8.2±2.6^{a}	2.3±0.49^{b}	0.99±0.08^{b}	1.0±0.20^{b}	2.2±1.1^{b}	2.8±0.13^{b}	***
AK10	octadecane	1800	A	nd	0.76±0.20	0.13±0.19	0.25±0.19	0.32±0.45	0.75±0.17	*
	Monoterpenes									
M1	α-thujene	932	B[6]	10±1.8^{a}	4.8±0.42^{b}	2.7±0.39^{b}	3.7±0.49^{b}	4.2±0.49^{b}	5.0±0.45^{b}	***
M2	α-pinene	941	A	22±2.9^{a}	24±2.1^{a}	6.2±0.97^{b}	8.5±0.80^{b}	19±1.8^{a}	20±2.8^{a}	***
M3	camphene	958	A	5.6±0.59^{a}	6.0±1.3^{a}	2.0±0.13^{b}	2.5±0.25^{b}	4.3±0.46^{ab}	5.4±0.81^{a}	***
M4	sabinene	980	A	34±5.5^{a}	18±5.9^{b}	5.8±1.1^{b}	8.7±1.3^{b}	12±1.1^{b}	19±6.8	**

M5	β-pinene	987	A	110±15^{ab}	122±23^{ab}	70±12^{b}	86±12^{b}	120±8.2^{ab}	145±23^{a}	**
M6	myrcene	990	A	799±67^{a}	100±9.0^{bcd}	42±4.4^{d}	59±7.7^{cd}	149±24^{bc}	173±25^{b}	***
M7	p-mentha-2,8-diene	1005	B[7]	2.5±1.1	5.2±0.89	nd	nd	3.3±1.1	4.3±0.64	*
M8	α-phellandrene	1013	A	19±2.6^{a}	14±2.6^{ab}	6.3±0.87^{c}	5.5±1.1^{c}	9.6±2.1^{bc}	17±0.80^{a}	***
M9	delta-3-carene	1019	A	1.2±1.6	nd	nd	0.82±0.19	nd	nd	ns
M10	α-terpinene	1024	A	30±5.6^{a}	14±1.9^{b}	8.0±0.89^{b}	11±3.0^{b}	8.1±2.7^{b}	14±2.4^{b}	***
M11	ortho-cymene	1030	A	469±11^{a}	190±22de	128±20^{e}	213±0.16^{cd}	299±37^{b}	267±14^{bc}	***
M12	limonene	1037	A	6524±207^{a}	3259±236^{b}	1188±89^{d}	1285±84^{d}	2371±246^{c}	3638±441^{b}	***
M13	β-(E)-ocimene	1048	B[8]	54±6.2^{a}	63±2.3^{a}	13±0.89^{c}	5.1±0.95^{c}	34±8.6^{b}	45±7.2^{ab}	***
M14	γ-terpinene	1065	A	1455±112^{a}	732±127^{b}	329±39^{c}	539±96^{bc}	389±89^{bc}	689±179^{bc}	***
M15	*p*-cymenene	1095	A	nd^{b}	19±2.6^{a}	nd^{b}	nd^{b}	nd^{b}	7.0±9.9^{ab}	**
M16	terpinolene	1096	A	38±4.6^{a}	nd^{c}	7.0±0.48^{bc}	6.5±1.0^{bc}	14±3.9^{b}	11±7.6^{bc}	***
M17	β-thujone	1119	A	1.9±1.3	0.58±0.82	0.45±0.32	0.13±0.18	nd	nd	ns
M18	allo-ocimene	1130	B[9]	150±16^{ab}	177±13^{a}	30±3.2^{c}	9.2±0.74^{c}	106±20^{b}	144±17^{ab}	***
M19	*p*-1,3,8 menthatriene	1134	B[10]	6.2±8.7	11±7.7	2.4±1.7	1.2±0.05	13±2.0	8.7±6.1	ns
M20	trans-allo-ocimene	1144	B[11]	81±5.9^{a}	79±8.6^{a}	20±2.3^{bc}	12±2.9^{c}	42±11^{b}	78±11^{a}	***
M21	camphor	1157	A	nd^{c}	2.2±0.16^{b}	nd^{c}	nd^{c}	1.9±0.39^{b}	3.2±0.28^{a}	***
M22	pentylcyclohexa-1,3-diene	1161	B[12]	3.3±0.64^{b}	5.4±1.2^{b}	16±1.1^{ab}	17±2.0^{ab}	56±13^{a}	25±7.1^{ab}	*
M23	cis-dihydrocarvone	1206	A	4.1±0.95^{a}	1.9±0.41^{b}	1.3±0.86^{b}	0.91±0.19^{b}	1.9±0.34^{b}	2.7±0.32^{ab}	**
M24	safranal	1215	A	11±2.6^{a}	4.6±0.69^{bc}	1.5±0.63^{c}	2.5±0.68^{c}	2.7±0.98^{c}	7.9±0.44^{ab}	***
M25	β-cyclocitral	1235	A	3.6±0.79^{a}	1.9±0.50^{ab}	0.73±.0.19^{b}	1.0±0.29^{b}	0.81±0.61^{b}	3.5±0.35^{a}	***

M26	L-carvone	1251	A	2.5±0.86^{ab}	2.1±0.57^{ab}	nd^{c}	0.89±0.18^{bc}	1.5±0.39abc	2.9±0.64^{a}	***
M27	D-carvone	1259	A	3.5±0.31	2.9±1.2	1.5±0.51	1.4±0.23	1.7±0.39	3.4±0.77	*
M28	carvacrol	1318	A	nd^{b}	nd^{b}	0.12±0.17^{b}	0.42±0.09^{b}	0.51±0.39^{ab}	1.1±0.15^{a}	**
	Monoterpenoid Alcohols									
MA1	p-mentha-2,8-dien-1-ol	1124	A	5.0±1.1^{a}	5.5±0.35^{a}	0.95±0.17^{b}	0.15±0.21^{b}	4.7±0.97^{a}	4.0±0.15^{a}	***
MA2	fenchol	1127	A	0.55±0.76	nd	nd	0.14±0.19	nd	0.87±0.64	ns
MA3	trans-carveol	1225	B[12]	9.8±4.5^{a}	1.9±0.18^{c}	0.99±0.10^{d}	1.4±0.10^{cd}	1.7±0.13^{c}	3.0±0.26^{b}	***
MA4	cis-carveol	1238	A	3.3±0.10^{a}	2.3±0.18^{a}	0.63±0.48^{b}	0.63±0.18^{b}	0.45±0.63^{b}	2.6±0.16^{a}	***
MA5	(Z)-8-hydroxy linalool	1346	B[12]	2.7±0.43^{a}	0.76±0.08^{c}	0.27±0.19^{c}	0.59±0.14^{c}	0.50±0.37^{c}	1.7±0.12^{b}	***
	Sesquiterpenes									
S1	α-ylangene	1387	B[10]	3.1±1.1^{a}	3.0±0.65^{a}	1.7±0.16^{ab}	0.69±0.09^{b}	1.1±0.39^{b}	1.8±0.17^{ab}	**
S2	α-copaene	1392	A	nd^{e}	9.2±0.11^{a}	6.2±0.18^{b}	2.0±0.18^{d}	1.8±0.30^{d}	4.5±0.43^{c}	***
S3	(E)-β-caryophyllene	1427	B[13]	2.2±0.42^{a}	0.25±0.35^{b}	0.49±0.05^{b}	0.33±0.07^{b}	nd^{b}	0.87±0.68^{b}	**
S4	β-caryophyllene	1442	A	217±9.8^{a}	71±1.3^{c}	60±1.2^{cd}	46±4.5^{d}	44±8.4^{d}	97±11^{b}	***
S5	(+)-aromadendrene	1461	A	2.2±0.10^{ab}	1.2±0.38^{cd}	2.7±0.42^{a}	0.21±0.30^{d}	0.98±0.32^{cd}	1.5±0.14^{bc}	***
S6	curcumene	1470	B[14]	3.3±0.15^{a}	nd^{b}	0.78±0.11^{b}	0.72±0.13^{b}	nd^{b}	0.59±0.83^{b}	***
S7	α-humulene	1477	A	19±1.2^{a}	12±0.69^{b}	4.5±0.10^{c}	6.3±0.66^{c}	6.1±1.3^{c}	11±0.89^{b}	***
S8	γ-himachalene	1493	B[15]	2.8±0.33^{a}	2.1±0.16^{ab}	1.1±0.05^{c}	0.92±0.14^{c}	1.3±0.35^{bc}	2.3±0.19^{a}	***
S9	β-selinene	1511	B[16]	192±14^{a}	31±0.93^{c}	24±0.82^{c}	24±1.9^{c}	29±4.7^{c}	59±4.9b	***
S10	valencene	1515	A	261±31^{a}	3.5±1.5^{b}	3.6±0.16^{b}	1.6±0.16^{b}	34±4.4^{b}	33±2.4^{b}	***
S11	α-selinene	1519	B[17]	22±1.3^{a}	5.4±0.16^{bc}	3.7±0.19^{c}	3.2±0.27^{c}	3.8±0.64^{c}	7.4±0.71^{b}	***

S12	(E)-nerolidol	1540	A	nd^{d}	2.3±0.19^{a}	1.7±0.05^{b}	0.91±0.21^{c}	0.21±0.29^{d}	1.2±0.11^{bc}	***
S13	kessane	1555	B[12]	200±39^{a}	2.3±0.30^{b}	0.51±0.04^{b}	0.51±0.09^{b}	26±3.1^{b}	27±1.9^{b}	***
S14	liguloxide$	1561	B[18]	5.2±0.89^{a}	nd^{b}	nd^{b}	nd^{b}	0.67±0.11^{b}	0.66±0.47^{b}	***
S15	rosifoliol	1588	B[19]	nd^{c}	0.45±0.32^{abc}	0.16±0.23^{bc}	0.70±0.09^{ab}	0.41±0.29^{abc}	0.99±0.04^{a}	**
S16	β-eudesmol	1633	B[20]	nd	nd	nd	0.29±0.19	0.65±0.92	nd	ns
	Oxides									
O1	caryophyllene oxide	1608	A	2.0±0.26^{a}	0.30±0.23^{d}	0.39±0.05^{d}	0.59±0.08^{cd}	1.2±0.02^{bc}	1.7±0.23^{ab}	***
	Phthalides									
P1	3-propylidene phthalide	1603	A	7.7±0.91^{a}	0.87±0.37^{b}	0.54±0.03^{b}	nd^{b}	0.46±0.33^{b}	nd^{b}	***
P2	3-n-butylphthalide	1675	B[21,22,23]	18±7.8^{a}	8.7±2.9^{ab}	3.8±1.3^{b}	3.4±0.70^{b}	13±1.4^{ab}	13±1.7^{ab}	*
P3	sedanenolide	1747	B[21,22,23]	58±4.0^{a}	16±2.9^{c}	5.2±0.50^{d}	4.5±0.35^{d}	25±3.4^{b}	21±2.2^{bc}	***
P4	trans-neocnidilide	1754	B[12]	2.7±0.24^{a}	2.8±0.33^{a}	1.3±0.12^{b}	1.8±0.08^{b}	2.7±0.05^{a}	2.9±0.19^{a}	***
P5	(Z)-ligustilide	1763	B[21,22,23]	4.0±0.49^{a}	0.41±0.08^{b}	0.21±0.08^{b}	0.24±0.04^{b}	1.0±0.79^{b}	0.77±0.10^{b}	***
	Unknowns									
U1	unknown 1	840		2.6±0.79	nd	3.1±0.71	2.0±0.23	nd	4.5±3.5	ns
U2	unknown 2	1076		nd^{b}	19±5.5^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	***
U3	unknown 3	1084		15±2.0^{a}	nd^{b}	nd^{b}	2.7±0.54^{b}	11±3.3^{a}	nd^{b}	***
U4	unknown 4	1141		2.2±0.38^{a}	1.4±0.98^{ab}	nd^{b}	0.30±0.25^{ab}	1.6±0.35^{ab}	1.4±0.98^{ab}	*
U5	unknown 5	1189		1.2±1.7	0.62±0.88	1.2±1.7	0.15±0.21	0.35±0.49	nd	ns
U6	unknown 6	1243		2.4±0.16	2.0±1.1	0.93±0.12	1.2±0.23	2.0±0.37	3.4±1.3	ns
U7	unknown 7	1276		7.3±1.5^{a}	4.1±2.1^{ab}	1.0±0.29^{b}	0.66±0.09^{b}	2.2±0.88^{b}	3.2±0.71^{b}	**

U8	unknown 8	1450		12±3.8[a]	3.3±0.53[b]	nd[b]	2.0±0.34[b]	1.9±0.48[b]	4.3±0.50[b]	***
U9	unknown 9	1543		2.0±1.7	0.38±0.53	nd	0.22±0.31	0.36±0.50	nd	ns
U10	unknown 10	1652		5.5±0.70[a]	1.3±0.35[bc]	3.2±0.62[b]	1.2±0.86[c]	1.3±0.31[bc]	1.7±0.17[bc]	***
U11	unknown 11	1710		2.0±0.50[a]	nd[b]	nd[b]	nd[b]	nd[b]	nd[b]	***
U12	unknown 12	1758		2.1±1.2[a]	0.27±0.20[b]	0.18±0.06[b]	0.19±0.08[b]	0.87±0.38[ab]	0.44±0.31[ab]	*
U13	unknown 13	1842		1.4±0.07[a]	0.69±0.10[b]	0.11±0.16[c]	nd[c]	0.55±0.10[b]	nd[c]	***

[a] Linear retention index on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; [1] Mumm et al. (2004); [2] Zhao et al. (2006); [3] Radulovic et al. (2010); [4] Czerny & Schieberle, (2002); [5] Bader et al. (2003); [6] Adams et al. (2005); [7] Mosayebi et al. (2008); [8] Sabulal et al. (2007); [9] Havlik et al. (2006); [10] Bylaite & Meyer, (2006); [11] Javindnia et al. (2006); [12] Andriamaharavo, (2014); [13] Boulanger et al. (1999); [14] Cao et al. (2011); [15] Su et al. (2006); [16] Yu et al. (2007); [17] Zeng et al. (2007); [18] Pripdeevech & Saansoomchai, (2013); [19] Ruberto et al. (2002); [20] Loayza et al. (1995); [21] Turner et al. (2021b); [22] Turner et al. (2021c); [23] Turner et al. (2021d); [$] tentatively identified, spectral quality value of 70 was used for this compound. [c] Estimated quantities (mg) collected in the headspace of celery samples containing 0.5 mL of saturated calcium chloride and filled up to 5 mL with HPLC-grade water, calculated by comparison with of 100 mg/L propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; nd - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. Tukey's HSD - means not labelled with letters are not significantly different ($p < 0.05$) according to genotype.

Phthalides have been shown to contribute to strong celery-like odours and in addition to being the most odour active compounds within celery crop. Upon completing aroma extraction dilution analysis (AEDA), Kurobayashi, Kuono, Fujita, Morimitsu & Kubota (2006) detected phthalide compounds including 3-n-butylphthalide and sedanenolide, also identified within this study, to contribute the most to the celery odour. This was further confirmed by Lund, Wagner, and Bryan (1973) whereby sedanenolide, 3-n-butylphthalide and hexahydro-3-n-butylphthalide imparted strong celery odour characteristics. Genotype 12 displayed the highest abundance of phthalide compounds (Table 7.1) including sedanenolide and 3-n-butylphthalide and hybrids 12x22 and 22x12 also displayed a high abundance of phthalides within their aroma profile. As these compounds consist of strong celery odour notes (Turner et al., 2021b), these celeries will consist of a typical celery flavour.

The maternal inheritance of compounds from parent to hybrid was observed most clearly between genotype 25 and hybrid 25x12 whereby similarities between the presence and absence of compounds within the aroma profile as well as the abundance of compounds was apparent (Table 7.1). Monoterpene, sesquiterpene and phthalide abundances for these celery samples were the lowest out of the six samples and for example camphor and p-mentha-2,8-diene were both not identified in genotype 25 and 25x12. Furthermore, apart from 3-propylidene phthalide, the relative abundances of phthalide compounds were not significantly different between 25 and 25x12. The influence of the female counterpart of the crop is clear, with 25x12 inheriting more similarities from the female parent, 25 than male parent 12. This is less clearly observed when both parents, 12 and 22, were used in the hybrids 12x22 and 22x12. The relationship of these genotypes is unknown but if there is a close relation, genetically, then this would explain the fewer significant differences observed between these hybrids (Table 2). m-Tolualdehyde was only identified in genotype 22 and 22x12 and other aldehydes such as (E,E)-2,4-octadienal and hexanal were either only expressed in 12, 12x22 and 22x12 or were expressed in high abundance in these samples. The chemical inheritance of monoterpenes and sesquiterpene compounds appeared to be less clear, however, β-selinene and β-caryophyllene were expressed in a high relative abundance in genotype 12 and 22x12, displaying a stronger influence from the male parent, 12. Genotype 12 also displayed a high influence over the phthalide content for the hybrids 12x22 and 22x12, where both expressed a higher relative abundance for phthalide compounds than genotype 22.

7.5.1.1. Principal Component Analysis of the volatile content of three celery parents and their three hybrids

Principal component analysis was used to visualise graphically the differences in the volatile composition of three parental genotypes and their hybrids and to examine any correlations occurring between genotypes (Figure 7.1). Using only the significant compounds according to the one-way ANOVA, a separation between genotypes was observed. Principal components one (PC1) and two (PC2) explained 69.79% of the total variation present within the data. Samples 12, 25, 25x12 and 12x22 were separated across F1, whereas samples 12, 22 and 22x12 along F2, respectively. The observation plot confirmed the findings presented in Table 2, where samples 12 and 22x12 expressed a strong association with many volatile compounds due to the high abundance identified. Conversely, samples 25 and 25x12, observed on the opposite side of the observation plot, displayed little or weak association with all volatile compounds (Figure 7.2).

Due to the low abundance of volatile compounds, these genotypes would be perceived as less aromatic when compared to the other genotypes. The hybrid 12x22 was positioned in the middle of the observation plot, displaying a stronger association with volatile compounds than genotype 25 and its hybrid 25x12; however, the relative abundance expressed within this hybrid remains consistently lower than 22x12 in all compound groups, except for phthalides. Thus, the hybrid (12x22) was less aromatic than 22x12 but still had the typical, distinctive celery aroma. Comparing the aroma profile between the three parental genotypes and the hybrid lines, genotype 12 and hybrid 22x12 expressed the highest relative abundance of volatile compounds and it can be hypothesised that these will be more aromatic genotypes in comparison to the other samples. The current results (Table 7.1) confirmed previous work where genotype 12 was shown to be very aromatic with strong flavour associations but low scoring in mouthfeel attributes such as crunchy and moist yet scored high for stringiness. Genotype 25 was reported to be less aromatic with a distinct cucumber flavour but was profiled as very crunchy, moist and with a firm first bite.

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(A)

(B)

(C)

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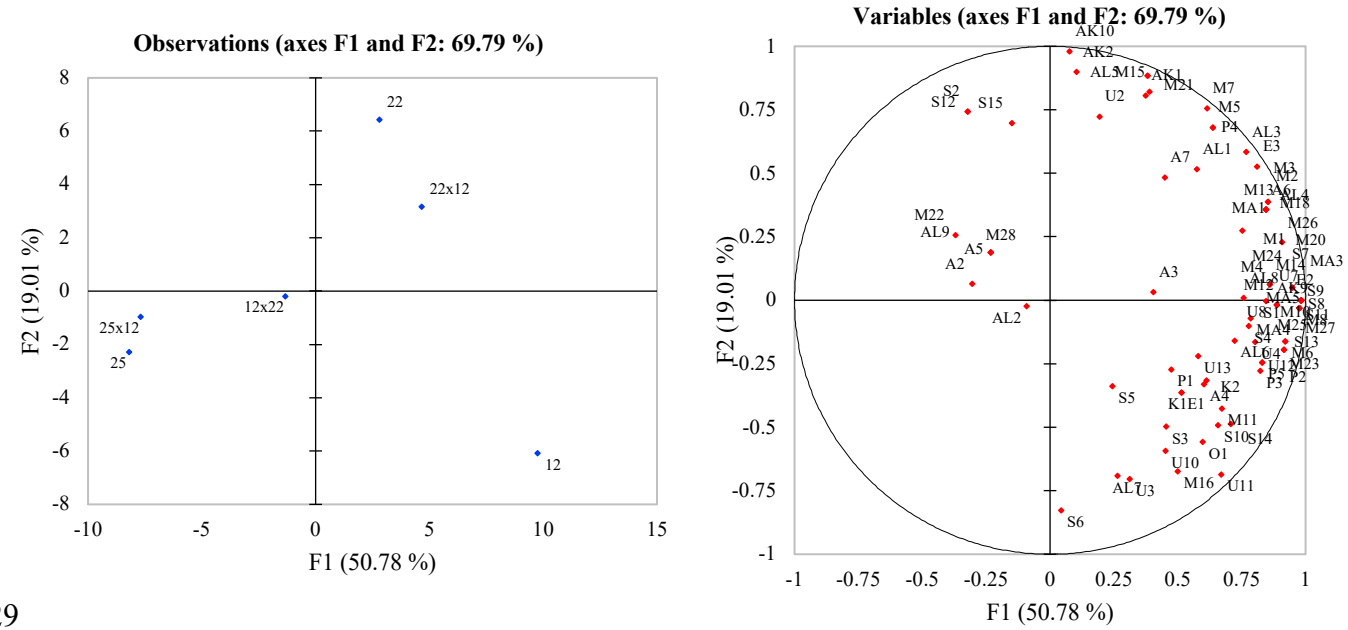

A2	pentanol	M22	pentyl cyclohexa-1,3-diene
A3	(Z)-3-hexenol	M23	cis-dihydrocarvone
A4	(E)-3-hexenol	M24	safranal
A5	hexanol	M25	β-cyclocitral
A6	octanol	M26	L-carvone
A7	(Z)-3-nonenol	M27	D-carvone
AL1	hexanal	M28	carvacrol
AL2	benzaldehyde	MA1	p-mentha-2,8-dien-1-ol
AL3	octanal	MA3	trans-carveol
AL4	phenylacetaldehyde	MA4	cis-carveol
AL5	m-tolualdehyde	MA5	(Z)-8-hydroxy linalool
AL6	(*E*,*E*)-2,4-octadienal	S1	α-ylangene
AL7	(*E*,*E*)-2,6-nonadienal	S2	α-copaene
AL8	(E)-2-nonenal	S3	(E)-β-caryophyllene
AL9	undecanal	S4	β-caryophyllene
E1	allyl hexanoate	S5	(+)-aromadendrene
E2	(E,Z)-3,6 nonadienol acetate	S6	curcumene
E3	(Z)-3-hexenyl butanoate	S7	α-humulene
K1	acetophenone	S8	γ-himachalene
K2	(Z)-jasmone	S9	β-selinene
AK1	nonane	S10	valencene
AK2	decane	S11	α-selinene
AK9	heptadecane	S12	(E)-nerolidol
AK10	octadecane	S13	kessane
M1	α-thujene	S14	liguloxide
M2	α-pinene	S15	rosifoliol
M3	camphene	O1	caryophyllene oxide
M4	sabinene	P1	3-propylidene phthalide
M5	β-pinene	P2	3-n-butylphthalide
M6	myrcene	P3	sedanenolide
M7	p-mentha-2,8-diene	P4	trans-neocnidlide
M8	α-phellandrene	P5	(Z)-ligustilide
M10	α-terpinene	U2	unknown 2
M11	ortho-cymene	U3	unknown 3
M12	limonene	U4	unknown 4
M13	β-(E)-ocimene	U7	unknown 7
M14	γ-terpinene	U8	unknown 8
M15	p-cymenene	U10	unknown 10
M16	terpinolene	U11	unknown 11
M18	allo-ocimene	U12	unknown 12
M20	trans-allo-ocimene	U13	unknown 13
M21	camphor		

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5033 **Figure 7.2.** Principal component analysis of six celery samples showing correlations with volatile compounds. (A) Projection of the samples; (B) Distribution
5034 of variables; (C) Compound codes as appear in plot (B)

Overall, genotype 25 and hybrid 25x12 displayed clear maternal inheritance within the volatile content in terms of the compounds identified and their relative abundance. The high abundance of volatile compounds identified in genotype 12 appeared to have been inherited by hybrids 22x12 and 12x22 (Table 2). We hypothesised that the parental genotypes would perform as previously (Turner et al. 2021c; Turner et al. 2021d) and maternal and paternal inheritance patterns become clearer upon sensory assessment, identifying phenotypic similarities between the parents and hybrids. Therefore, sensory evaluation was performed using a trained panel to further investigate these assumptions.

7.5.2. Sensory evaluation of fresh celery samples

The sensory profile of the three parental genotypes and hybrids was generated by a trained panel who came to the consensus of 28 terms for the quantitative assessment of celery samples and mean panel scores for these attributes are presented in Table 7.3. Out of the 28 attributes that were profiled, 15 of these were identified to be significantly different between genotypes. Few significant assessor x sample interactions were identified, suggesting that the panellists scored the samples in a consistent manner (Lignou, Parker, Baxter & Mottram, 2014).

Appearance and mouthfeel attributes expressed the highest number of significant differences between genotypes. The appearance of the celery samples can be found in Figure 7.1. Genotype 12 was scored high for appearance attributes (CA, RA) and hybrids descended from this genotype appear to have inherited these phenotypic characteristics, as high scores for both colour and ribbed were apparent. Their resemblance is also clear as shown in Figure 7.1. Hybrid 22x12 displayed less prominent ribs and the scoring of this attribute was further decreased for 25x12 hybrid. Clearly, genotype 25 had a stronger influence on 25x12, where lower scores were observed for appearance. In terms of mouthfeel attributes, genotype 12 was shown to be the least crunchy, most stringy, with the driest petiole with a soft first bite. The genetic crosses appear to have these altered mouthfeel attributes, expressing higher scores for crunchiness, stringiness, and moistness. Hybrids 12x22 and 25x12 exhibited higher moistness and lower stringiness scores when compared to genotype 12. Regardless of the maternal or paternal parent, this relationship provides evidence that by combining genotype 12, a genotype expressing a dry mouthfeel,

stringiness and ribbed appearance, with a genotype that exhibited these characteristics to a lesser extent, the said characteristics are also expressed to a lesser extent (Table 7.2).

Seven out of the ten odour and flavour attributes evaluated showed no significant differences between genotypes apart from grass odour and fresh parsley odour and flavour. Genotype 12 was scored significantly higher for grass and fresh parsley odour and flavour followed by genotype 22. The resemblance in scoring is reflected by the volatile content between these parents whereby fewer significant differences were observed (Table 7.1). Although the genetic code of these genotypes was not revealed, these parents may be closely related as they share several characteristics. Investigating their hybrids, 12x22 displayed a high score for grass odour, like genotype 12, whereas 22x12 was scored high for fresh parsley odour and flavour as genotype 22. The maternal genotype is closely associated with the descendent hybrid, expressing similar appearance, odour, and flavour characteristics (Table 7.2).

Table 7.2. Mean panel scores for sensory attributes of six celery samples

		Scores[A]						
Code	Attribute	12	25	22	25x12	22x12	12x22	P-value[B]
	Appearance							
CA	Colour	66.9 [a]	31.1 [d]	62.9 [ab]	51.1 [c]	59.6 [abc]	55.6 [bc]	***
STA	Stalk thickness (depth of cross-section)	25.2 [c]	61.2 [a]	60.0 [a]	58.4 [a]	45.4 [b]	49.3 [ab]	***
RA	Ribbed well-defined ribs)	77.3 [a]	52.5 [d]	61.1 [bc]	58.5 [cd]	65.1 [bc]	68.9 [b]	***
	Aroma							
FFA	Fresh fennel	16.3	14.2	18	15.9	13.1	20	ns
GGA	Grassy/green	34.5 [a]	19.9 [b]	31.3 [ab]	28.9 [ab]	29.5 [ab]	32.9 [a]	**
FPA	Fresh parsley	23.7 [a]	12.3 [b]	22.3 [ab]	13.1 [ab]	23.4 [ab]	16.8 [ab]	**
FCA	Fresh coriander	14.5	10.5	16.9	16.7	13.2	14.2	ns
	Taste/flavour							
BT	Bitter	44.5 [a]	26.0 [c]	36.1 [ab]	28.6 [bc]	32.1 [bc]	34.1 [bc]	***
ST	Sweet	3.4 b	11.7 [a]	7.9 [ab]	7.5 [ab]	8.9 [ab]	9.1 [ab]	*
SAT	Salt	19.1	14.9	17.6	17.3	17.9	17.6	ns
UT	Umami	2.7	4	2.9	3.7	3.3	3.6	ns
FFF	Fresh fennel	15.8	12	20.3	15.7	15.7	23.5	ns
RF	Rocket	4.8	1.1	2.5	3.9	3.4	2.9	ns
FCF	Fresh coriander	16.1	14.5	18.9	18.7	13	16.8	ns
FPF	Fresh parsley	25.9 [a]	9.8 [b]	20.9 [ab]	16.3 [ab]	20.7 [ab]	16.5 [ab]	*
SF	Soapy	18.6	10.5	13.4	16.8	15.3	15.9	ns
GGF	Grassy/green	28.4	26.5	26.5	24.4	24.4	30	ns
	Mouthfeel							
CM	Crunchy	54.7 [a]	55.4 [a]	63.8 [a]	65.7 [a]	59.3 [a]	63.2 [a]	*

SM	Stringy	68.1 [a]	45.2 [b]	44.5 [b]	55.3 [ab]	54.4 [b]	55.5 [ab]	***
MM	Moist	42.6 [c]	70.7 [a]	67.5 [a]	66.1 [a]	53.6 [b]	61.3 [ab]	***
FM	Firmness of first bite	50.5 [b]	54.5 [ab]	62.3 [ab]	62.2 [ab]	54.4 [ab]	65.2 [a]	**
	After-effects							
CAE	Celery residue in the mouth	40.4 [a]	29.9 [b]	29.8 [b]	31.9 [b]	30.5 [b]	34.5 [ab]	***
NAE	Numbness	21.7 [a]	10.3 [b]	17.6 [ab]	16.4 [ab]	16.2 [ab]	15.4 [ab]	**
BAE	Bitter	31.9 [a]	16.8 [b]	23.9 [ab]	22.9 [b]	21.2 [b]	22.3 [b]	***
UAE	Umami	3.2	3.3	3.1	1.4	3.2	3.5	ns
SAE	Salty	13.5	11.7	11.8	12.9	12.6	13.4	ns
SOAE	Soapy	11.7	9.3	9.5	13.3	12.3	12.5	ns
GGAE	Grassy/green	27.1	21.2	21.9	20.8	21.5	24	ns

[A] Means are from two replicate samples; differing small letters (a, b, c,) represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$); nd, not detected. [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

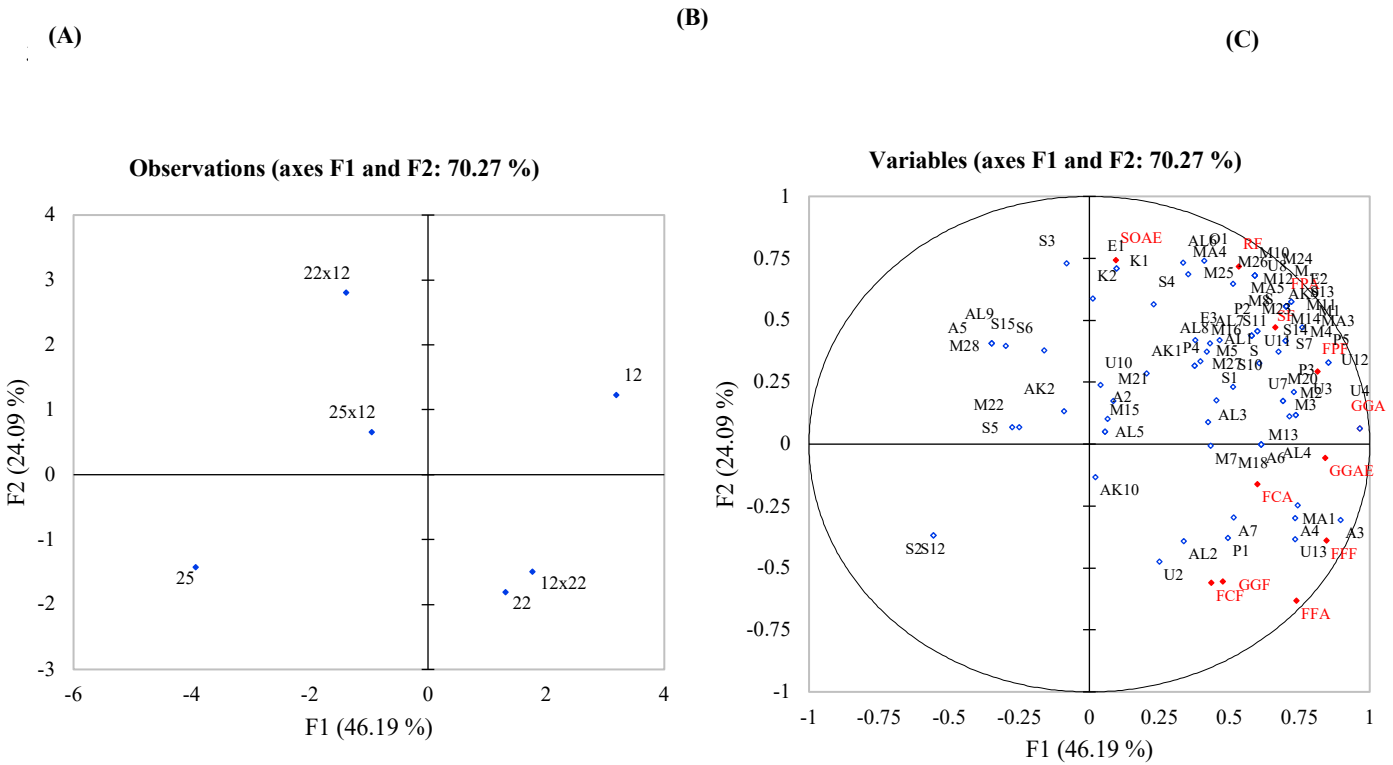
7.5.2.1. Principal Component Analysis of the fresh celery sensory profile and volatile composition

PCA was used to visualise the sensory and chemical differences observed across the genotypes and hybrids with the volatile compounds identified (Table 7.1) and odour and flavour attributes (Table 7.2) used as variables (Figure 7.3). Principal components one (PC1) and two (PC2) explained 70.27% of the total variation present within the dataset where the first axis separated genotypes 22, 25 and 12x22 and the second axis separated genotypes 12, 22 and 12x22, respectively. Genotypes 12 and 25 were displayed as opposites with genotype 12 expressing associations with many aroma compounds due to the high relative abundance identified and genotype 25 displayed no association with any flavour attribute due to its low relative abundance (Table 7.1). The profiling of genotype 12 and 25 reflects previous studies whereby both 12 and 25 were profiled as high and low extremes when grown in different geographical locations and across multiple years (Turner et al. 2021c; Turner et al. 2021d). Throughout these experiments, these genotypes have represented the most significantly different genotypes for all sensory attributes as well as behaved consistently in terms of their volatile profile when grown in different geographical locations and across multiple years. For this reason, they were recommended as "stable" genotypes for fresh produce growers (Turner et al. 2021b; Turner et al. 2021c; Turner et al. 2021d). Genotypes 12, 22 and 12x22 were mostly associated with flavour and odour attributes including fresh fennel, coriander, and parsley and with most of the volatile compounds.

Hybrid 25x12 expressed lower associations with these flavour attributes due to its lower relative abundance of monoterpenes, sesquiterpenes and phthalides and low scoring by the trained panel (Table 7.1, Table 7.2).

The grass odour observed in the hybrid 12x22 was inherited from its female parent genotype 12, both expressing high relative abundance in (Z)- and (E)-3-hexenol, (Z)-3-hexenyl butanoate and (E,Z)-3,6-nonadienol acetate, compounds observed to express a fresh, grass-like odour. Whereas the fresh parsley odour observed in hybrid 22x12 was inherited from the female parent genotype 22, both expressing a high relative abundance of monoterpene compounds also identified in fresh parsley including α-pinene, camphene, p-mentha-2,8-diene and β-pinene (Orav et al. 2003; Farouk, Ali, Al-Khalifa, Mohsen & Fikry, 2017) (Table 7.2). Along with this, genotype 12 was positively correlated with soapy flavour and the associations to flavour and odour attributes, combined with the high abundance of many volatile compounds (Table 7.1) confirms that genotype 12 is very aromatic. On the other hand, genotype 25 expresses no close association with any of the flavour and odour attributes confirming the previous statement that this genotype is not aromatic compared to genotype 12 or 22. Similar odour and flavour characteristics of genotype 25 were displayed in hybrid 25x12 (Figure 7.3, Table 7.2).

In terms of the sensory attributes, grass odour and flavour and parsley flavour were positively correlated with genotype 12, 22 and their hybrids. Alcohols (A3, A4), monoterpenes (M6, M11), sesquiterpenes (S13, S14) and phthalides (P3, P4) also displayed positive correlation with these samples and attributes. Fresh parsley odour and flavour that was scored highly in genotype 22 and 22x12 expressed a positive relationship with each other accompanied by; esters (E1, E2), monoterpenes (M1-M4, M6, M8, M10, M12, M14, M20, M23-27), sesquiterpenes (S7-S9, S11, S13) and phthalides (P2, P3) (Figure 7.3). Many compounds displayed a positive correlation with fresh parsley which was expected due to similarities between the celery and parsley aroma composition. Genotype 25 and 25x12 displayed the lowest scores of fresh parsley aroma and flavour due to the lower relative abundance of these compounds that were identified (Table 7.1).



A2	pentanol	M22	pentyl cyclohexa-1,3-diene
A3	(Z)-3-hexenol	M23	cis-dihydrocarvone
A4	(E)-3-hexenol	M24	safranal
A5	hexanol	M25	β-cyclocitral
A6	octanol	M26	L-carvone
A7	(Z)-3-nonenol	M27	D-carvone
AL1	hexanal	M28	carvacrol
AL2	benzaldehyde	MA1	p-mentha-2,8-dien-1-ol
AL3	octanal	MA3	trans-carveol
AL4	phenylacetaldehyde	MA4	cis-carveol
AL5	m-tolualdehyde	MA5	(Z)-8-hydroxy linalool
AL6	(*E*,*E*)-2,4-octadienal	S1	α-ylangene
AL7	(*E*,*E*)-2,6-nonadienal	S2	α-copaene
AL8	(E)-2-nonenal	S3	(E)-β-caryophyllene
AL9	undecanal	S4	β-caryophyllene
E1	allyl hexanoate	S5	(+)-aromadendrene
E2	(E,Z)-3,6 nonadienol acetate	S6	curcumene
E3	(Z)-3-hexenyl butanoate	S7	α-humulene
K1	acetophenone	S8	γ-himachalene
K2	(Z)-jasmone	S9	β-selinene
AK1	nonane	S10	valencene
AK2	decane	S11	α-selinene
AK9	heptadecane	S12	(E)-nerolidol
AK10	octadecane	S13	kessane
M1	α-thujene	S14	liguloxide
M2	α-pinene	S15	rosifoliol
M3	camphene	O1	caryophyllene oxide
M4	sabinene	P1	3-propylidene phthalide
M5	β-pinene	P2	3-n-butylphthalide
M6	myrcene	P3	sedanenolide
M7	p-mentha-2,8-diene	P4	trans-neocnidlide
M8	α-phellandrene	P5	(Z)-ligustilide
M10	α-terpinene	U2	unknown 2
M11	ortho-cymene	U3	unknown 3
M12	limonene	U4	unknown 4
M13	β-(E)-ocimene	U7	unknown 7
M14	γ-terpinene	U8	unknown 8
M15	p-cymenene	U10	unknown 10
M16	terpinolene	U11	unknown 11
M18	allo-ocimene	U12	unknown 12
M20	trans-allo-ocimene	U13	unknown 13
M21	camphor		

Figure 7.3. Principal component analysis of six celery samples showing correlations with volatile compounds and sensory profiling. (A) Projection of samples; (B) Distribution of variables, sensory attributes are highlighted in red. (C) Compound codes as appear in plot (B)

The results presented in Table 7.1 and Table 7.2 showed significant differences in the aroma composition and sensory characteristics between the parental genotypes and hybrids and inherited characteristics were observed between parents and their offspring. Whether these celery hybrids meet the desires of the consumer, if there is a more preferred hybrid and what are the drivers of preference in celery was determined through the completion of a consumer trial whereby the consumer acceptability of these hybrids and parental genotypes was investigated.

7.5.3. Consumer evaluation of celery samples

One hundred and eighteen consumers evaluated the celery samples, and the demographic data is summarised in Table 7.3. A higher proportion of the consumers were female (63.6 %), and the mean and median ages were 34.9 and 30, respectively. Close to half of the consumers were working (48.3 %) and 47.5 % were students. In total, 43.2 % of consumers related to the food and nutrition department at the University of Reading. The largest ethnic group was White (English, Welsh, Scottish, Northern Irish or British) making up 42.4 % of the sample population. Most consumers taking part stated that they liked celery (70.3 %) and the most frequent consumption was less than once a month (45.8 %).

The mean liking scores of the celery samples are presented in Table 7.4a. The results demonstrated a significant difference in appearance, aroma, texture, and overall liking for all the samples that were tested, with results ranging from dislike slightly to like slightly. No significant difference was identified in taste liking for all samples and all samples were scored with an average score of 5; ‘neither like nor dislike’. While consumers did not like the celery samples extremely, the attributes of the hybrids, particularly 25x12 and 12x22, were scored higher for appearance, aroma and texture liking than the parental genotypes. Genotype 12 was scored the lowest for overall liking. When consumers were asked to rank the hybrids from the most liked (1) to least liked (3) no significant difference was observed; samples were scored around 2 which demonstrated no significant preference.

Consumers were also asked to rank a list of six attributes that they found most important when consuming celery. The list that was presented to them contained attributes that are common in celery and in some cases, were very prominent in the samples such as the smooth exterior (not stringy). The attribute ‘crunchy’ was ranked as the most important followed by sweet taste, whereas the attribute

bitter taste ranked as the least important when consuming celery (Table 7.4b). Although ranked as least important, bitterness should still be considered an important characteristic to celery taste as the compounds that inflict bitterness and astringency often possess multiple health benefits upon consumption including antioxidant, anti-inflammatory, and anti-cancer properties. These are predominately from non-volatile compounds such as phenolic acids and flavonoids. (Drewnowski & Gomez-Carneros, 2000; Guerra, Carrozzi, Goñi, Roura & Yommi, 2010; Sung, Chung & Kim, 2016).

Table 7.3 Consumer demographics and characteristics of the consumer panel

Consumers	Number	Percentage (%)
Total number of volunteers	118	
Age		
mean	34.9	
median	30	
min	19	
max	71	
Gender		
male	42	35.6
female	75	63.6
prefer not to say	1	0.84
Working Status		
working	57	48.3
unemployed	3	2.5
student	56	47.5
other	2	1.7
working in food/nutrition/sensory sector	51	43.2
Ethnic group		
White	73	61.9
Mixed or Multiple ethnic groups	2	1.7
Asian or Asian British	21	17.8
Black, African, Caribbean or Black British	15	12.7
other ethnic group	7	5.9
Celery liking		
yes	83	70.3
no	35	29.7
Consumption Frequency		
less than once a month	54	45.8
once a month	19	16.1
2 to 3 times per month	19	16.1
once a week	13	11
2 to 4 time per week	9	7.6
once a day	4	3.4
Purchase Frequency		
once a month	80	67.8
once a week	17	14.4
never	21	17.8
Method of consumption		

I do not eat celery	15	12.7	5172
raw (on its own)	25	21.2	
raw (with condiments)	49	41.5	5173
raw (in salads)	42	35.6	
cooked (boiled, roasted, fried, on its own)	47	39.8	5174
cooked (in soups, stocks or sauces)	68	57.6	5175
other	6	5.1	5176

5177

5178 **Table 7.4a.** Liking scores and preference ranking for celery samples

5179

	Liking[A]					Ranking[B]
Samples	Appearance	Aroma	Taste	Texture	Overall	
12	5.7 [bc]	6.2 [a]	5.0	4.7 [c]	4.7 [b]	-
25	5.0 [c]	5.5 [b]	5.3	6.0 [ab]	5.5 [a]	-
22	6.3 [ab]	6.1 [a]	5.3	6.6 [a]	5.5 [a]	-
25x12	6.1 [b]	6.1 [ab]	5.4	6.1 [ab]	5.6 [a]	2.0
22x12	6.3 [ab]	6.1 [ab]	5.4	5.8 [b]	5.4 [ab]	2.0
12x22	6.8 [a]	6.2 [ab]	5.4	6.1 [ab]	5.6 [a]	2.1
p-value	***	*	ns	***	**	ns

5180 [A] Means not labelled with the same letters (a,b,c,d) are significantly different ($p < 0.05$); means are from 118 consumers on a
5181 9-point hedonic scale (from dislike extremely to like extremely). B Mean rank (1: most preferred to 3: least preferred); ns, no
5182 significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant
5183 at 0.1% level.

5184

5185 **Table 7.4b.** Consumer ranking for celery samples

Attributes	Ranking[A]
Crunchy texture	2.3 [a]
Sweet taste	2.8 [ab]
Moist texture	3.8 [c]
Smooth exterior (not stringy)	3.4 [bc]
Strong aroma	4.1 [d]
Bitter taste	4.6 [cd]

5186 [A] Mean rank (1: most important to 6: least important).

5187

5188 **7.5.3.1 Internal preference mapping and agglomerative hierarchical cluster analysis of**
5189 **consumer data**

5190 Agglomerative hierarchical cluster (AHC) analysis was completed to identify relatively
5191 homogeneous groups of consumers based on their overall liking scores. Three clusters of consumers
5192 were identified and the mean liking scores of the clusters are presented in Table 7.5. Consumers in
5193 cluster 1 (43.2%) neither liked or disliked hybrids 25x12 and 22x12 and expressed a moderate dislike

for genotype 12. Cluster 2 (38.9%) behaved in a similar manner to cluster 1, liking slightly genotypes 25, 22 and 25x12 and neither liked or disliked genotype 12 and hybrid 22x12. Opposing clusters 1 and 2, consumers in cluster 3 (17.8%) liked slightly genotype 12 and moderately disliked 25x12 due to its strong flavour attributes.

Table 7.5. Overall liking of the celery samples for the cluster of consumers obtained from agglomerative hierarchical clustering.

Cluster/Percentage of Consumers	Samples [1]						*p* Value	Overall Liking per Cluster [2]
	12	25	22	25x12	22x12	12x22		
1 (43.2%)	3.5 ^{c,AB}	4.6 ^{ab,ABCD}	4.5 ^{b,ABC}	5.5 ^{a,CDEFGH}	5.2 ^{ab,CDEF}	5.0 ^{ab,CDE}	***	4.7 ^{c}
2 (38.9%)	5.4 ^{b,CDEFG}	6.8 ^{a,H}	6.8 ^{a,H}	6.7 ^{a,GH}	5.7 ^{b,CDEFGH}	6.1 ^{ab,EFGH}	***	6.2 ^{a}
3 (17.8%)	6.5 ^{a,FGH}	4.8 ^{bc,BCDE}	5.2 ^{ab,CDEF}	3.3 ^{c,A}	5.1 ^{ab,CDEF}	6.0 ^{ab,DEFGH}	***	5.1 ^{b}
Overall liking per sample [3]	4.7 ^{b}	5.5 ^{a}	5.5 ^{a}	5.6 ^{a}	5.4 ^{ab}	5.6 ^{a}		

[1] Significant difference for the means per cluster ($p < 0.05$) within a row are denoted by differing small letters $^{(abc)}$; means are from 51 consumers for cluster 1, 46 consumers for cluster 2 and 21 consumers for cluster 3, respectively; significant differences from the interaction (sample x cluster) are denoted by differing capital letters ($^{ABCDEFGH).}$ [2] Mean for overall liking per each cluster was significantly different with $p < 0.0001$. [3] The mean for overall liking per sample is from 118 consumers and it was significantly different with $p = 0.0004$. Significant interaction between sample x cluster was observed as calculated by two-way ANOVA ($p < 0.0001$); *** significant at 0.1% level

Labelling each participant present within each cluster as a liker or non-liker, 60.8, 82.6 and 57.1% were celery likers in clusters 1, 2 and 3. Interestingly, cluster 3 contained the highest proportion of celery non-likers and they liked the most genotype 12, a genotype that expressed a high abundance of volatile compounds and profiled as very aromatic with a strong bitter taste, whereas 25x12 was the least liked and profiled as less aromatic (Table 7.2). On the other hand, hybrid 25x12 was the most liked of the hybrids according to clusters 1 and 2. One reason might be the high score of crunchiness and moist mouthfeel by the trained panel (Table 7.2); both attributes ranked as important according to consumers (Table 7.4a). There was also significant interaction between sample x cluster for overall liking confirming that consumers scored differently the samples in each cluster (Table 7.5).

Sensory attributes assessed by the trained panel (Table 7.2) were regressed onto the first two principal components of the consumer overall liking data to form an internal preference map (Figure

7.4). Principal component one (F1) and two (F2) explained 47.63 % of the variation in the data with hybrids and genotype 22 separated from genotypes 12 and 25 across F1, driven by sweet taste (ST), moist mouthfeel (MM) and stalk thickness (STA) attributes. Genotypes 12 and 25 were separated across F2 with genotype 12 being positively correlated with grass/green flavour (GGF), bitter taste (BT) and stringy mouthfeel (SM) attributes.

Cluster 1 displayed no significant relationship with any sensory characteristics (Figure 7.4), therefore, confirming that celery not possessing a strong aroma such as hybrids 22x12 and 25x12 (Tables 7.1 and 7.2), were more liked. Genotypes 25 and 22 and hybrid 25x12 were scored highly for stalk thickness (STA), moist mouthfeel (MM) and had a firm first bite (FM) with a sweet taste (ST) as discussed during sensory profiling (Table 7.2) and these attributes were closely associated to the most liked genotypes within cluster 2. Both clusters expressed no significant correlation with any flavour or odour attributes and preferred the celery that expressed low relative abundance of the volatile compounds (Table 7.1). For this reason, genotype 12 was the most disliked celery sample for clusters 1 and 2. Genotype 12 expressed a high relative abundance of volatile compounds (Table 7.1) in addition to scoring significantly higher in grass/green flavour (Table 2). Ribbed appearance (RA), grass/green aroma (GGA), bitter taste (BT) and fresh parsley aroma and flavour (FPA and FPF) were attributes positively correlated with this genotype.

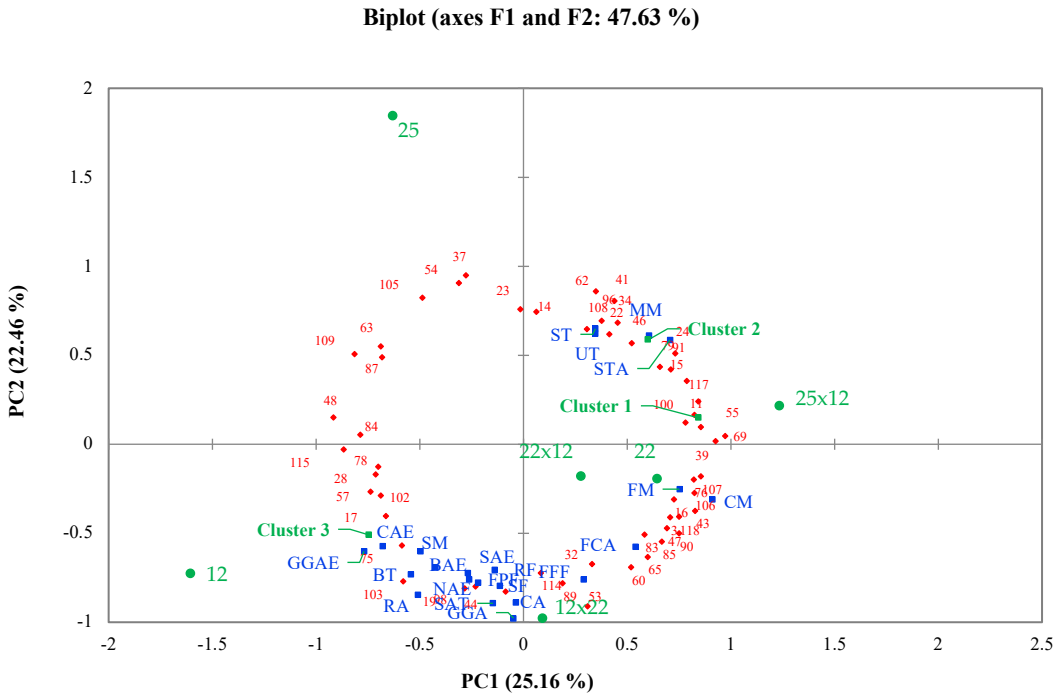


Figure 7.4. Internal preference map of six celery samples. Sensory attributes and consumer cluster means were regressed onto the consumer preference matrix generated by PCA. Blue squares - sensory attributes, codes correspond to those in Table 7.3. Green squares - clusters 1, 2, 3, mean liking positions of three clusters from AHC (Table 7.6). Red circles: overall liking scores of each consumer.

Penalty analysis was used to relate Just-About-Right (JAR) data to liking scores and explain drivers of overall liking in relation to aroma, sweetness, bitterness, flavour and stringiness intensity and the results are presented in Table 7.5. When the attributes are not at the optimum intensity for a consumer, this may influence the overall liking. Sweetness was ranked by the consumers as the second most important characteristic, and this was reflected in Table 7.6 whereby for all genotypes and hybrids there was a negative impact on the overall liking when the sweetness of the samples was considered too low. This agreed with over 50 % of the consumers in all samples. On the other hand, there was a significant drop in the liking of all samples when the bitter taste intensity was “too much” by the consumers with the genotypes 12 and 22 perceived the most bitter and genotype 25 the least bitter. Hybrid samples were scored in between the parent genotypes. Interestingly, regarding the flavour intensity attribute, it can be observed that there was a significant drop in the liking for almost all samples when the flavour intensity of the samples was considered either “too little” or “too much”. Where significant drops were observed for flavour intensity attribute, no significant drop in overall liking was observed for aroma intensity, too little or too much, displaying that consuming celery is more important

for deciding preference than just smelling the sample. Stringiness, which expressed a negative correlation with crunchy texture by the sensory panel (Table 7.2), displayed significant drops in overall liking if samples were "too much" in genotype 12 and all the hybrids. Genotype 12 and 12x22 were considered to be the most stringy, and a mean drop of 1.3 and 0.9 in the overall liking occurred respectively. Although scored lower, the stringiness scored by the panel of 12x22 was like genotype 12 (Table 7.2). The maternal inheritance of the ribbed appearance is clearly demonstrated from genotype 12 in 12x22. As texture was scored as an important attribute for consumers (Table 7.4b), we would recommend to breeders to use a female parent that expresses the desirable appearance and textural attributes as a strong maternal inheritance has been observed in this study.

Table 7.6. Mean Just-About-Right ratings and penalty analysis showing the influence on overall liking rating

Samples	Overall [A]	Significance of Sample (*p*-Value) [B]	Penalty Analysis			
			Too Little		Too Much	
			Mean Drop	Frequency (%)	Mean Drop	Frequency (%)
	JAR Aroma					
12	2.9 [a]	**	0.69	24.6	1.15	17.0
25	2.5 [b]		0.49	48.3	3.30	7.6
22	2.8 [a]		0.70	29.7	1.54	11.9
25x12	2.7 [ab]		0.39	31.1	1.32	13.6
22x12	2.8 [a]		0.61	30.5	1.62	13.6
12x22	2.9 [a]		0.74	28.0	1.55	15.3
JAR Bitterness						
12	3.4 [a]	**	1.15	15.3	2.09 *	45.8
25	2.9 [b]		0.72	28.0	2.17 *	22.9
22	3.3 [a]		1.45	14.4	2.09 *	40.7
25x12	3.1 [ab]		0.60 *	21.2	1.98 *	30.5
22x12	3.2 [ab]		0.52	21.2	1.56 *	33.9
12x22	3.2 [ab]		0.51	21.2	2.22 *	30.5
JAR Sweetness						
12	2.2	ns	1.18 *	66.1	0.53	1.7
25	2.5		1.55 *	50.9	0.06	4.2
22	2.4		1.31 *	52.5	-	0.0
25x12	2.4		1.69 *	50.9	0.41	2.0
22x12	2.4		1.73 *	54.2	2.36	0.9
12x22	2.4		1.76 *	46.6	1.44	0.9
JAR Flavour						
12	3.3 [a]	***	1.11	17.8	2.26 *	41.5
25	2.8 [b]		1.37 *	38.1	2.75	15.3
22	3.0 [ab]		1.26 *	23.7	2.28 *	40.7
25x12	3.1 [ab]		1.10 *	24.6	2.39 *	28.8
22x12	3.0 [ab]		1.16 *	22.9	1.96 *	25.4
12x22	3.1 [ab]		1.26 *	22.0	2.39 *	30.5

JAR Stringiness						
12	4.0 [a]	***	1.76	5.1	1.33 *	70.3
25	3.2 [cd]		0.71	19.5	0.60	30.5
22	3.0 [d]		−0.57	22.9	0.59	22.0
25x12	3.4 [bc]		0.24	15.3	0.88 *	42.4
22x12	3.5 [b]		−0.19	14.4	0.90 *	49.2
12x22	3.3 [bcd]		0.62	11.9	1.64 *	35.6

[A] Means not labelled with the same letters are significantly different ($p < 0.05$). [B]* Represents a significant difference ($p < 0.05$) within a sample in overall liking compared with mean liking rating when the sample was considered Just-About-Right

Additional comments on the samples provided by the participants contained both positive and negative points and these are shown in Table 7.7. Although bitter and sweet taste have been identified as drivers of disliking and liking, the results from the consumer evaluation of celery samples demonstrated that consumers could not identify differences in taste (Table 7.4a) whereas the trained panel clearly identified significant differences between all samples in sweetness and bitterness (Table 7.2).

Table 7.7. Examples of participants' comments (three positive and three negative comments) relating to the celery samples used in this study.

Sample	Comments and Participants Details
12	Very different from any other celery I had before. This is very yummy (IP12). Flavours were balanced and texture and appearance were good and appealing (IP120). It is very good fresh smell (IP63). Would not be pleased if I had bought this Did not finish it (IP3). I was unable to break it in two due to the fibres. It was excessively stringy, and the flavour was too strong too (IP32). It was very stringy. The aroma and taste was herbal (IP62)
25	Had a slight salty taste which I liked (IP117). This one is very juicy (IP65). Good texture and light overall flavour (IP19). Looked very pale. Bland flavour (IP51). Too pale in colour (IP112). I would not buy this because of the colour (IP88).
22	Very juicy in texture (IP14). This sample will be a good quality celery that I'm expecting when buying one (IP31). what I would expect from a good celery stick (IP49). No distinct flavour (IP59). Unpleasant after taste (IP110). Really bitter and salty (IP77)
25x12	Beautiful sample of celery (IP52). Overall good celery to taste and flavour (IP30). Crunchy and juicy (IP96). Very sweet and aromatic. Too stringy (IP116). Too stringy and rather boring overall (IP28). Too bitter, unpleasant (IP98).
22x12	Attractive celery, good cross section and colour. Good crunch and mouthfeel not as stringy as many (IP09). I enjoyed this one was quite good and not as stringy as some of the other flavour was good and have a nice crunch (IP70). It looks more appealing (IP21). Flavour too strong and too stringy (IP7). This sample is stringy for me. Some fibers are left in mouth (IP40). This one is too stringy and bitter (IP75).
12x22	Very strong aroma and flavour. Texture and lack of strings was good. Nice colour (IP11). Really liked this sample, Tastes of what celery to me should taste like (IP28). Good texture and flavour. My favourite (IP122). The intense taste bothered me. It tasted bitter at the first bite (IP83). Tasted very chemical-like (IP44). Very bitter aftertaste (IP36).

Overall, there was no hybrid that was significantly preferred by the consumer with all hybrids scoring between 2.0 and 2.1 (Table 7.4a). Both 25x12 and 22x12 were scored in a similar manner in preference ranking (Table 7.4a) as well as in sensory analysis however, upon combining the data collected from liking (Table 7.4a), importance of attribute ranking (Table 7.4b), cluster analysis (Table 7.5) and JAR (Table 7.6), with further developing, 25x12 holds the potential to be a new hybrid that matches most of the consumers' desire. Expressing characteristics including a crunchy and moist mouthfeel, low stringiness and an odour and flavour that was not scored too highly by the panel (Table 7.1, Figure 7.1, Table 7.2, Figure 7.2). Contrastingly, hybrid 12x22 expressed high abundance of volatile compounds (Table 7.1) and was scored accordingly by the panel, with strong associations to fresh parsley flavour (Figure 7.3, Figure 7.4). The maternal inheritance was clear in both 12x22 and 25x12, with the characteristics of both female parents displayed within the hybrids. This was less apparent in hybrid 22x12, whereby the possibility of these genotypes being closely related causes difficulties with matching parental characteristics. The overall liking score for genotype 12 was the lowest (Table 7.4a), the sample expressed a stringy and dry mouthfeel attributes yet high scoring flavour attributes such as soapy, fresh parsley and grass (Table 7.2). This genotype was also scored as the most bitter and least sweet. Bitterness was an attribute ranked as least important and sweetness was ranked as second most important for consumers (Table 7.4b). 25x12 was the only hybrid that expressed a mean drop in liking if an increase or decrease in bitterness occurred (Table 7.6) indicating that the bitter intensity of this crop is at an acceptable level for 21 % of consumers. This hybrid contains genetic material from both genotype 25 and 12, the most sweet and bitter parental genotypes, and we can clearly see that the favourable attributes of both genotypes have been passed on; the preferred mouthfeel attributes of genotype 25 combined with the distinct flavour of genotype 12 without being overpowering. The taste characteristics have been combined to produce a less bitter hybrid.

7.6. Conclusions

The present study aimed to explore the sensory characteristics of new celery hybrids and their parental genotypes, identifying similarities and differences between the parents and offspring, and to

evaluate consumer liking and perceptions of celery hybrids. Significant differences between parental genotypes and hybrids were observed in the aroma composition, sensory profiling, and consumer liking. In addition, non-significant differences were observed in parent genotypes and their hybrid off-spring highlighting the potential for maternal and paternal inheritance of phenotypic characteristics.

The hybrids in this study were grown in Spain (2021) and before we can confirm with confidence that we have developed a celery variety that meets the consumer demands, these hybrids must be grown in different scenarios and investigate any variation occurring within the aroma composition and changes in the sensory characteristics. Growing these hybrids in different geographical locations and over multiple years will identify the stability of these hybrid lines and examine how variables including air temperature, soil type, water composition and different agronomical techniques might influence the aroma profile. Following this up with sensory profiling will identify the impact of these variables upon the aroma composition and consumer preference for the hybrids.

The findings from this study combined with previous studies completed by the authors will contribute to further understanding how changes in the aroma and sensory profile may influence consumer acceptability and preference. This work provides knowledge and pinpoints the importance of attributes that drive consumer preference which in turn is useful to fresh produce growers and breeders. Furthermore, the information on the maternal inheritance of characteristics in celery has been displayed in this paper will aid breeders in the understanding of inheritance in celery, ultimately leading to the production of new celery hybrid lines that are consumer preference-driven based on their metabolite and sensory profile.

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CHAPTER 8: Overall Discussion, Future Work and Final Remarks

8.1 Overall Discussions and Conclusions

Celery is a crop that is grown and consumed globally, appearing as a key ingredient within may cuisines including French, Italian and Cajun. This is due to its strong, distinct flavours that, when combined with carrots, onions, bell peppers or tomatoes, forms part of the "holy trinity". The distinct celery flavour is attributed to the range of volatile compounds that constitute the aroma profile but predominantly, phthalides including sedanenolide, butylphthalide and ligustilide which have been labelled countless times in literature to be the characteristic compounds of celery odour. Although less important, monoterpenes and sesquiterpenes are also commonly reported within celery and contribute citrus, fresh, woody, and floral notes. These compound groups were identified in all harvests and both locations, displaying the importance of these compounds to celery.

The results presented in the preceding chapters highlighted the potential for new celery hybrids to be developed further into high quality varieties for human consumption. The thesis also identified the influence of factors that are common in the growing environment of celery on aroma composition and thus how perceived flavour and sensory characteristics are consequentially altered. Using a diverse range of genotypes with varying origins, appearances and uses, across two geographical locations and over the duration of four years, we have observed how genotype, temperature, water and micronutrient availability, maturity and field placement are all involved in the determination of celery flavour through the regulation or synthesis of secondary metabolites. There have been limited investigations examining the influence of abiotic and biotic factors on the aroma profile of celery and therefore, any relationships that have been discussed between the aroma composition and the environment can only be hypothesised by using examples of alternative crops behaving in a similar manner.

The results presented in this project demonstrate that both internal and external factors have a significant influence over the aroma composition of celery. Genotype has been observed to consistently play a role in predetermining the aroma composition observed, as well as influencing the changes in profile throughout crop development, through the synthesis of new compounds over time. As discussed in chapter 3 where significant differences were observed in the climate, we concluded that due to the

environmental stresses such as high temperatures, relative humidity and minimal rainfall experienced by the crop, secondary metabolites were synthesised as a defensive mechanism to protect the crop. On the other hand, when comparing different geographical locations such as in chapter 4, we hypothesised that factors such as altitude, field placement and relative humidity apply environmental stress to the crops, leading to variation in the synthesis of volatile compounds as a protective or adaptive mechanism. Growing in Spain introduced new variables for discussion including the differences in soil composition as well as water availability and salinity as discussed in chapter 4. We hypothesised that the differences in water and soil sources led to differences in water and soil composition, particularly the micronutrients available for uptake by the plant during growth, ultimately leading to differences in the synthesis of primary and secondary metabolites. We hypothesised further that these compositional differences explain the presence of ketones and aldehydes identified in Spanish grown celery that were not previously observed in UK-grown celery. Finally, upon investigating the development of aroma across maturity through the examination of volatile abundance using two genotypes (12 and 22), we concluded that genotype influences and regulates the rate of synthesis of volatile compounds in celery.

Additionally, sensory profiling revealed deviations in the scoring of the eight genotypes throughout the project. Celery grown in the UK in 2018b was scored with a flavour profile closely associated to soapy, grass green and rocket whereas fresh coriander and parsley attributes were scored higher in the UK 2020. Spanish celery was scored to be more closely associated with the herbal attributes in addition to fresh fennel. Changes in taste characteristics were also observed; salt taste was detected by the trained panel in both Spanish harvests, here we hypothesised that the use of desalinated sea water and saline soils present would be the cause of this taste characteristic. Throughout sensory profiling, appearance and mouthfeel attributes expressed the most significant differences between genotypes, particularly observed between genotypes 12 and 25. Many correlations between these attributes were exposed, for example, a ribbed petiole appearance expressed a strong positive correlation with a stringy mouthfeel and a negative correlation with a firm first bite and moist mouthfeel. A darker petiole colour expressed a positive correlation with bitterness and rocket flavour and bitterness expressed a negative correlation with sweet taste. Fresh fennel and coriander expressed

positive correlations with their aroma counterpart in addition to soapy flavour, an attribute that is characteristic of both crops.

We observed certain genotypes to behave in a similar manner regardless of their environment, specifically, genotype 12, 22 and 25. These three genotypes remained similar throughout the project, especially 12 and 25, which were the most opposing genotypes when regarding their sensory profile and aroma composition. Genotype 12 expressed a high relative abundance of chemical compounds and associated with grass/green flavours and bitter taste, whereas genotype 25 expressed low relative abundance of chemical compounds and displayed associations with a cucumber flavour and sweet taste. The mouthfeel attributes of these two genotypes were also significantly different with genotype 12 displaying prominent ribs which was correlated with stringy mouthfeel and genotype 25 was scored with a thick petiole, correlating with a moist mouthfeel and low stringiness. Observing the relative abundance of volatile compounds in genotype 22, these remained significantly lower than genotype 12 and significantly higher than genotype 25 throughout the project. The sensory panel profiled the mouthfeel attributes of genotype 22 in a similar manner to genotype 25 in addition to a fresh fennel flavour. For these reasons, it was decided that genotypes 12, 22 and 25 and their hybrids would be taken forward for consumer acceptance.

Collecting the consumer acceptance and celery preference data, we identified that mouthfeel and sweet taste are drivers of liking whereas bitter taste and strong aroma were drivers of disliking in celery. Completing Agglomerative Hierarchical Cluster (AHC) analysis combined with internal preference mapping (Chapter 7), we identified three clusters of consumers. Cluster 1 (43.2 %) and 2 (38.9 %) both expressed similarities in their celery preferences, with the hybrid 25x12 displaying the attributes that drove their preference including moist mouthfeel and sweet taste. Conversely, cluster 3 (17.8 %) preferred celery associated with a strong aroma and bitter taste, attributes that genotype 12 expressed. With further research and development including investigating the differences in preference when grown in the UK and various seasons, hybrid 25x12 has the potential to become the first celery to be developed with consumer preference in mind and display the attributes that were identified as most important.

8.1.1. Answering Research Questions

- What are the key aroma compounds and what aroma do they contribute to celery?

Using GC/MS, the aroma composition of celery was identified to mainly be composed of monoterpenes, sequiterpenes and phthalides, agreeing with what was previously identified in the literature. A range of alcohols, aldehydes, ketones, and esters were also identified in this project, but these were observed to vary considerably depending on the maturity, harvest year and geographical location. Combining observations in the literature with our own findings, phthalides, particularly sedanenolide and butylphthalide, were the most prominent phthalides in addition to being the characteristic compounds by contributing strong celery and herbal odours. Monoterpene compounds expressed odour characteristics that were associated with fresh, citrus, and earthy odours and sesquiterpenes were detected to exhibit woody, floral and damp odours. This information was discussed in chapter 6.

- What are the key contributors to differences in the aroma composition?

Upon completing the project, genotype, harvest year and geographical location were all observed to have a significant influence on the aroma composition of celery. Compiling all the data together and performing principal component analysis, the effect of location was clear with Spanish-grown celery expressing a vastly different profile to UK grown celery; this difference was caused by the presence of ketones and aldehydes that were not previously identified in the UK crop. Growing in different locations introduced more variables including the water availability, the composition of water and soil and the field placement (angle of the slope, altitude of the field, direction of the field and distance from the sea). These factors would lead to differences in the availability of micronutrients or apply environmental stress on the crop, causing a change in the secondary metabolite production.

- Can changes in the aroma composition lead to noticeable changes in the sensory profile?

The trained panel worked closely with us throughout the project and before each scoring session, several vocabulary and training sessions were completed to ensure good repeatability and accurate

scoring of the samples. Additionally, the panel were also exposed to celery through G's Fresh Ltd who employed the panel to score several products of their own, comparing their results to an inhouse panel and an E-nose. Although statistical comparison between all harvests was not possible, chapters 3 and 4 display the differences in perceived sensory characteristics. The panel identified significant differences from each harvest and profiled them differently; the UK harvest in 2018 was profiled as more grass and rocket-like whereas the UK harvest in 2020 was profiled to be more associated with the herbal attributes including fresh parsley, coriander, and fennel. The Spanish harvest of 2019 and 2021 also were profiled differently to one another. Compositional differences caused by genotype and other environmental variables was determined by the panel.

- What attributes do consumers find desirable in celery?
- What are the drivers of preference in celery?

When completing the consumer trial, we presented the volunteers with a list of six common attributes in celery including sweet taste, crunchy mouthfeel, smooth exterior, moist mouthfeel, strong aroma, and bitter taste and asked them to rank them in order of importance. A crunchy mouthfeel was ranked as the most desirable attribute in celery followed by a sweet taste whereas a bitter taste was perceived as the least desirable characteristic followed by a strong aroma. Completing statistical analysis from our JAR and penalty analysis revealed to us that the drivers of liking was sweet taste and flavour whereas bitter tasting was a driver of disliking and by combining AHC, overall liking and sensory data, we identified that the drivers of preference changed according to clusters.

- Can we create a new hybrid of celery based on its metabolite profile that displays the potential to meet the consumer demand?

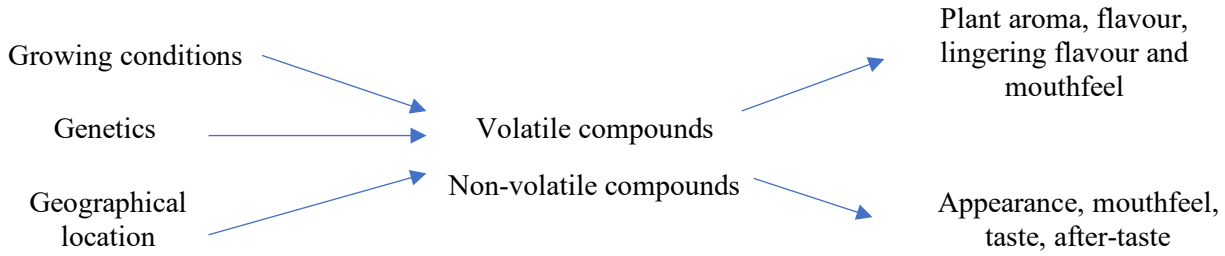
We have developed a hybrid that has the potential to meet the desires of the consumer however more development on the hybrid will be required before we can confirm this. Cluster 1 and 2 expressed preference towards the mouthfeel attributes, especially moist mouthfeel, and firmness in addition to sweet taste, therefore focussing on these attributes will meet the demands of most of the consumers (82

%). Furthermore, we must continue research on these hybrids, investigating their performance in different geographical locations, especially in the UK and during different seasons.

8.2. Industrial Relevance, Application and Future Work

8.2.1. Key findings for Industry

The celery genotypes used in this project were chosen by the industrial supervisor, Dr Frances Gawthrop, Tozer Seeds, due to their differences in origin, characteristics and uses, plus several of these genotypes are the parents of commercial hybrids that are currently available on the market (see Appendix V). Any decisions made on this project were discussed thoroughly with Tozer Seeds and regular meetings were held with the growers used in this project, G's Fresh Ltd. The project findings have educated and advised G's Fresh on the variables of celery and how they influence the aroma composition and sensory profile of celery. This has led to regular taste panels being held, using an in-house tasting panel to assess certain sensory attributes for their fresh produce. The information gathered from this project will be provided to breeders and growers to educate on environmental and location factors on the aroma composition of the crop. Particularly with the increased risk of global warming and increased temperatures/increased frequency of extreme weather events, it is likely that the crops will be more frequently exposed to warmer temperatures and irregular weather. Below, a summary of the key findings of the project can be seen. In the diagram, the left side displays the factors that influence the sensory attributes of celery can be observed and on right, the outcomes are displayed



- Genotype determines the crop's ability to produce volatile compounds including the plant's response to changes in the growing environment such as temperature, rainfall and geographical location. These all significantly influenced the aroma composition of celery, leading to

significant sensory differences in appearance, aroma, taste and mouthfeel attributes. We recommend that growers and breeders select varieties that have been specifically developed for growth in the required environment. This will increase the probability of producing a quality product, rather than using a hybrid that may be more susceptible to disease or degradation due to growth in unfavourable conditions.

- Celeries of various genotypes will mature differently, synthesising different compounds that will lead to significant changes in the sensory profile – harvesting pre-mature or after optimal-harvest will lead to changes in the aroma profile. This can be advantageous to growers as they firstly, utilise crops that will maintain their flavour profile for longer during optimal maturity and secondly, growers can offer a variety of celery products. For example, harvesting earlier will lead to celery with lower quantities of phthalides but more terpenes, producing celery that will be perceived as more fresh, floral and citrus. On the other hand, harvesting later will lead to a variety with higher phthalides, aldehydes and ketones, producing a more woody, herbal and stronger celery tasting variety.
- Within the three clusters identified in the final chapter, there were conflicting drivers of preferences. From this, it is important that consumers are offered a choice on the produce available, not just in celery but in other fresh produce. This is already present in dairy products like cheese and milk and with the variation available within celery, why not offer a celery that is strong in taste (such as genotype 12), great for cooking or a variety that is moist in mouthfeel and sweet in taste, presenting a suitable vessel for holding condiments.
- According to consumers, mouthfeel and sweetness are the most important attributes when it comes to driving preference in celery – if celery breeders wish to development a consumer-driven hybrid, focusing on the removal of the ribs that appear on the celery petiole would lead to a less stringy variety. This would be achieved by ensuring the maternal parent of the hybrid expresses less prominent ribs.

- There is strong evidence in this project of the maternal parent on the phenotype in celery. Therefore, when considering breeding programmes, it is important that the maternal parent expresses the desirable traits. Although still present, paternal influence is much less significant.

8.2.2. Future work

Moving forward, we propose to investigate how the factors studied in the project impact the non-volatile content of celery including sugars and phenolic acids. This can be completed on the freeze-dried material that is currently stored in airtight containers out of sunlight. It is expected that growing in an environment that would be considered "harsh" would lead to a change in phenolic acids or flavonoid compounds. As observed in chapter 3 and 4, the synthesis of compounds in response to changes in the geographical location and climate is genotype dependent and this was similarly observed by Shamloo et al. (2017) whereby the secondary metabolite profile was significantly influenced by genotype in various wheat genotypes including phenolic acids, flavonoids, and fatty acids. Due to climate change and increasing temperatures, investigating how these factors would impact the plant composition would provide useful information to growers and breeders as to how to adapt their methods and approach to growing fresh produce. Consumer analysis identified bitter taste and sweet taste as drivers of liking, therefore studying these non-volatile compounds would provide a deeper understanding on how these taste characteristics are influenced and vary according to the growing environment. Furthermore, by studying the phenolic compounds, we can also examine the discolouration that occurs within celery and whether the browning or pinking that develops overtime varies between genotypes.

Organic farming is becoming more popular with retailers as well as consumers. Throughout our meetings with G's Fresh, it was often mentioned that organic celery tastes better and with investigations that G's have previously carried out comparing their conventionally grown celery against organic celery in their taste-panels, organic was scored "better". We would like to examine the differences of organically-grown celery against conventionally-grown celery by completing volatile analysis and linking with sensory profiling, using the same methods as chapters 3 and 4. This has previously been examined in literature with D'Antuono, Neri and Moretti (2002) and Rożek, Nurzyńska-Wierdak, Sałata and Gumiela (2016) both identifying differences in the aroma composition

due to the use of organic fertilizer, however sensory analysis was not carried out and therefore we do not know whether these significant differences in volatile composition have a significant effect on the sensory characteristics. Finally, by using a consumer panel, we can identify whether consumers can detect a difference between organic and non-organic celery along with whether organic is preferred or not. In addition to this, we can further investigate the impact of different production methods such as indoor lighting and growing hydroponically on celery. These methods will inflict alternative stresses on the crop.

Finally, a mentioned throughout the thesis, celery is a vegetable that is not only consumed raw, in salads, but cooked, forming part of many soups, stocks and sauces. There has been little research investigating the changes of celery flavour during cooking and by conducting this experiment using the hybrids created in this project, we can identify whether we have created a variety that would be better suited for cooking. Here, we can continue to observe the patterns in volatile changes and how the variables studied throughout the project would impact the flavour further.

8.3. Final Remarks

In the preceding chapters, we confirmed the significant influence of genotype, growing environment and maturity in celery, highlighting the importance of selecting genotype according to environment. Genotype predetermined the aroma composition, as observed in chapter 1, but fundamentally, growing environment including geographical location, temperature, relative humidity, and rainfall lead to significant changes in the aroma composition. We are confident that the completion of this project will provide a greater understanding to fresh produce growers and breeders on the factors that influence celery and its flavour, aiding in the development of new hybrid lines that are consumer driven, as well as in producing a stable, high-quality product.

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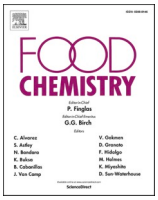

Investigating the factors that influence the aroma profile of *Apium graveolens*: A review

Lucy Turner [a], Stella Lignou [a,*], Frances Gawthrop [b], Carol Wagstaff [a]

[a] Department of Food and Nutritional Sciences, University of Reading, Harry Nursten Building, Pepper Lane, Whiteknights, Reading RG6 6DZ, United Kingdom
[b] A.L. Tozer Ltd, Pyports, Downside Bridge Road, Cobham KT11 3EH, United Kingdom

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ABSTRACT

Celery *(Apium graveolens)* is a regularly consumed vegetable, providing strong, distinct flavours to dishes as well as health benefits. Constituents of the aroma profile of celery include a range of volatile compounds (terpenes, phthalides and aldehydes) that contribute to its characteristic odour and flavour. Vast amount of research has been completed on the aroma profile of celery. However, there is limited information stating the cultivar, origin and geographical location, despite that research on a plethora of other crops has indicated that these are key factors driving crop performance and quality attributes. This paper characterises the underlying biochemistry that determines the aroma profile of celery, whilst investigating the genetic and environmental influences leading to its variation. We make recommendations for minimum standards (MIAPAE: Minimum Information About a Plant Aroma Experiment) that should be adopted by the scientific community prior to publication of data relating to flavour and aroma characterisation of crops.

profile of *Apium graveolens*: A review

1. Introduction

Celery is a member of the Apiaceae or Umbelliferae family, known for the shape of their aromatic flowers called umbels. Crops belonging to this family exhibit distinct flavours including parsley, carrot, fennel, dill and coriander (Terry, 1989). Celery is most frequently used during cooking as well as consumed in its raw state in salads or with condiments (Rożek, 2007). Celery is thought to be part of the "holy trinity" in many cuisines, combined with bell peppers and onions to form Cajun holy trinity or combined with carrots and onions to form "Soffritto" in Italian cooking.

There are three main subspecies of *A. graveolens*: leaf celery (*Apium graveolens* L. subsp. *secalinum*), stalk celery (*Apium graveolens* L. subsp. *dulce*) and root celery, also known as celeriac (*Apium graveolens* L. subsp. *rapaceum*). Stalk celery and celeriac are consumed often as vegetables globally, whereas leaf celery or Chinese celery is commonly cultivated and consumed in East Asian countries. Currently on the market, there is an assortment of celery produce available for consumption which is presented in a variety of formats; prepacked whole celery (the celery base, long petioles and leaves, often cut below any knuckles), prepared celery sticks (chopped petioles with no leaves or knuckles) and celery hearts (chopped, with inner petioles; exposing the heart of the celery). Furthermore, celery can be grown as a white, green or pink variety. Varieties can also be found in a range of heights and appearances including noticeable ribs along the petioles, low knuckles or bowing petioles.

Studies have shown that petioles and leaves share similar volatile compounds, however it is often seen that the leaves are much more aromatic than the petioles and a higher yield of essential oil is gained from the leaves (Li, Hou, Wang, Tan, Xu & Xiong, 2018). Typically, it is the celery petioles that are often consumed in the UK; however, the leaves are consumed in other countries and form part of salads or as a garnish for traditional dishes. Conversely, the aromatic herb coriander, also a member of the Apiaceae family, is used regularly in cooking but the seeds and leaves are utilised.

Celery is a versatile plant grown for many functions; the seed, which commonly undergoes extraction to obtain essential oil, can be used as a flavouring agent but also for medicinal uses. The seed has been reported to have excellent anti-inflammatory and antioxidant potential. Kaufman, Cseke, Warber, Duke, and Brielmann (1999) identified over two dozen compounds having the above properties including a range of phthalides, chlorogenic acids, flavonoids (apigenin and luteolin) as well

* Corresponding author.
E-mail addresses: l.turner@pgr.reading.ac.uk (L. Turner), s.lignou@reading.ac.uk (S. Lignou), frances.gawthrop@tozerseeds.com (F. Gawthrop), c.wagstaff@reading.ac.uk (C. Wagstaff).

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as terpenes. Celery is consumed as a salad vegetable and regularly used as a flavouring agent in stock, soups and bouillons (Malhotra, 2012); its distinct flavour is made up of a combination of volatile compounds that are responsible for the grassy, herbal aroma. These compounds range from aldehydes and esters to terpenes and phthalides, the latter found to contribute most significantly to the characteristic odour of *A. graveolens L.* (Macleod, MacLeod & Subramanian, 1988). These compounds, along with low molecular weight sugars, organic acids and flavonoids, are responsible for perceived taste and flavour (Rowan, 2011).

While celery has been the focal point in a plethora of literature reviews, the majority of these have been general reviews and not focused on collating data from previous studies to identify differences in the aroma profile and what may influence this. For example, a widespread and thorough review completed by Sowbhagya (2014) looked at the chemical, technological and nutraceutical functions of celery, however, there was limited focus on the aroma and the impact of variety or different environmental conditions on aroma. Conversely, Li et al. (2018) published a critical review on the advances in celery research providing an in-depth review discussing the current technologies as well as the developments in genetic breeding, genomics research and function genes in celery.

Predominately, research investigating celery flavour utilises the seed or essential oil, with fewer publications looking at the flavour of fresh samples. The flavour profile will change depending on the chemical composition which in turn will change as a result of genotype, season, the part of the plant that is consumed, the geographical region it is grown, the stage and the quality of harvest (Malhotra, 2012) as well as soil type, methods of extraction and analysis of the volatile components. This review aims to examine and elucidate current literature investigating the aroma compounds present in leaf and stalk celery (*Apium graveolens* L. subsp. *secalinum; Apium graveolens* L. subsp. *dulce*), determine how these compounds contribute to flavour and identify factors that play a role in influencing the aroma, thus showing the need for minimum standards to be adopted by the scientific community, allowing for the creation of a repository with potentially replicable and high quality data.

2. Methodology

In order to carry out the review, the scientific search engines that were used were Web of Science, ScienceDirect and Google Scholar. Web of Science was mainly used as it offers access to a broader variety of scientific datasets which can be searched singly or simultaneously, including; BIOSIS Previews, Data Citation Index and Food Science and Technology Abstracts (FSTA). Articles were sorted in accordance to relevance of the search string used.

Table 1
Key words and synonyms used for searching databases.

Main Key word	Synonym
Celery	• *Apium graveolens* • Umbelliferae • Apiaceae • Cultivar • Crop
Aroma profile	• Volatile • Essential oil • Flavour • Odour • Terpenes • Phthalides • Secondary metabolites
Postharvest	• Maturity • Ripening • Shelf-life • Quality
Environment	• Geographical location • Season

The following keywords were identified: celery, aroma, postharvest, environment (Table 1). These key words were either used in conjunction or separately. Search operators and search strategies were adopted including key word synonyms, truncation and wildcard symbols in order to help to refine or widen the search. Search strategies were vital for the refinement of the journals used for this review as a vast quantity of journals have previously investigated celery, with close to 3000 journals available for use (Table 2).

There were no limitations on dates of papers used, the majority of papers found were published from 1969-present and references were exported to Mendeley reference manager. Furthermore, peer-reviewed journals and journals where access was available through the University of Reading library services were preferred. Originally, papers were considered for evaluation depending on the information they included such as harvest date, cultivar used and cultivar origin, however, this meant many papers were eliminated due to the absence of information of this nature.

3. Volatile compounds contributing to aroma and flavour

Within nature, volatiles are comprised of a diverse range of organic compounds that occur naturally, performing multiple functions; from plant and insect signalling through pheromones to food whereby flavour compounds influence organoleptic properties (Pichersky & Gershenzon, 2002). In plants, a range of biosynthetic pathways occur leading to the formation of different products. It has been identified that agents of primary metabolism are the original precursors for the biosynthetic pathways that lead to volatile synthesis. These include carbohydrates, fatty acids and amino acids Croteau & Karp, 1991; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008). For example, amino acid degradation will lead to the synthesis of phenylpropanes and benzenoids, these are the precursors involved in the synthesis of aromatic alcohols, aldehydes and esters. Whereas in food, flavour compounds can be synthesised through a number of pathways for example, cooking methods such as grilling or roasting, causing the formation of flavour compounds through the Maillard reaction.

Table 3 shows a collection of volatile compounds including terpenes, alcohols, aldehydes and phthalides that have been identified in celery from published data. This is accompanied by Table 4, which contains the environmental and genotypic data that was included in the studies to build Table 3.

It can be seen in Table 3 that there is a variety of compounds present in celery that contribute to its aroma. Although the vast majority of literature focuses on the terpene and phthalide content, the number of other compounds present in celery including alcohols, esters and aldehydes should not be ignored as these are responsible for fresh, grassy and green notes. The reporting levels of these compounds remain relatively low in comparison to terpenes and phthalides, with *(E)*-2-hexen-ol, *(Z)*-3-hexenal, and hexanol only being reported a handful of times.

Completing the review has shown that the aroma compounds present in *A. graveolens* differ considerably depending on cultivar, geographical location, processing, extraction method and the material used. Table 3 shows the compounds most commonly reported, and these are: limonene (17 times), 3-*n*-butylphthalide (15 times), β-pinene (14 times), α-pinene and myrcene (13 times), *(Z)*-caryophyllene and β-selinene (12

Table 2
Key words search results in Web of Science.

Search string	Full text available online	Relevant
Celery	2,925	3
Celery aroma profile	6	2
Volatile content of celery	11	2
Volatiles of celery essential oil	25	12
Phthalide content of celery	36	13
Celery postharvest	16	2

Table 3
Summary of volatile compounds identified in celery as reported in studies since 1963.

Compound Name	Aroma descriptor[a]	Reference[b]																					Concentration range (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total	
Aldehydes																								
hexanal	green, fatty, leafy		X							X		X					X						4	0.1–2.7
3-methylbutanal	fruity, chocolate, fatty		X					X															2	tr – 0.87
2-methylbutanal	musty, cocoa, nutty		X																				1	0.l7 – 0.45
furfural	sweet, almond, baked bread		X																				1	0.35–1.1
(*Z*)-3-hexenal	green												X				X						2	n/a
benzeneacetaldehyde	honey, floral rose, sweet				X																		1	tr – 0.13
heptanal	green, herbal, fatty											X					X						1	0.1
octanal	citrus, orange peel, green											X					X						2	tr
nonanal	waxy, aldehydic, fresh				X							X											1	tr – 0.26
undecanal	waxy, soapy, floral																X						1	n/a
dodecanal	waxy, soapy, citrus																X						1	n/a
citronellal	waxy, floral, herbal																X						1	n/a
(*E*)-2-nonenal	green cucumber, aldehydic												X										1	n/a
Alkane																								
2-methylpentane												X											1	0.1
3-methypentane												X											1	0.1
hexane												X											1	0.1
octane												X											1	0.1
nonane												X											1	0.3
Alcohols																								
(*Z*)-3-hexenol	green												X			X	X	X			X	X	6	tr – 3.96
1-hexanol	green, fruity, apple															X	X	X					3	tr – 0.36
2-hexanol														X									1	1.2–1.3
heptanol	musty, leafy, herbal																X						1	n/a
(*E*)-2-hexen-ol	green, leafy, fresh, grassy																	X					1	n/a
linalool	citrus, floral			X				X				X			X								3	tr – 0.80
(*E*)-2,8-p-menthadiene-1-ol	fresh, minty							X										X					2	tr – 0.20
(*Z*)-2,8-p-menthadiene-1ol	fresh																	X					1	n/a
borneol	balsam, camphor, herbal			X																			1	1.4
geraniol	floral, fruity, rose			X																			1	0.6
thymol	herbal, thyme, phenolic			X					X														2	0.70–6.1
terpinene-4-ol	menthol, woody		X									X				X							3	tr – 1.19
dihydrocarveol	green, minty, sweet																	X					1	n/a
α-terpineol	citrus, woody, lemon											X				X		X					3	tr – 0.1
(*Z*)-carveol	spicy, caraway							X								X		X					3	tr – 3.4
carvacrol	spice, woody, camphor								X														1	1.9–3.4
limonene-1,2-diol	cool, minty																	X					1	n/a
(*E*)-carveol	spicy, caraway, spearmint																	X					1	n/a
(*E*)-p-mentha-1(7),8-dien-2-ol	camphor, menthol, phenol																	X					1	n/a
(*E*)-1(7)8-p-menthadiene-2-ol																		X					1	n/a
eugenol	sweet, warm			X				X															2	0.1–3.0
citronellol	floral, leather, waxy				X																		1	0.12
Globulol	floral, rose				X																		1	3.56
Alkene																								
(E,Z)-undeca-1,3,5-triene	fresh, green, greasy											X	X										2	tr
pentylcyclohexadiene			X					X	X			X											4	0.2–4.5
Esters																								
2-octen-1-ol acetate	green, citrus, vegetable				X			X															2	tr – 5.38
(E)-3-hexenyl-1-acetate	sharp, fruity, green																		X				1	0.25
carvyl acetate	green, spearmint, herbal						X		X	X							X		X				4	tr – 25
bornyl acetate	woody, pine, herbal								X														1	tr – 0.2

(*continued on next page*)

Table 3 (*continued*)

Compound Name	Aroma descriptor[a]	Reference[b]																					Concentration range (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total	
α-terpinyl acetate	sweet, herbal, bergamot			X													X						2	0.1
phenylethyl propanoate	floral, red rose, fruity				X																		1	0.61
(*Z*)-3-hexenyl pyruvate	green, oily, melon																X						1	n/a
(E)-pinocarvyl acetate								X											X				1	tr – 1.0
Monoterpenes																								
α-thujene	woody, green,		X	X			X					X		X									5	tr – 7.5
α-pinene	fresh, woody		X	X		X	X	X	X		X	X		X	X	X				X	X		13	tr – 9.59
camphene	citrus, cooling		X			X	X		X			X		X	X					X	X		9	tr – 0.29
sabinene	citrus, pine, spicy		X			X	X	X	X		X	X		X									9	tr – 1.72
β-pinene	green, nutmeg,		X			X	X	X	X		X	X		X	X	X				X	X	X	14	tr – 11.51
myrcene	balsam, fruity,		X	X	X	X	X	X	X		X	X	X	X	X	X	X				X		13	tr – 20.97
α-phellandrene	citrus, herbal, green										X			X							X		3	0.1–0.28
d-3-carene	citrus, pine, herbal				X										X	X							4	tr
α-terpinene	terpenic, pine			X								X		X									3	0.1–0.5
p-cymene	cumin, lemon		X				X	X		X	X	X		X		X							8	tr – 0.31
limonene	citrus, pine, minty		X	X	X	X	X	X	X	X	X	X		X	X	X	X			X	X	X	17	tr – 84
β-phellandrene	minty, terpenic							X				X											2	tr – 0.6
β–(*E*)-ocimene	sweet, herbal		X				X		X		X	X		X	X					X			8	0.1–12.50
β–(Z)-ocimene	warm, floral, herbal						X	X	X		X										X		5	tr – 10.1
γ-terpinene	sweet, citrus		X	X		X	X	X			X	X		X		X				X			10	tr – 78.24
dihydrocarvone	herbal, minty, mentholic							X								X			X				3	tr – 50.0
L-carvone	spearmint, herbal, minty					X				X									X				3	0.19–10.0
p-mentha-1,3,8-triene	terpenic, camphoreous									X	X	X											3	tr – 2.3
Sesquiterpenes																								
α-copaene	woody, spicy, honey				X									X						X			3	tr – 0.82
(*E*)-caryophyllene	sweet, woody, spice				X		X		X		X												4	0.1–8.1
(*Z*)-caryophyllene	clove, pepper, woody		X	X	X	X		X				X		X	X	X				X	X	X	12	tr – 10.5
α-humulene	woody		X				X	X	X					X						X	X	X	8	tr – 8.3
ar-curcumeme							X	X				X											3	tr – 0.4
β-selinene	herbal		X	X	X	X		X	X	X		X		X	X					X		X	12	0.6–16.3
α-selinene	pepper, orange, amber		X			X	X	X		X		X		X	X	X				X			10	tr – 2.8
(Z)-β-guaiene	woody, spicy, powdery						X																1	2.6
cuparene	woody, cedar, floral				X																		1	0.64–2.11
(*E*)-β-farnesene	woody, citrus, herbal				X						X												2	0.1–1.27
kessane					X		X	X	X			X		X									6	0.6–5.34
liguloxide							X																1	tr
spathulenol	earthy, herby, fruity			X	X									X									2	tr – 4.43
Phthalides																								
alkyl phthalide												X											1	tr
3-butylhexahydrophthalide	celery		X						X			X							X			X	5	tr – 1.2
3-*n*-butylphthalide	celery, herbal, phenolic	X	X	X	X			X	X	X		X	X	X	X	X	X		X			X	15	tr – 20.0
(*Z*)-3-butylidenephthalide	celery, herbal	X	X	X					X			X				X	X						7	0.1–30.5
(E)-3-butylidenephthalide	herbal, lovage, celery	X			X							X											3	1.0–20.1
cnidilide	celery, herbal		X									X											2	tr – 41.0
Sedanenolide	herbal	X	X	X				X	X	X		X		X		X							9	0.2–39.5
(E)-sedanolide	herbal, celery											X											1	5
(Z)-sedanolide	herbal, celery											X											1	1.4
(*Z*)-ligustilide	herbal, celery		X		X		X		X			X				X							6	tr – 47.31
sedanolide	herbal, celery	X	X					X	X	X			X	X		X			X			X	11	0.2–45.2
(*E*)-ligustilide	sweet, spicy		X		X					X		X	X	X	X	X							9	0.1–6.95
Other compounds																								
2-pentyl furan	green, fruity, earthy				X					X						X							3	tr – 0.35
camphor	camphoreous			X												X							2	tr – 0.6
pentylbenzene					X			X		X						X							4	tr – 1.84
2-undecanone	waxy, fruity, fatty				X																		1	0.42–0.54

(*continued on next page*)

Table 3 (*continued*)

Compound Name	Aroma descriptor[a]	Reference[b]																						Concentration range (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total	
caryophyllene oxide	sweet, fresh, spicy parsley, herbal				X		X	X	X					X									4	tr – 4.11
apiole				X			X			X	X												4	0.1–23.2
Total Compounds Identified		**5**	**28**	**22**	**24**	**11**	**21**	**29**	**25**	**15**	**14**	**40**	**8**	**24**	**13**	**24**	**17**	**12**	**7**	**11**	**10**	**9**		

[a] Odour descriptors identified using The Good Scents Information System.

[b] (1) Uhlig et al., 1987 (2) van Wassenhove, Dirinck, Vulsteke, & Schamp, 1990a (3) Sellami et al., 2012 (4) Shojaei, Ebrahimi, & Salimi, 2011 (5) Sorour et al., 2015 (6) Rożek et al., 2016 (7) Philippe, Suvarnalatha, Sankar, & Suresh, 2002 (8) Marongiu et al., 2013 (9) MacLeod et al., 1988 (10) Orav et al., 2003 (11) MacLeod & Ames, 1989 (12) Kurobayashi et al., 2006 (13) Wolski et al., 2004 (14) Jian-Qin et al., 1990 (15) Tang et al., 1990 (16) Gold & Wilson, 1963 (17) Wilson, 1967 (18) Wilson, 1970 (19) Ehiabhi et al., 2006 (20) Deng et al., 2003. (21) Lund et al., 1973; tr = value was less than 0.1; n/a = data not available.

times). Out of alcohol, ester and aldehyde compounds, the highest reported compound is *(Z)*-3-hexenol (6 times) followed by linalool (4 times). Out of the 21 papers, Wilson (1967) and Gold & Wilson (1963) reported the highest number of aldehydes and alcohols.

Table 4 lists all the various isolation and analysis methods that have been used across the studies to construct Table 3. The most popular method of extraction is hydrodistillation (HD) followed by gas chromatography/mass spectrometry (GC/MS). Although HD is a traditional method of extraction that is regularly used throughout industry, the high temperatures used can contribute to the thermal degradation of some volatile components (Oreopoulou, Tsimogiannis & Oreopoulou, 2019). Victório, Riehl & Lage (2009) compared the volatile content using simultaneous distillation–extraction (SDE), HD and static headspace methods on *Aplinia zerumbet* (Pers). Although they found a difference in the composition of the essential oil between these processes, they concluded that all methods were suitable for the analysis of volatiles, however, SDE is more suitable for analysing smaller quantities of plant material (Victório, Riehl, & Lage, 2009).

Using a method where volatiles can be isolated from a matrix at room temperature under a vacuum, will prevent thermal degradation of compounds and improve recovery rates. MacLeod and Ames (1989) used low temperature high vacuum distillation and identified 40 compounds including 13 monoterpenes, 12 phthalides and five sesquiterpenes as well as several alcohols, alkenes and alkanes. Utilising high vacuum distillation allows for the separation of higher boiling compounds such as phthalides, which has been shown to be difficult to isolate and characterise in previous studies shown by Orav, Kailas and Jegorova (2003). Here six phthalides isomers were identified but the correct characterisation of these isomers could not be completed.

In terms of analysis, the majority of the studies (Table 4) used 1D GC in order to analyse celery volatiles. However, with this method, correct characterisation of phthalides was shown to be limited and even in some studies, no phthalides were identified. The utilisation of 2D GC has shown to aid in the correct separation of phthalides as well as the characterisation of phthalide isomers (Bartschat, Beck, & Mosandl, 1997; MacLeod & Ames, 1989; van Wassenhove et al., 1990a).

Only one study by Deng, Song, Zheng, Hu & Zhang (2003) analysed fresh celery samples by extracting the volatiles present in the headspace using solid phase micro-extraction (SPME) followed by GC/MS. However, investigating celery as an essential oil has shown to yield results with more identifiable compounds than SPME as shown by MacLeod & Ames (1989); van Wassenhovet et al. (1990a); Philippe et al. (2002) and Shojaei et al. (2011) (Table 3, reference 11, 2, 4 and 7).

Orav et al. (2003) and Sorour, Hassanen and Ahmed (2015) compared the differences in volatile content between fresh and dried celery material and concluded that processing the celery through methods such as freeze drying or air drying should not alter the presence of aroma compounds but only the abundance of certain compounds. This was confirmed by Orav et al., (2003) who investigated the difference of aroma profiles in fresh celery and air dried, oven dried and freeze-dried celery, showing that there was little difference between the processing methods in terms of the presence or absence of compounds; but differences were observed in terms of the concentrations of certain compounds (e.g. a decrease in limonene and a slight increase in phthalide concentration).

Table 3 also shows the variation in % composition between compounds. Although variation is expected when so many variables are involved, certain compounds show a extreme variation; the biggest occuring within the monoterpenes, particularly for limonene and γ-terpinene. Both of these compounds have been identified to be very common monoterpenes in celery as shown by van Wassenhove et al. (1990a), identifying limonene and γ-terpinene as the most abundant compounds across four varieties. A possible cause of this variation could be due to the influence of abiotic and biotic factors, such as maturity and environment, have upon these compounds. Thus, showing the importance of examining the same cultivar across different seasons in different

Table 4
Summary of environment × genotype using the references found in Table 3.

Ref[a]	Variety used	Cultivar origin	Geographical location of growth	Year(s) grown	Material tested	Extraction and analysis method
1	Utah 52–70, Giant pascal, Chinese Heug-Kunn, French dinant, Golden self-blanching, Camlyn, Florida 2–14, Clean-cut Harris	N/A	Michigan, USA	1985	Fresh	Solvent extraction and separated by HPLC and identified by GC/MS
2	Blancato, Avon Pearl, Golden Spartan, Loret	N/A	Roeselare-Rumbeke, Belgium	1986 and 1987	Essential oil	Extracted by simultaneous steam distillation–extraction (likens-Nickerson) and identified by high-resolution multi-dimensional gas chromatography with FID
3	N/A	N/A	Soliman, Tunisia	2008	Essential oil and fresh	Extracted with solvent extraction and hydrodistillation and identified using GC/FID
4	Wild Type	N/A	Koohrang, Bazoft and Samsami, Iran	2008	Essential oil	Extracted by hydrodistillation and identified using GC/MS
5	N/A	N/A	Agriculture Research Centre, Egypt	2013	Fresh and dried	Extracted by hydrodistillation and identified using GC/MS
6	Safir	Netherlands	Lublin, Germany	2019	Fresh	Extracted by steam distillation and identified using GC/MS/MS
7	Gaudich	Punjab, India	Kanpur and Punjab, India	N/A	Celery seed oil	Oils sourced for the study and identified using GC/MS
8	N/A	Europe	Italy and Portugal	N/A	Fresh	Extracted by SFE and hydrodistillation and identified using GC/FID and GC/MS
9	N/A	Libya	Libya, brought fresh	N/A	Fresh	Extracted by steam distillation and identified using GC/FID and GC/MS
10	N/A	Estonia	Brought fresh	N/A	Fresh and air-dried essential oil	Extracted by SDE and identified by capillary GC and GC/MS
11	Celebrity	N/A	Brought fresh	N/A	Fresh	Extracted by high vacuum-low temperature distillation and identified using GC/GC/FID, GC/MS and GC/OPA
12	N/A	N/A	Nagano Prefecture, Japan brought fresh	N/A	Fresh	Extracted by hydrodistillation followed by SAFE and identified using GC/FID, GC/MS and
13	N/A	N/A	N/A	N/A	Fresh	Extracted by solvent extraction and identified using GC/ITMS
14	N/A	N/A	N/A	N/A	Celery seed oil	Extracted by steam distillation and identified using GC/MS and GC/FTIR
15	N/A	N/A	Brought fresh	N/A	Fresh	Solvent extraction and identified using GC and GC/MS
16	N/A	N/A	Brought fresh	N/A	Celery juice	Extracted by steam distillation, fractions were collected in portions of the apparatus (column-bottom, chilled water trap, ice trap, salt and ice trap, dry-ice trap and liquid nitrogen trap). Identified using GC, GC/FID and GLC
17	N/A	N/A	N/A	N/A	Essential oil	Extracted by batch and continuous steam distillation followed by solvent extraction, and identified using GC/MS F&M
18	N/A	N/A	N/A	N/A	Essential oil	Extracted by batch and continuous steam distillation, identified using GC/MS
19	N/A	N/A	Nigeria	N/A	Essential oil	Extracted by hydrodistillation and identified using GC/MS
20	N/A	N/A	Research Centre for Plants, Shenghai	N/A	Fresh	HS-SPME-GC/MS was using for extraction and identification
21	Utah 5270 and Flormart		Florida	November 1972, April and July 1973	Essential oil	Extracted by steam distillation, volatile content determined by "Bromate Titration Method" and were separated using GLC.

[a] Refer to Table 3 for references.

geographical locations. Although not as vast, variation between the reported composition of phthalides can be seen, particularly with cnidilide, (*Z*)-ligustilide and sedanolide. Characterising phthalides and their enantiomers correctly have been shown to difficult using 1D GC and hydrodistillation techniques, this could explain the variation between extraction processes.

Furthermore, out of the 21 papers that were used to build Table 3, 13 papers mentioned the geographical region the cultivar under investigation was grown, seven provided the celery cultivar name, seven provided growing and harvesting dates, five mentioned the cultivar origin, three completed a multisite experiment, three used more than one cultivar and only one repeated the experiment the following year (Table 4). Not one paper used one single cultivar in a multisite experiment that was repeated the following season. The vast quantity of research that has been completed on celery and its aroma profile can only be described as partial and inconclusive. Clearly, there is variation in the aroma profile and simply studying one cultivar, grown in one location, in one year is not a sufficient sample size or experiment to conclude the following compounds are the only compounds to be present in celery. There was no compound that was detected in every study on celery.

It is clear from Table 4 that many authors do not record basic information regarding the provenance of their samples, this would enable some consideration of the genetic and environmental influences on aroma compounds. Other communities have developed standards for minimum information required for characterising raw materials used in experimental datasets and it is recommended that the flavour science community also adopts a similar approach.

Plant phenotyping experiments, and it could be argued that flavour and aroma are a subset of phenotype, are already required to adhere to

Table 5
Recommended attribute checklist for plant aroma experiments.

Checklist section	Attribute	Recommended information to include
Experimental design	Field	Replication, block design, harvest protocol
	Laboratory	Replication, analysis protocol including extraction protocol, use of standards, temperature programs, QCs and statistical analysis used
Sample information	Seed	Preparation, source, pre-treatments
	Plant	Taxon, common name, origin, cultivar, age and life stage at harvest
	Plant extract	Type of extract used e.g. essential oil, fresh or dried material
Timing and location	Timing	Start and duration of experiment, timings between the stages of harvest and processing
	Location	Growth, post-harvest, processing and storage location
Environment	Met data	Average day and night temperature (°C), rainfall (mm), day and night length (hours)
	Agronomic practices	Treatments, watering and irrigation (L)
	Nutrients	Fertiliser composition and amount added, soil salinity
	Postharvest	Temperature of storage (°C), transport between facilities, processing and storage conditions
Raw material collection, processing and storage	Collection	Plant organ of interest, method of collection
	Processing	Method of processing, duration, location and temperature
	Storage	Method of storage, duration, location and temperature

standards. The proposed guidelines for the correct handling of data from plant phenotyping experiments to allow for data reuse and combining are known as the "Minimum Information About a Plant Phenotyping Experiment" (MIAPPE). These guidelines contain a checklist of attributes that would aid in the understanding of the plant phenotypic data and how it was obtained. The checklist of attributes can be categorised into the following sections: general metadata, timings and locations, environments, treatments, experimental design, sample collection and processing and observed variables (Ćwiek-Kupczyńska et al., 2016). Similarly, MIAME: Minimum Information About a Microarray Experiment present six fundamentals that enable the correct interpretation of results and experimental repetition including: the raw data for each hybridisation as well as the final processed data for the set of hybridisations, essential sample annotation (experimental factors), experimental design, annotation of the array and essential protocols (laboratory and data processing) (Brazma et al., 2001).

Following a similar attribute checklist to MIAME and MIAPPE, Table 5 presents MIAPAE: 'Minimum Information About a Plant Aroma Experiment', describing the minimal information that would allow for accurate interpretation and correct repetition of the experiment. Including the attributes presented in Table 5 allows for sufficient information to be provided, ensuring experiments whereby the aroma of plants is profiled can be interpreted, verified and repeated correctly, with the ultimate goal of facilitating the formation of superior datasets.

The variation in compounds identified in celery between experiments investigating the aroma profile can be seen clearly (Table 3) and with different cultivars, experimental designs, processing methods and instrumental analysis, however, it is difficult to compare these results. Using the proposed MIAPAE standards, whereby information on the experimental design, sample collection, processing and testing is

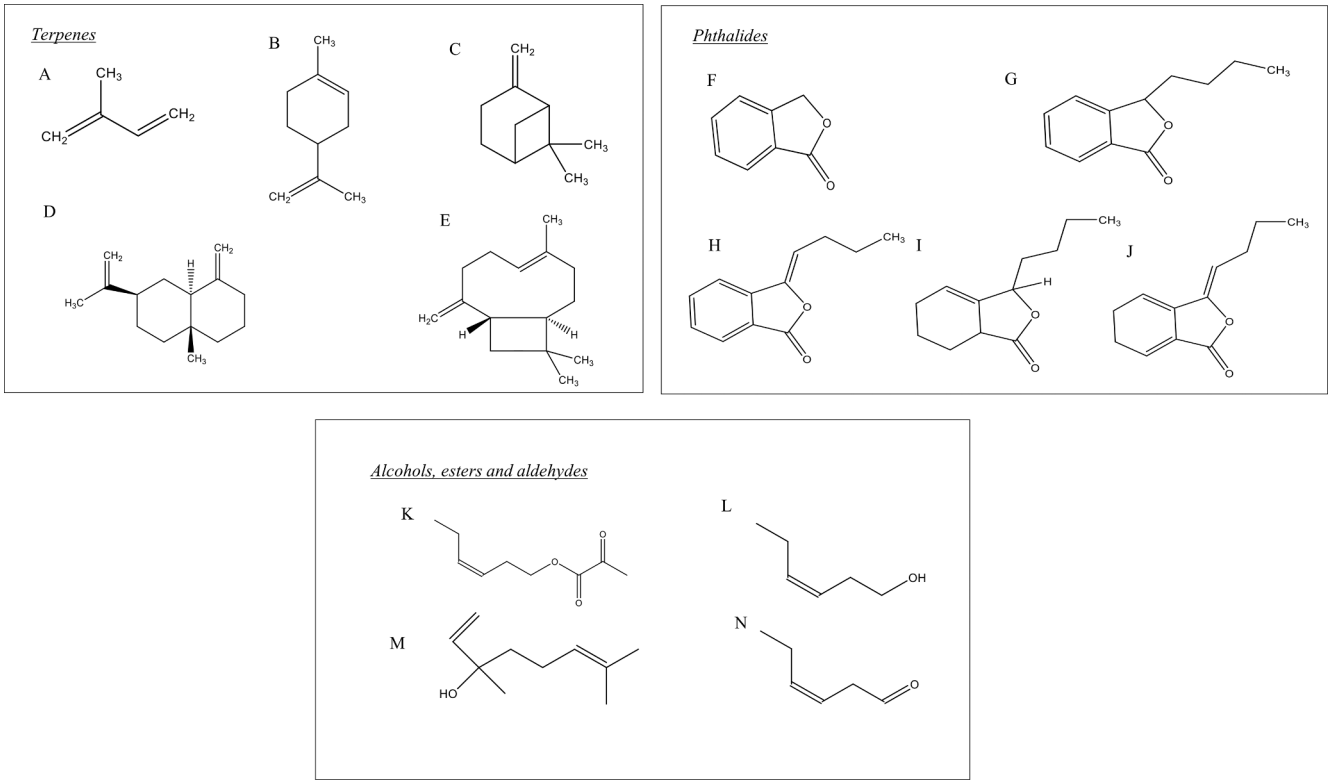

Fig. 1. A range of volatile compounds that occur and contribute to the typical aroma of celery; isoprene (A), limonene (B), β-pinene (C), β-selinene (D), β-caryophyllene (E), 1(3H)-isobenzofuranone (F), butylphthalide (G), 3-butylidenephthalide (H), (Z)-ligustilide (I), sedanenolide (J), (Z)-3-hexenyl pyruvate (K), (Z)-3-hexen-1-ol (L), linalool (M) and (Z)-3-hexenal (N).

included, experiments can either be replicated or variables changed/introduced to allow for further comparison, collation of datasets and eventually leading a possible public repository with the purpose of providing high-quality plant aroma data.

3.1. Terpenes

The aroma of raw celery is often described as fresh, herbal, woody and citrusy, and the main contributors to these descriptors are terpenoids, sesquiterpenes and monoterpenes. These are all major components that constitute the aroma profile in celery, as well as ubiquitous across many other flowers, herbs, spices and food stuffs. Terpenes play a diverse range of roles in nature and in industry, from insect and plant signalling to fragrances and flavourings.

Terpenes are mostly hydrocarbons and are constituents of essential oils. Isoprene, a unit made up of five carbons, is the building block for terpene synthesis and when biosynthesis occurs, isoprene forms either acyclic, cyclic or polycyclic compounds (Parker, 2015). Celery contains a range of monoterpenes, two isoprene units ($C_{10}H_{16}$), and sesquiterpenes, made up of three isoprene units ($C_{15}H_{24}$) and these can be cyclic or bicyclic in structure, including: isoprene, limonene, β-pinene, β-selinene and β-caryophyllene. The structure of β-caryophyllene includes a nine-membered ring that is fused to a cyclobutene ring (Fig. 1).

Biosynthesis of terpenes occurs from isopentane either through the mevalonic acid pathway (appendix 1, schematic 1) (MVA-pathway) from acetyl-CoA or the non-mevalonate pathway (appendix 1, schematic 2). During the MVA-pathway, the pyrophosphorylation of mevalonic acid leads to the production of mevalonic acid pyrophosphate (MVA-PP), decarboxylation and dehydration of MVA-PP will result in the formation of isopentenyl diphosphate (IPP). IPP can be isomerized to produce dimethylallyl diphosphate (DMAPP). The bonding of IPP with DPP leads to the synthesis of geranyl pyrophosphate (GPP), which is the precursor of monoterpenes, and then the bonding of a further IPP molecule forms farnesyl pyrophosphate, the precursor of sesquiterpenes (Schwab et al., 2008). Alternatively, isoprene can also be synthesised through the non-mevalonate pathway or the MEP/DOXP, which similarly to the MVA-pathway, leads to the production of IPP and DPP. However, the MEP/DOXP-pathway occurs more predominantly in green plants, operating in the plastids, utilising D-glyceraldehyde 3-phosphate bonding with pyruvate to form 1-deoxy-D-erythritol (DXP). This eventually leads to the production of DMAPP, IPP and GPP to synthesise predominantly monoterpenes and some sesquiterpenes. In contrast, the MVA-pathway operates in the cytosol and synthesises mostly sesquiterpenes, sterols and triterpenes (Kuzuyama & Seto, 2012).

Within *A. graveolens*, there has been a wide range of terpenes reported in literature including a variety of monoterpenes and sesquiterpenes. Monoterpenes such as d-limonene (62.4–70.3%) and *(I)*-β-ocimene (10.1–10.5%) contributed the largest proportion of volatiles present in fresh celery grown in Estonia (Orav et al., 2003) (Table 3, reference 10), whereas, Jian-Qin et al. (1990) (Table 3, reference 14) identified in celery seed oil d-limonene (72.16%), β-selinene (12.17%) and α-selinene (2.05%) as the most abundant terpenes.

Limonene (18,000–37,000 μg/kg), λ-terpinene (6,000–16,500 μg/kg) and β-pinene (436–1,205 μg/kg) were most abundant across the four varieties used in an investigation carried out by van Wassenhove et al. (1990a) using blanching varieties grown in Belgium (Table 3, reference 2). The variation across the four cultivars used in this study provides evidence that there is a genetic basis for flavour deviation between cultivars. Throughout literature, it can be seen that limonene is the most abundant terpene, with an odour often described as citrus, fresh and lemon. However, limonene is not a key characteristic aroma compound, with a reported odour threshold range of 0.50–0.59 ppb orthonasal and 0.46–0.62 ppb retronasal (Plotto, Margaría, Goodner, Goodrich & Baldwin, 2004).

A study carried out by Deng et al., (2003) utilised SPME GC/MS to analyse the volatile constituents making up celery, identifying many compounds including monoterpenes and terpenoids. Obtaining a cultivar grown in Shanghai, Deng et al. (2003) confirmed the high proportion of limonene present (32.22% relative contents), followed by α-pinene (16.56% relative contents), and β-ocimene (9.59% relative contents). These values differ considerably when comparing literature (Table 3) suggesting that multiple factors play a role in celery flavour including geographical location and cultivar (Deng et al., 2003).

3.2. Phthalides

Phthalides are naturally sourced in plants, being particularly abundant in *Ligusticum* and *Angelica* from the Apiaceae family (Karmakar, Pahari & Mal, 2014). Celery, celeriac and lovage are rich sources of phthalides and these compounds hold many health benefits; they are biologically active compounds playing roles on the central nervous system and cardiac performance, aiding in anti-thrombotic modulation and providing protection against cerebral ischaemia and high blood pressure (Lin, Chan, Chung, & Li, 2005). In 2002, synthesised *dl*-3-*n*-butylphthalide, established from 3-*n*-butylphthalide, was approved by the China Food and Drug Administration as a new drug for the treatment of strokes. Previous research shows a significant increase of cerebral blood flow in cerebral ischemia rats when *dl*-3-*n*-butylphthalide was used as treatment (Yan, Feng, & Zhang, 1998). More recently, a 90-day administration of *dl*-3-*n*-butylphthalide was completed, whereby the administration of *dl*-3-*n*-butylphthalide had significantly more favourable outcomes than Ozagrel, a drug commonly used to treat strokes (Cui et al., 2013).

Structures and biosynthetic pathways of phthalides have been suggested previously but they remain ambiguous and little is actually known about these compounds. One possible pathway way has been suggested by Karmakar et al. (2014) (appendix 1, schematic 3). They hypothesised that phthalide is originally synthesised from tetraketide **(2)** which in turn, is formed from the condensation of four acetic acid units **(1)** bonded by the action of polyketide synthase. According to Karmakar et al. (2014), dialdehyde **(8)** is synthesised through the condensation of the tetraketide unit to orsellinic acid **(3)** though various enzymes (ketoreductase, cyclases and aromatases). Then, orsenllic acid is subject to methylation, regiospecific oxidation and decarboxylation **(4–7)**. An intramolecular Cannizzaro reaction **(9)** occurs producing phthalide **(10)** from dialdehyde. Phthalides are classified according to their substitution at C-3 and the oxidation occurring within the benzene ring (Karmakar et al., 2014). This can be seen in Fig. 1, where the double bonds within the benzene ring change along with the arrangement present at C-3 to produce a different compound.

To date, all naturally occurring phthalides are derived from 1(*3H*)-isobenzofuranone consisting of one benzene ring bonded with a γ-lactone between carbon atoms. 1(*3H*)-Isobenzofuranone has the most simple phthalide structure, $C_8H_6O_2$ (Lin et al., 2005). Multiple phthalides have been identified in celery including: phthalide, 3-butylphthalide, 3-butylidenephthalide, (*Z*)-ligustilide and sedanenolide (Fig. 1).

Using enantioselective multidimensional gas chromatography, Bartschat et al. (1997) analysed 3-butylphthalide enantiomers and eight 3-butylhexahydrophthalide stereoisomers in celery, celeriac, celery seed and fennel extracts. From this, 3-butylphthalide enantiomers (3*S* and 3*R*) were identified with 3*S* enantiomer showing to be the preferred configuration in all extracts. Furthermore, 3-butylhexahydroxyphthalides (3*R*,3a*R*,7a*S* and 3*S*,3a*R*,7a*S*) were detected and shown to be generated in high enantiomeric purity in celery and celeriac extracts. Bartschat et al. (1997) stated that the high enantiomeric purities of these compounds suggest that they may be synthesised with high stereoselectivity; originating from partially hydrogenate phthalides such as sedanolide and sedanenolide, known key contributors to *A. graveolens* odour.

Often in literature, the stereochemical aspects of these phthalide compounds have been neglected including the impact these have upon sensory characteristics. MacLeod and Ames (1989) analysed the volatile

components present in supermarket purchased celery and celeriac using GC, GC/MS and GC odour port assessment (GC/OPA) and positively identified 12 phthalides in both extracts including two 3-butylhexahydrophthalide isomers. Although the stereochemistry was not taken into consideration, these two isomers were shown to possess different odours according to GC/OPA. The first isomer identified exhibited a "sweet, sickly, cooked celery" and "braised celery, peppery, smoky" in celery and celeriac respectively. The second isomer was not identified in celery but was described as "celery, fruity, fragrant" in celeriac. MacLeod and Ames (1989) discussed how having a substitution of an alkyl group at C_3 would lead to a less celery odour compared to an alkylidene substitution whereby a more intense celery odour due to the alkylidene group increased from C_1 to C_4. This is in agreement with findings by Gold & Wilson (1963) who identified four alkylidene phthalides in celery juice distillate fractions that possessed a strong characteristic celery odour and were identified as the principal odour components of celery.

There has been conflicting evidence on whether phthalides are truly present as earlier studies were unable to separate and characterise phthalide compounds including 3-butylhexahydroxyphthalides enantiomers and the sedanolides. Uhlig et al. (1987) investigated the effect of phthalides on the flavour of celery using eight different cultivars of varying origins but grown in Grand Rapids, Michigan (Table 3, reference 1). DCM extracts of celery stem tissue were separated by HPLC and identified using GC/MS. The peak area per gram of total solids of butylphthalides (butylphthalide, *trans*- and *cis*- butylidene phthalide), sedanenolide and sedanolide were identified. Sedanolide was absent in six out of eight cultivars tested and they suggested that this result could be due to technical error, as the HPLC was unable to resolve minute quantities of sedanolide from sedanenolide. Within the cultivars, there was over six-fold variation in the abundance of different compounds, with butylphthalide abundance ranging from 250 to 1540 peak area per g total solids (Uhlig et al., 1987). In Uhlig's study, five phthalides were identified, almost half of the phthalides identified by MacLeod and Ames (1989).

For sensory evaluation, Uhlig presented the plant tissue from the samples diluted in water to six trained panellists, whereby the intensity of celery flavour was evaluated on a nine-point hedonic scale (1 = no celery flavour and 9 = extremely strong celery flavour). These flavour scores were correlated with the phthalide content, leading Uhlig to conclude that the variation of phthalide content across cultivars resulted in significant differences in the perception of celery flavour (Uhlig et al., 1987).

Phthalides, although lower in abundance in than terpenes, are much more odour-active, exhibiting flavour dilution factors of around 15,000 before the limit of detection is reached and can be seen to be characteristic compounds of celery aroma (Kurobayashi et al., 2006). Sedanenolide has an odour threshold value of 0.14 – 0.60 ppm depending on the enantiomer (Oguro & Watanabe, 2011) and 3-*n*-butylphthalide has a value of 0.00001 ppm (Bartschat et al., 1997). Furthermore, Lund, Wagner, and Bryan (1973) identified the odour threshold of phthalide compounds that expressed a celery-like odour. These included sedanolide (1 ppm), 3-*n*-butylphthalide (10 ppm) and hexahydro-3-*n*-butylphthalide (2 ppm) as well as β-selinene (1 ppm), although the latter were identified to not exhibit a characteristic celery odour when compared with sedanolide and 3-*n*-butylphthalide, they were still considered to be contributors to the fresh celery aroma. Out of these compounds, sedanolide was identified as the most characteristic compound to the celery odour.

3.3. Alcohols, aldehydes and esters

In plants, alcohols, aldehydes and esters originate from saturated and unsaturated fatty acids such as linolenic acid and are formed predominately by three processes: *α*-oxidation, *β*-oxidation and the lipoxygenase pathway. Initially, saturated and unsaturated fatty acids are bound to acyglycerols as triacylglycerides and are released as free fatty acids via enzymatic oxidative (acyl hydrolase) degradation of lipids. The lipoxygenase pathway, which leads to the synthesis of short-chain aldehydes and alcohols (C_6 and C_9), involves multiple enzymes including lipoxygenase (LOX), hyperoxide lyase (HPL) and alcohol dehydrogenase (ADH). LOX catalyses the conversation of linolenic acid to 9-hydroperoxide or 13-hydroperoxide.

With the use of enzymes or *β*-oxidation; aroma compounds are formed such as 3-*(Z)*-hexenol, *(E)*-jasmone and 3-*(Z)*-hexenyl acetate. For example, hexanal is a linolenic acid-derived aldehyde with a fatty, green odour, it is synthesised through a series of enzymatic reactions using LOX, HPL, 3Z,2E-enal isomerase and alkenal oxidoreductase (Schwab & Schreier, 2002; Stumpe & Feussner, 2006). Fig. 1 shows the compound structure for: (*Z*)-3-hexenyl pyruvate, (*Z*)-3-hexen-1-ol, linalool and (*Z*)-3-hexenal, these are just a selection of alcohols, aldehydes and esters that have been identified in celery. Compounds known as green leaf volatiles (GLVs) are synthesised in the plant when subject to biotic and abiotic stresses. These include compounds such as 3-(*Z*)-hexanol, 3-(*Z*)-hexenyl acetate and hexanal, these compounds often have green, fatty odours, important to celery aroma.

Few published papers focus on the presence of other volatiles such as alcohols, esters and aldehydes. These compounds are vital to the aroma, with odours described as green, fresh, citrus and floral. Shojaei et al. (2011) studied the chemical composition of three ecotypes of wild celery (Bazoft, Koohrang and Samsami) grown in three different regions of Iran in 2008 and identified a range of aromatic compounds using GC–MS analysis (Table 3, reference 4). Within the three ecotypes, at least 22 compounds were identified and phthalides made up the majority of the chemical composition. Compounds such as 2-octen-1-ol acetate, pentylbenzene and 2-undecanone were reported at much lower abundances, yet at similar concentrations to sesquiterpenes. Gold and Wilson (1963) investigated the volatile flavour substances present in celery juice, identifying 38 compounds comprising of aldehydes, esters, alcohols, terpenes and phthalides (Table 3, reference 16). Gold and Wilson identified the ester (*Z*)-3-hexenyl pyruvate as a principle odour constituent using a dry ice trap, with odour descriptors such as green, vegetative and floral green tea (Gold and Wilson, 1963).

Wilson (1967) identified and quantified the alcohol composition of celery essential oil using column chromatography on two celery essential oils. Using this method of separation allowed him to identify that the two essential oils were comprised of 10 to 15% alcohol, including hexan-1-ol, *(Z)*-3-hexene-1-ol and *(E)*-2-hexene-1-ol as well as terpene alcohols; *(E)*- and *(Z)*-2,8-p-menthadiene-1-ol (Table 3, reference 17). He concluded that although these alcohol compounds did not possess aromas that were typical of celery, they were still important contributors to the overall aroma and flavour (Wilson, 1967).

4. Genetics and the aroma of celery

Over the years, there has been a focus on improving yield to increase product availability as well as to decrease cost paid by the consumer. However, this means that there has been a lack of focus on the quality of crops and therefore, important traits such as flavour have been ignored. Key aspects of quality include nutritional content, post-harvest quality, being free of disease and eating quality. There has been a lot of focus on developing disease-resistant celery lines, particularly to Fusarium yellows (*Fusarium oxysporum* f. sp. *apii*) which is one of the biggest diseases to threaten celery production worldwide. It was Orton, Hulbert, Durgan, and Quiros (1984) who developed the first Fusarium-resistant celery line using a celeriac accession (Orton et al., 1984). Furthermore, breeding of late bolting or slow bolting variety has also been emphasised to improve yield, particularly during the winter-spring season to extend the season (Li et al., 2018).

There are multiple reasons as to why emphasis on breeding for flavour has been low. Breeders carry out taste tests during the development phase whereby taste attributes such as bitterness and sweetness

are scored, and lines are rejected if unpalatable. Nevertheless, breeders do not have the tools available to select for flavour, in addition to the need to select for the maintenance and consistency of flavour (Klee, 2010). Determining the flavour would require sensory profiling analysis to be completed on a whole breeding population using a trained panel, as well as laboratory work to identify and quantify the aroma compounds present. This can be a lengthy and expensive process. Using transcriptome sequencing could help identify genes that are being expressed in the same cultivar that has been taken into different environments and grown, providing information on the differences in gene expression. However, genetics only show the potential flavour of the crop, factors such as the environment, handling and damage and cooking will alter the flavour profile and taste (Klee, 2010).

Conversely, work completed by Thappa et al. (2003) investigating the variation of aroma compounds in celery seed and leaf oil, particularly focused on reducing the limonene and increasing the phthalide content to improve the flavour quality for consumption. Although this study concentrated on seed varieties, the success in producing a genetically improved celery expressing a reduced limonene content shows that *A. graveolens* can be modified to exhibit desired properties (Thappa et al., 2003).

Although there have been advances in biotechnology, the celery genome remained unconstructed only until recently, whereby previously, the genome of the carrot was the only member of the Apiaceae family with the genome constructed. Li et al. (2020) reported the genome sequence of *A. graveolens L.* with a total sequence length of 2.21 Gb and 34,277 predicted genes which is larger than the carrot sequence. The completion of this work allowed Li et al. (2020) to identify significant genes involved in disease resistance and secondary metabolite synthesis and metabolism. Focusing on terpenoid synthase family genes, three developmental stages were monitored using previous transcriptome data to analyse the expression of these terpenoid synthase proteins. During the first two stages of development, these proteins were seen to be expressed at a higher abundance than stage 3, signifying that terpenoid metabolism is involved in the growth and development of celery (Li et al., 2020).

5. Abiotic factors and the aroma of celery

It is difficult to predict the flavour profile of a crop at the point of consumption as multiple factors and interactions between the environment and genotype will contribute to any variations that may occur. Although the genotype will determine the capacity of the crop to synthesise the chemical components of the flavour profile, environmental factors play an important role in determining the phenotype (or chemotype). This in turn influences flavour, causing crops of the same variety to develop different secondary metabolite profiles such as polyphenols and volatiles, in different growing environments (Raffo, Sinesio, Moneta, Nardo, Peparaio & Paoletti, 2006). A response to abiotic stress is to synthesise aromatic compounds that protect the crop, which ultimately affects postharvest quality (Yan, Yu, Xu, Gu & Zhu, 2014). This means that edge effects in the field can impact on volatile content. Crop plants grown on the borders of the field may exhibit a different volatile content to individuals of the same cultivar grown in the middle of the field, where there is more protection from pests and unfavourable weather conditions. Short chain aldehydes and alcohols (C_6 and C_9) are known to be produced by plants in response to wounding occurring during harvest and storage. These compounds are GLVs and are important contributors to the characteristic aroma of celery but also play an important role in the plant defence strategies though intra and interplant volatile signalling. The evidence suggests that once damage has occurred, GLVs form, released and detected by other plants, evoking a defence system in response (Matsui, 2006; Scala, Allmann, Mirabella, Haring, & Schuurink, 2013).

A study carried out by Yan et al. (2014) showed that celery grown in soil in a drier climate or 'more stressful' environment could impose a higher bitterness through increased polyphenols to protect the crop against abiotic and biotic stresses. Yan et al. (2014) utilised a deep sequencing method to identify how miRNAs interact under heat stress, recognising that, although different varieties of celery have similar morphology, the miRNA population being expressed in order to withstand biotic and abiotic factors of their surroundings (Yan et al., 2014). Furthermore, the colour of the petiole can be manipulated through placement of planting and white celery can be produced by planting seeds in a shaded area. Here, the crop is away from direct sunlight and thus the production of chlorophyll is inhibited, and the crop remains white in colour (Sowbhagya, 2014).

Exposure to alternative environmental conditions and sequencing the genes expressed will help identify which parts of the genome respond to different environmental stimuli such as; soil composition, season and climate (Stoop & Pharr, 1994). From this, it can be identified which genes expressed are also connected to flavour compounds.

D'Antuono, Neri and Moretti (2002) found that changing the nitrogen levels in the soil can lead to a change in the flavour profile of celery. Using the cultivar Darklet and varying nitrogen concentrations, they found that higher doses of nitrogen led to a higher sedanenolide and lower monoterpene (limonene) content (D'Antuono et al., 2002). Thappa et al. (2003) reported that a high limonene content may lead to an unpalatable celery and a celery exhibiting higher phthalide content can be more desirable. Conversely, the application of nitrogen fertiliser on celery crop was shown to have a negative influence over the volatile composition of the crop, as identified by van Wassenhove, Dirinck, Schamp, and Vulsteke (1990b). Applying organic and mineral nitrogen fertiliser to two different varieties of celery saw a large decrease in the volatile content, particularly in the phthalide compounds.

Furthermore, the influence of irrigation on the chemical composition of the essential oil of *A. graveolens* was investigated by Rożek, Nurzyńska-Wierdak, Sałata, & Gumiela (2016), whereby an increase in a range of monoterpenes (α-pinene, cymene, limonene) can be seen in the petioles. However, a decrease can be seen in compounds such as myrcene, caryophyllene and *(Z)*-β-ocimene. In terms of phthalides, only (*Z*)-ligustilide was identified in the petioles of celery at 0.05% when no irrigation was used, it could not be identified when irrigation was applied (Rożek et al., 2016).

On the other hand, Khalid & Hussein (2012) investigated the effect of cattle and liquid manures on the essential oil content of celery grown at the Experimental Farm of National Research Centre, Egypt across two seasons. The essential oil was extracted using hydrodistillation and analysed using GC/MS. Overall, statistical differences were observed when using a liquid manure and it was concluded that the use of a combination of liquid and cow manure gave the "best essential oil production". Although an increase in the phthalide content was witnessed, a closer look shows that there was no statistically significant change and in fact there was a decrease in the monoterpene content. An increase in acetate esters including *trans*-pinocarvyl acetate and *cis*-carvyl acetate can be seen, as well as in sesquiterpenes such as β-selinene, β-humulene and β-caryophyllene (Khalid & Hussein, 2012). While there was a positive influence on the essential oil content (%) and yield when using liquid and cow manures, there was minimal influence on the essential oil constituents and the impact these manures had on the flavour profile could be questioned (Kokotkiewicz and Luczkiewicz, 2016).

Finally, the time of harvest could have an influence on the aroma of celery, although it has been shown that this is only minimal. Lund et al. (1973) were able to show seasonal and varietal differences from the oils recovered from celery waste from a packinghouse in Florida, using two varieties and taking waste trimmings and stalks in different seasons (November, April and July). A slight difference was observed in the composition of the waste trimmings from all cuts; sedanolide and β-selinene, identified as important compounds to the celery odour in this study and exhibited a decrease from 3.09% and 4.00% in November to 2.68% and 3.67% in April respectively. Limonene was not detected at all

in the April harvest. They attributed this difference to the higher proportion of stalks in the waste in April rather than leaf trimmings and concluded that using an oil with a higher leaf content leads to a better quality of oil for flavouring. Varietal differences are more obviously observed, whereby compounds marked as celery-like odour compounds are shown to either be lower or not detected in the second variety used in this study, it can be expected that this variety will have a less "typical" celery odour (Lund et al., 1973).

6. Post-harvest environment and the aroma of celery

The flavour of the crop can be influenced post-harvest due to poor harvesting techniques, incorrect handling or storage conditions. The optimum storage conditions for celery include a temperature of 0 °C with a high relative humidity of 95% (Malhotra, 2012). This maintains the desired organoleptic properties and appearance qualities over storage, however when the temperature is increased to 10 °C, these desired properties start to change. Viña and Chaves (2003) studied the textural differences and changes in fresh cut celery stored at 0 °C and 10 °C for 27 days. Sampling occurred at day 0, 7, 14, 21 and 27. Firstly, after seven days, strong yellow discolouration of the petioles was witnessed, and texture changes described as a "loss of crispiness" occurred. They further acknowledged the development of "off-odours" when samples were stored at 10 °C for 21 days, accompanied by rot and micro-organism decay. Twenty-one days is not a typical duration for the supply chain and these senescence characteristics would not be experienced by the consumer. Furthermore, this assessment was only completed through visual inspection (Viña & Chaves, 2003). It is likely that these off-odours were produced earlier on in the experiment, but not at a noticeable level to be detected by the human nose until day 21. Without the use of a fully trained nose, this becomes a very subjective method of monitoring organoleptic property changes. Using a GC/MS method would confirm the presence and identification of the off odours that were produced.

Preservation methods such as drying (freeze-drying and convection drying) and their influence on the aroma profile on the essential oil of two cultivars of celery were investigated by Nurzyńska-Wierdak, Gruszeck & Kosior (2018). Using convection drying, a larger number of compounds were retained including limonene and β-selinene, whereas freeze-drying allowed a higher retention of myrcene. The effect of drying on the phthalide content is unclear as they were not identified in either cultivars. Although it is clear that harvest time and cultivar used had an impact on the essential oil content, they concluded that convection drying allows for a higher yield of essential oil than freeze-drying (Nurzyńska-Wierdak et al., 2018). Overall, freezing has been shown as the optimum preservation method in terms of retaining the volatile constituents of celery essential oil when comparing to fresh celery (Kokotkiewicz & Luczkiewicz, 2016; Roslon, Osińska, & Gajc-Wolska, 2010; Roslon, Osińska, & Wajs-Bonikowska, 2013).

It is known that vegetables belonging to the Apiaceace family are capable of synthesising furanocoumarins, these being responsible for the production of off-odours, due to unfavourable conditions such as UV radiation, temperature changes and bacterial infections (Chaudhary, Ceska, Warrington & Ashwood-Smith, 1985). Furanocoumarins are secondary metabolites present in a limited number of plant families including: Moraceae, Apiaceae and Rutaceae and are involved in plant defence and environmental adaptation (Dugrand-Judek et al., 2015). Chaudhary et al. (1985) identified levels of furocoumarins was at its highest in celery that showed signs of fungal infections after 22 to 29 days of storage. There was a statistically significant increase in the levels of 5-methoxypsoralen, 8-methoxypsoralen and psoralen compared with fresh celery. These furocoumarins are defence compounds with antimicrobial properties, synthesised in response to the biotic stress (Chaudhary et al., 1985).

A review completed by Forney (2008) identified processes during postharvest handling on fresh-cut produce that caused significant flavour loss. Forney identified two kinds of mechanisms that cause flavour loss, the first being metabolic changes due to the synthesis of flavour compounds and these could be off odours as well. Metabolic changes are subject to the crop physiology, which in turn is influenced mainly by environmental factors. The second mechanism is diffusional changes in product flavour, whereby the volatile compounds transfer out of the crop. Where metabolic changes are dependent on the plant physiology, diffusional changes are reliant on the chemical and physical properties of the flavour compound itself. The determination of the flavour of celery post-harvest is dependent on these two mechanisms which in turn, are dependent on the environment in which the crop is kept (Forney, 2008).

7. Conclusion

Using the data that has been collated in Table 3, showing the aroma compounds in various celery varieties, it can be seen that the aroma profile of celery is complex, consisting of an assortment of compounds ranging from terpenes and phthalides to alcohols and aldehydes. Terpenes and phthalides are most consistently reported throughout literature, with less emphasis placed upon other compounds such as alcohols, esters and aldehydes. However, this does not mean the latter are any less significant contributors to the aroma of celery.

Given the vast amount of work that has been already completed, there is rarely a dataset that states the variety of celery used, the season and location in which it was sampled and whether repetitions were completed over multiple time points in multiple sites. Therefore, very few papers provide insight into the aromatic variance that may be attributed to environmental factors, as distinguished to those due to the genetic influence of variety. When the cultivar variety is specified, it is clear that there is an impact of genetics on aroma, since all sources express different aroma compounds. Providing minimal standardised information such as geographical location of growth and cultivar could help build a bigger and better library to help understand the impact these factors have upon the aroma profile of celery and we recommend the adoption of MIAPAE standards for flavour and aroma publications on all crops.

Preference of celery flavour by consumers is an area that needs further investigation to help improve the quality of celery that is produced, alongside an understanding of how the postharvest environment further changes the organoleptic profile of the crop as it moves through the supply chain. Furthermore, linking sensory profiling and consumer liking with flavour chemistry is an untouched topic and making this connection will provide information for producers and retailers on how celery quality is perceived and how important sensory attributes, such as flavour and aroma, are to influencing consumer preference. The availability of the celery genome sequence now makes targeted breeding for these biochemically driven traits a realistic possibility for vegetable plant breeders to pursue so that lines can be developed that have distinct flavour profiles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128673.

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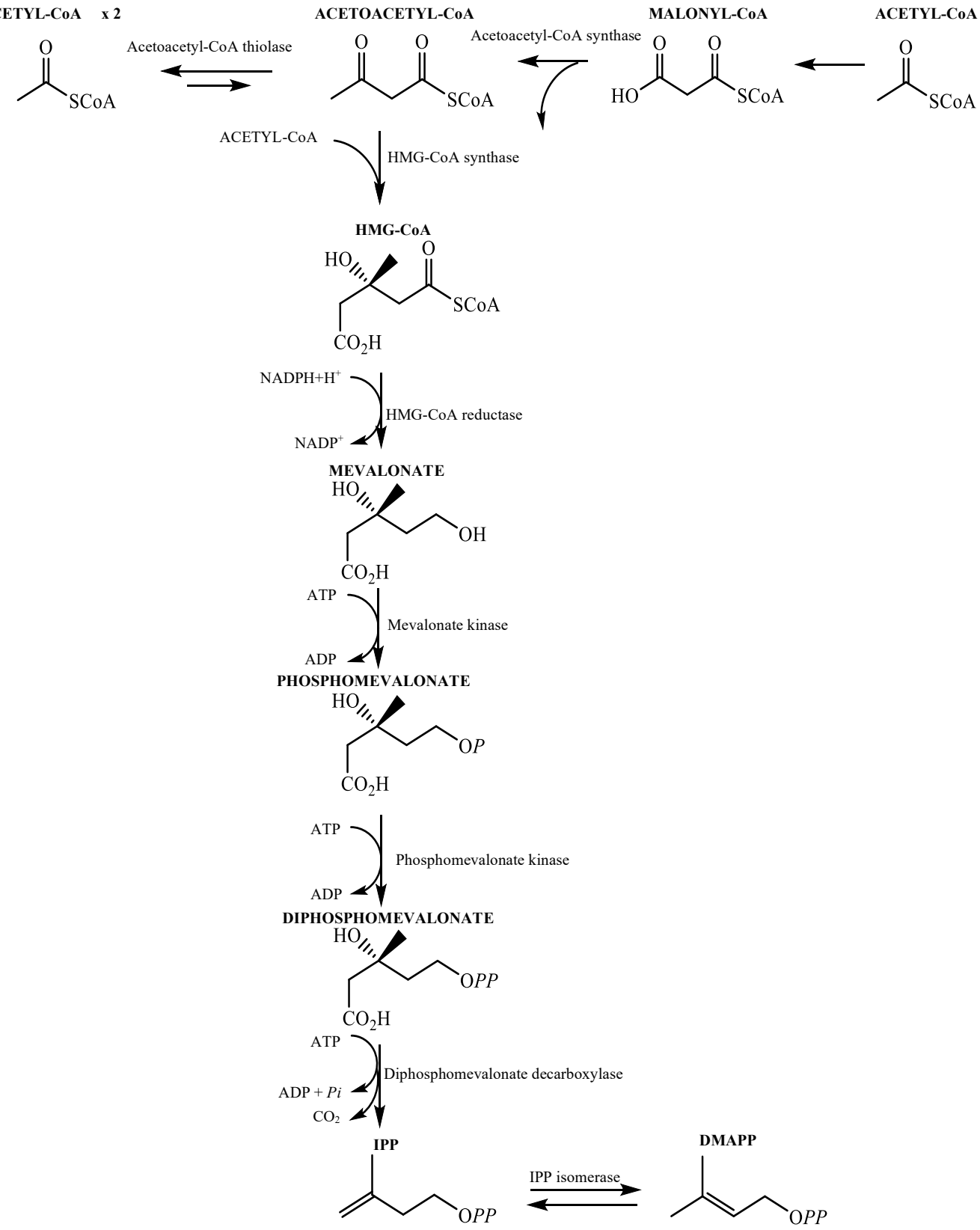
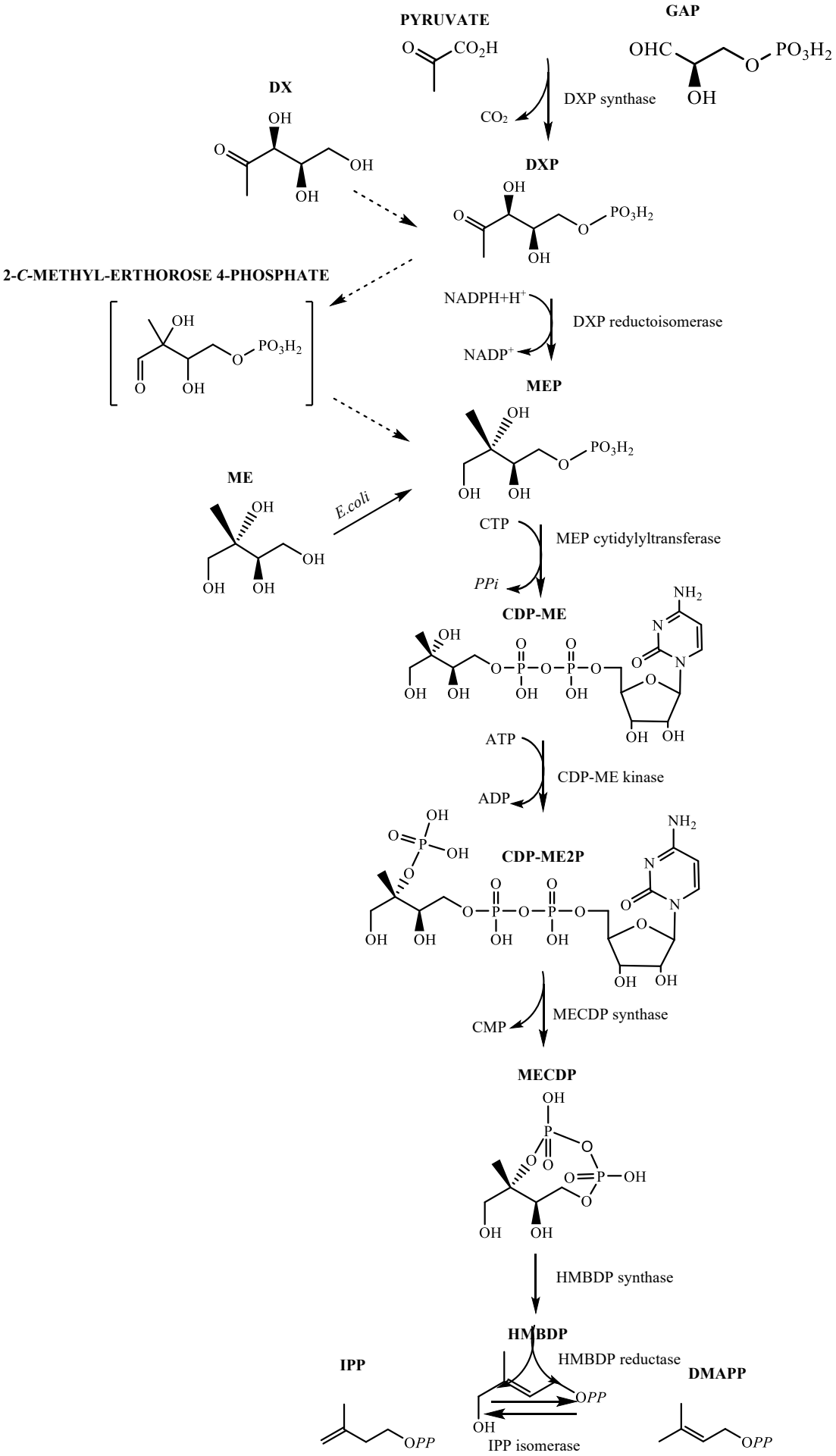
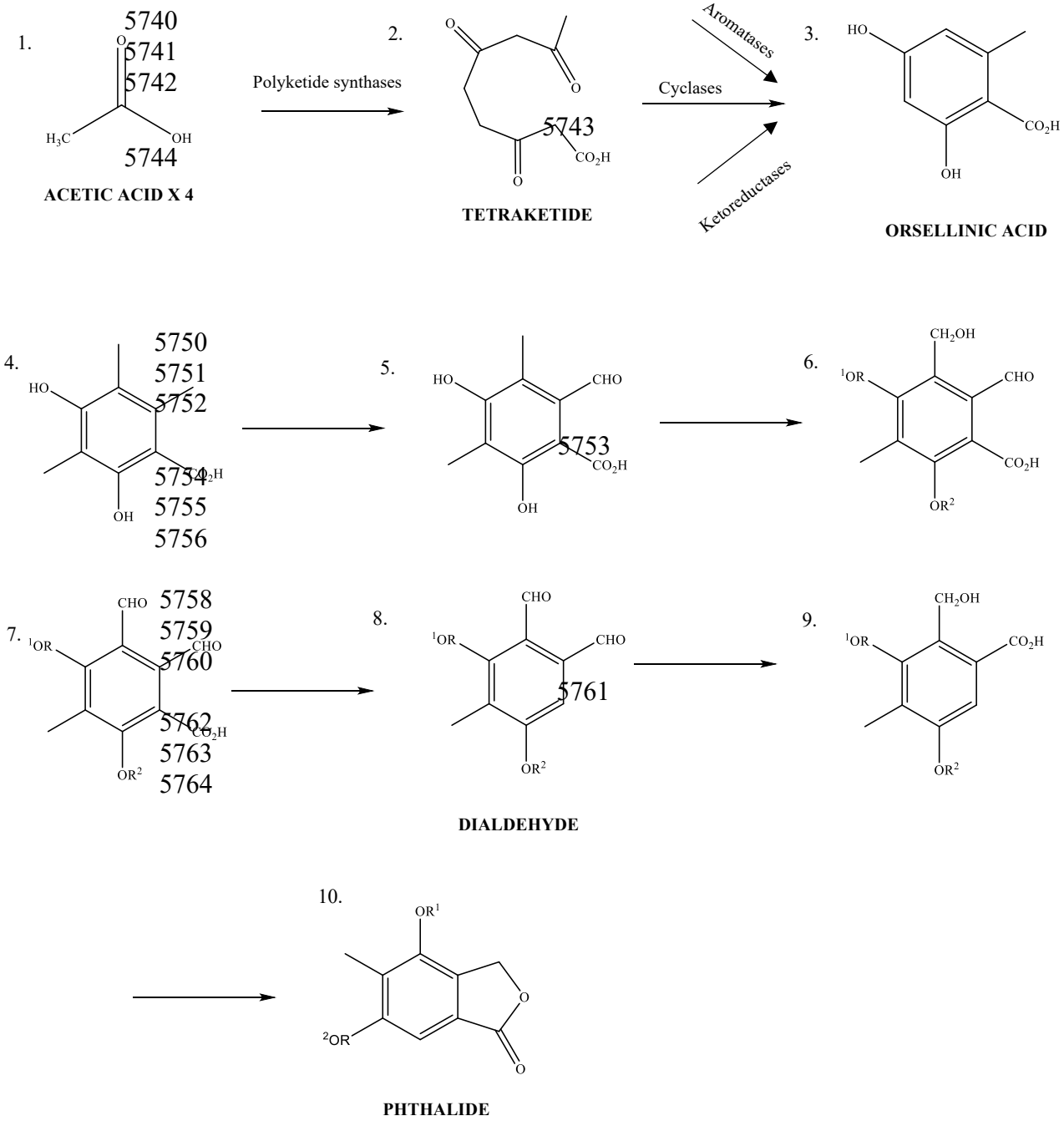
Appendix II - Schematic of Mevalonate Pathway for IPP and DMAPP Synthesis




PYRUVATE
GAP
DX
DXP synthase
DXP
2-C-METHYL-ERTHOROSE 4-PHOSPHATE
NADPH+H+
DXP reductoisomerase
NADP+
MEP
ME
E.coli
CTP
MEP cytidylyltransferase
PPi
CDP-ME
ATP
CDP-ME kinase
ADP
CDP-ME2P
CMP
MECDP synthase
MECDP
HMBDP synthase
HMBDP
IPP
HMBDP reductase
DMAPP
IPP isomerase

Appendix IV – Phthalide synthesis



Appendix V- Table of 24 celery genotypes and their origins

Genotype no.	Origin
1	Florida, USA
2	California, ISA
3	N/A
4	N/A
5	USA
6	USA
7	USA
8	Australia
9	Australia
11	UK
12	UK
13	Californian, USA
14	N/A
15	California, USA
16	N/A
17	UK
18	France
19	California, USA
20	Chinese
21	California, USA
22	Michigan, USA
23	UK
25	EU
26	N/A
29	USA

foods

MDPI

Article

Investigating the Relationship of Genotype and Climate Conditions on the Volatile Composition and Sensory Profile of Celery (*Apium graveolens*)

Lucy Turner [1], Stella Lignou [1,*], Frances Gawthrop [2] and Carol Wagstaff [1]

[1] Department of Food and Nutritional Sciences, Harry Nursten Building, University of Reading, Whiteknights, Reading RG6 6DZ, UK; L.Turner@pgr.reading.ac.uk (L.T.); c.wagstaff@reading.ac.uk (C.W.)
[2] A.L.Tozer Ltd., Pyports, Downside Bridge Road, Cobham KT11 3EH, UK; frances.gawthrop@tozerseeds.com
* Correspondence: s.lignou@reading.ac.uk; Tel.: +44-(0)118-378-8717

Abstract: *Apium graveolens* is a biennial crop grown across the globe for its stalks, leaves and seed and is known for its distinct flavour and strong taste. Various extraction methods on fresh and dried celery and its essential oil are reported in the literature examining the aroma profile of this crop and demonstrating that its volatile composition is determined by variables including cultivar, season, geographical location and agronomic practices. This study investigated the volatile and sensory profile of eight celery genotypes grown over two years (2018 and 2020) in the same location in the UK. Solid-phase-micro-extraction followed by gas chromatography-mass spectrometry were used to determine the volatile compounds present in these genotypes and sensory evaluation using a trained panel to assess the sensory profile of fresh celery. Significant differences ($p < 0.05$) in the volatile composition and sensory profile were observed and influenced by both genotype and harvest year. Two genotypes exhibited similar aroma composition and sensory profile between the years. Celery samples harvested in 2018, which possessed air temperatures that were considerably warmer than in 2020, exhibited higher proportions of sesquiterpenes and phthalides and we hypothesise that the higher proportions were generated as a response to heat stress. Studying the relationship between the genotype and the environment will provide clear information to guide growers in how to consistently produce a higher quality crop.

Keywords: celery; aroma; volatile compounds; SPME GCMS; phthalides; terpenes; preharvest

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1. Introduction

Celery is a vegetable that belongs to the Apiaceae family which is grown across the globe and consumed regularly and forms part of the "holy trinity" or "Soffritto" in cooking, used raw in salads or with condiments [1]. The investigation of the aroma and flavour of celery has been studied using a range of extraction techniques, such as solvent assisted flavour extraction (SAFE) and solid phase microextraction (SPME), combined with instrumental analysis, such as gas chromatography/mass spectrometry (GC/MS) on celery leaf, petiole and seed. The consensus is that terpenes (monoterpenes and sesquiterpenes) and phthalides make up the majority of compounds present in the flavour profile. Phthalides, in particular, have been shown to be key contributors to typical celery aroma (3-n-butylphthalide, sedanenolide and (E)- ligustilide and (Z)-ligustilide) and possess odour descriptors such as "celery", "herbal" and "green" [2,3]. The composition of alcohol, aldehyde and ester compounds have been poorly represented in literature. Although they are not characteristic compounds to celery odour, their importance should not be neglected as these compounds contribute to green, fresh and woody notes that are important to the overall celery aroma. Wilson [4] identified and quantified 13 alcohols in celery essential oil using gas chromatography including n-hexanol, cis-3-hexene-1-ol and dihydrocarveol. Wilson commented on the pleasant aroma of these compounds and concluded that although

they are not characteristic compounds of celery, they complete the typical flavour and aroma of celery [4].

In a recent review by the authors [5], the complexity of the aroma profile is discussed and the variation within reported datasets caused by differences in cultivar, geographical location of growth, agricultural techniques as well as extraction and analysis techniques are highlighted. In order to overcome these variances, Turner et al. [5] recommended the use of Minimum Standards About a Plant Aroma Experiment (MIAPAE), ultimately leading to a repository of data whereby accurate interpretation of results and correct experimental repetition can occur. Importantly, it was demonstrated that the genotype alone does not determine the final flavour outcome, but other factors during preharvest (cultivar, climate and agronomy) and postharvest (harvest techniques and storage conditions) simultaneously influences the final composition [5,6]. The application of alternative agronomic practices, including varying nitrogen levels in soil, the use of irrigation systems and inorganic/organic fertilisers, as well as growing celery in different geographical regions have all been shown to influence the aroma composition of celery [7–11]. Rożek, Nurzyńska-Wierda and Kosior [12] explained the consequences of agricultural techniques on the volatile composition of leaf celery essentials, while van Wassenhove, Dirinck, Schamp and Vulsteke [13] concluded that the use of fertiliser (organic and/or inorganic) resulted in a decrease in terpene and phthalide content.

Limited research has been conducted on the impact of the environment on the volatile composition of celery, with few studies using the same cultivar over multiple sites and seasons that are compliant to MIAPAE [5]. van Wassenhove, Dirinck, Vulsteke and Schamp [14] investigated the volatile composition of four celery cultivars grown in two seasons (1986 and 1987) on sandy loam fields in Belgium. Although differences in the composition were observed, their focus was not on the variation of composition but more on the validity of their method to identify and separate terpenes and phthalides in celery. Genotypic and seasonal differences were observed in the total terpene and phthalide content of all four cultivars [14]. Lund, Wagner and Bryan [15] also reported differences in the oil composition of celery (Utah 5270) waste trimmings between November 1972 and July 1973, yet no seasonal significant differences were shown. Conversely to van Wassenhove et al. [14], a much smaller group of compounds were investigated by Lund et al. [15] that numbered around 12 compared to the 33 compounds identified by van Wassenhove et al. [14]. This suggests that the harvest year has minimal impact over the volatile composition. Alternatively, Shojaei, Ebrahimi and Salini [10] showed the impact of the environment on the volatile composition by testing one species of wild celery (*Kelussia odoratissima*) sampled across three different regions of Iran. They identified trans-ligustilide as the main compound from the three locations contributing various percentages—47.31%, 37.55% and 33.73%. There were also variations in the presence of compounds throughout three ecotypes; the Bazoft ecotype was found to contain fewer compounds than the ecotypes grown in Koohrang and Samsani [10].

The aim of this study was to investigate the relationship between genotype and the environment on the volatile composition of eight celery genotypes grown in the UK across two different years (2018 and 2020). In addition, sensory evaluation using a trained panel was used in order to understand how chemical and physiological changes lead to differences in organoleptic perception and used to identify interactions between compounds groups and climate. Ultimately, this information could assist breeders and growers to develop and select cultivars that are optimal for specific growing climates and to allow for the production of a consistent quality product.

2. Materials and Methods

2.1. Celery Material and MIAPAE Standards

2.1.1. Sample Information

The eight genotypes used in this study were chosen based on their differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the

exact genetic identity of each genotype used in this study, the origins of these parental breeding lines and their images postharvest can be found in the Supplementary Materials Table S1.

2.1.2. Timing, Location and Environment

The celery seeds (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd. (Cobham, UK) were grown in commercial conditions and harvested in Cambridgeshire (UK) by G's Fresh Ltd. (Ely, UK, 52°21′12.9″ N 0°17′15.6″ E) during the spring/summer of 2018 and 2020. The celery was grown in a field with commercial celery products and treated by the same agronomic techniques and conditions as commercial celery, including identical fertiliser application and exposure to water. For both years, 20–25 mm of overhead irrigation was used and standard commercial fertiliser, pest and disease control regimes were applied. In 2018, plugs were transplanted mid-June after growing in the nursery for 22 days and then harvested 91 days later. The average daily air temperature was 18.2 °C with an average soil temperature of 23.8 °C, 0.2 mm of rainfall daily and an average relative humidity of 88.1%. In 2020, the plugs were transplanted late April after growing in the nursery for 24 days and were harvested 76 days later. The average daily air temperature was 14.3 °C with an average soil temperature of 15.4 °C, 0.05 mm daily rainfall and an average relative humidity of 74.8%. Prior to the harvest, the celery is tested regularly in-field to ensure standards for commercial quality are met, including visual and taste tests. The celeries were harvested within a close time-frame compared to the commercial produce also being grown in the field.

2.1.3. Raw Material Collection, Processing and Storage

The celery was grown in three randomised blocks in the centre of the field to reduce any influence from edge effects at a density of 10 plants m^{-2} and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles and then sealed in labelled bags for transportation to the University of Reading (United Kingdom). Celery samples used for sensory evaluation were refrigerated for one day, while samples for aroma analysis were immediately frozen at −80 °C for one week and subsequently freeze-dried for five days. Samples were then milled into a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ, USA) and then stored in an airtight container for a maximum of two weeks before analysis with gas chromatography-mass spectrometry (GC/MS).

2.2. Chemical Reagents

For GC/MS analysis, calcium chloride and the alkane standard C_6–C_{25} (100 μg/mL) in diethyl ether were obtained from Merck (Poole, UK).

2.3. Solid Phase Microextraction (SPME) Followed by GC/MS

Celery (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and filled to 5 mL using HPLC-grade water in a 15 mL SPME vial fitted with a screw cap. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA). The SPME fibre stationary phase was composed of 75 μm divinylbenzene/Carboxen™ on polydimethylsiloxane, Supelco (Bellefonte, PA, USA). Equilibration was set for 10 min at 37 °C before exposing the fibre to the sample headspace for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm and kept at 37 °C. After extraction, the SPME device was inserted into the GC injection port and desorbed for 5 min. An Agilent capillary column HP-5MS (30 m × 250 μm × 0.25 μm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The temperature program used was: 2 min at 80 °C isothermal, an increase of 4 °C/min to 250 °C and 6 min at 250 °C isothermal. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature of the injector, interface and detector was 250 °C and the sample injection mode was splitless. Mass

spectra were measured in electron ionization mode with an ionization energy of 70 eV, the scan range from 29 to 250 m/z and the scan rate of 5.3 scans/s. The data were recorded using HP G1034C Chemstation system.

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading) or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C6–C25 n-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions as described by Turner et al. [16].

2.4. Sensory Evaluation of Fresh Celery Samples

Sensory evaluation was carried out using quantitative descriptive analysis (QDA^{TM}) to determine the sensory characteristics of the eight celery samples and the characteristics were estimated quantitatively. The trained sensory panel at the Sensory Science Centre (University of Reading, n = 12; 11 female and 1 male) was used to develop a consensus vocabulary to describe the sensory characteristics of the eight celery genotypes. During the development of the sensory profile, the panelists were asked to describe the appearance, odour, taste, flavour, mouthfeel and aftereffects of the samples in order to produce as many descriptive terms as seemed appropriate. References were used to help confirm the characteristics of certain attributes including fresh and dried fennel, salad rocket, flat leaf parsley and fresh coriander. The terms were discussed by the panelists as a group, with the help of the panel leader, and this led to a consensus of 22 and 24 attributes for the 2018 and 2020 harvest, respectively. The sensory assessment of the samples was carried out in a temperature-controlled room (22 °C) under artificial daylight and in isolated booths, each equipped with an iPad. Celery petioles were chosen to be as uniform as possible. The first outer petioles were removed and discarded. The next ring of petioles were used and these were washed with filtered water and cut to 15 cm petiole length prior to serving to the panellists at room temperature. The panellists scored in duplicate for each sample in separate sessions. Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Guelph, ON, Canada) was used to acquire the data. Samples, coded with three-digit random numbers, were provided in a monadic balanced order, with sample sets randomly allocated to panelists. The panellists were asked to assess the appearance first; to break the petiole in half to assess the odour; to bite from the middle for taste, flavour and mouthfeel; and then after 30 s delay to assess the aftereffects. The intensity of each attribute for each sample was recorded on a 100 point unstructured line scale. Between samples, the panellists cleansed their palate with water and crackers.

For the 2020 harvest, due to the COVID-19 pandemic restrictions, the trained panel assessed the samples from home in July 2020. Vocabulary refreshment and training sessions occurred prior to scoring virtually on the Teams platform. Samples were prepared similarly to 2018 but were sent out to panellists using chilled transport couriers. The panellists completed their scoring simultaneously using Compusense Cloud software whilst on video on Teams.

2.5. Statistical Analysis

The percentage composition was calculated from the data collected by SPME GCMS analysis. Quantitative data for each compound identified in the SPME GC/MS analysis were analysed by both one-way and two-way analysis of variance (ANOVA) and principal component analysis (PCA) using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine the sample means that differed significantly ($p < 0.05$) between harvest maturities and the celery genotypes. These data are shown in Table 1. Only those compounds exhibiting significant differences between

harvest year, genotype and their interaction (harvest year × genotype) were included in the principal component analysis.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out ANOVA of sensory panel data. The means from sensory data were taken over assessors and correlated with the percentage composition means from the instrumental data via PCA using XLSTAT.

3. Results and Discussion

3.1. Volatile Composition

In total, 86 compounds were identified in the headspace of the eight celery genotypes in both harvest years (2018 and 2020) and listed in Table 1. Sixty-five compounds were identified in 2018 across eight genotypes, including: 22 monoterpenes, ten sesquiterpenes, eight aldehydes, five alcohols (three of which are classified as monoterpenoid alcohols) and five phthalides. Nine additional compounds were identified in the headspace of the same genotypes from the 2020 harvest including: 22 monoterpenes, 13 sesquiterpenes, five phthalides and five alcohols (including three monoterpenoid alcohols).

Quantitative differences were observed between the two harvest years (E) as well as the eight genotypes (G) used in this study. Two-way ANOVA revealed more significant differences between aroma composition caused by the harvest year compared to the genotype, although differences caused by the genotype were still observed. The majority of alkanes and compounds including nonanal, α-thujene, camphene, sabinene, (+)-cis-p-mentha-2,8-dien-1-ol, α-ylangene, (E)-β-caryophyllene and trans-neocnidilide expressed no significant difference in the relative amount between 2018 harvest and 2020 harvest.

Table 1. Percentage composition of volatile compounds identified in the headspace of eight celery genotypes using SPME GC/MS and harvested in 2018 and 2020.

Code	Compound	LRI_{expt} [a]	ID [b]	Percentage Composition (%) [c]														p [d]		
				2018								2020								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	E [e]	G [f]	GxE [g]
	Alcohols																					
A1	3-methyl-3-buten-1-ol	730	A	0.42± 0.08 ^{b}	0.31± 0.04 ^{ab}	0.94± 0.27 ^{c}	0.35± 0.14 ^{ab}	0.22± 0.07 ^{ab}	0.23± 0.06 ^{ab}	0.30± 0.12 ^{ab}	0.39± 0.06 ^{b}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***
A2	(E)-2-penten-1-ol	758	A	0.73± 0.28 ^{ab}	0.42± 0.16 ^{ab}	0.64± 0.04 ^{ab}	0.23± 0.08 ^{a}	0.32± 0.09 ^{a}	0.65± 0.23 ^{ab}	1.2± 0.54 ^{b}	0.50± 0.22 ^{ab}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	0.12± 0.05 ^{a}	tr± 0.01 ^{a}	0.15± 0.03 ^{a}	tr± 0.05 ^{a}	tr± 0.03 ^{a}	tr± 0.01 ^{a}	***	***	***
A3	1-pentanol	763	A	0.21± 0.06 ^{ab}	0.11± 0.04 ^{a}	0.31± 0.20 ^{ab}	0.13± 0.10 ^{a}	0.23± 0.15 ^{ab}	0.39± 0.14 ^{ab}	0.63± 0.25 ^{b}	0.28± 0.08 ^{ab}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	tr± 0.03 ^{a}	tr± 0.01 ^{a}	0.10± 0.03 ^{a}	0.14± 0.02 ^{a}	0.12± 0.03 ^{a}	0.10± 0.02 ^{a}	**	**	**
	Total Aldehydes			1.4	0.84	1.9	0.71	0.77	1.3	2.1	1.2	0.07	0.06	0.18	0.03	0.25	0.21	0.3	0.13			
AL1	hexanal	800	A	9.7± 0.8	1.3± 0.46	2.6± 0.32	0.65± 0.29	2.0± 0.39	8.9± 2.7	13± 5.5	6.3± 1.2	0.16± 0.05	0.11± 0.02	0.22± 0.1	0.14± 0.03	0.24± 0.03	0.35± 0.25	0.22± 0.05	0.26± 0.15	*	ns	*
AL2	(E)-2-hexenal	849	A	0.18± 0.11	tr± 0.02	tr± 0.02	tr± 0.01	tr± 0.03	0.15± 0.11	0.20± 0.08	0.11 ± 0.05	nd	nd	nd	nd	nd	nd	nd	nd	**	ns	**
AL3	heptanal	901	A	tr± 0.03	nd	0.28± 0.15	0.16± 0.13	0.25± 0.16	0.23± 0.14	0.29± 0.08	0.25± 0.15	nd	nd	nd	nd	nd	nd	nd	nd	**	ns	**
AL4	(E)-2-heptenal	954	A	0.10± 0.22 ^{a}	1.6± 0.55 ^{abc}	1.6± 0.23 ^{abc}	0.5± 0.04 ^{ab}	1.5± 0.10 ^{abc}	3.2± 1.5 ^{bc}	4.2± 1.3 ^{c}	1.8± 0.97 ^{abc}	0.18± 0.05 ^{a}	0.2± 0.07 ^{a}	0.28± 0.10 ^{a}	0.36± 0.04 ^{ab}	0.54± 0.06 ^{ab}	0.53± 0.16 ^{bc}	0.46± 0.11 ^{a}	0.03± 0.04 ^{a}	***	***	***
AL5	n-octanal	1003	A	0.10± 0.07	nd	0.49± 0.06	0.27± 0.06	0.39± 0.19	0.51± 0.26	0.51± 0.17	0.51± 0.23	0.18± 0.02	0.16± 0.05	0.22± 0.04	0.25± 0.02	0.19± 0.03	0.24± 0.03	0.25± 0.14	0.15± 0.03	*	*	*
AL7	m-tolualdehyde	1086	B [17]	0.33± 0.07 ^{ab}	0.24± 0.02 ^{a}	4.0± 0.28 ^{d}	1.1± 0.28 ^{c}	0.95± 0.02 ^{bc}	0.19± 0.02 ^{a}	0.26± 0.05 ^{a}	1.6± 0.29 ^{c}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	tr± 0.01 ^{a}	nd ^{a}	***	***	***
AL8	nonanal	1105	A	0.33± 0.14	0.12± 0.02	0.20± 0.03	tr± 0.01	0.17± 0.03	0.16± 0.1	0.22± 0.17	0.19± 0.09	0.10 ± 0.02	tr± 0.05	0.21± 0.01	tr± <0.01	tr± 0.01	0.11± 0.02	0.14± 0.01	tr± 0.01	ns	ns	ns
AL9	(E,E)-2,6-nonadienal	1156	A	0.21± 0.04 ^{c}	0.30± 0.03 ^{c}	0.18± 0.02 ^{bc}	0.18± 0.04 ^{bc}	0.17± 0.03 ^{bc}	0.16± 0.08 ^{a}	tr± 0.03 ^{ab}	0.22± 0.08 ^{c}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***
	Total Esters			11	3.6	9.4	3	5.5	14	19	11	0.65	0.57	0.94	0.82	1.1	1.3	1.1	0.52			
E1	methyl butanoate	717	A	tr ± 0.03	tr± 0.01	tr± 0.02	tr± <0.01	tr± 0.02	tr± 0.04	tr± 0.05	tr± 0.01	nd	tr± <0.01	nd	tr± <0.01	tr± <0.01	tr± <0.01	tr± <0.01	tr± <0.01	ns	ns	ns
E2	1-octen-3-yl-acetate	1108	B [18]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr± 0.02 ^{a}	tr± 0.01 ^{a}	0.11 ± 0.03 ^{c}	tr± 0.01 ^{ab}	tr± 0.01 ^{a}	tr± 0.01 ^{ab}	nd ^{a}	tr± 0.02 ^{b}	***	***	***
E3	(E)-pinocarvyl acetate	1310	B [19]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.36± 0.18 ^{ab}	0.38± 0.19 ^{ab}	0.43± 0.12 ^{ab}	0.14± 0.01 ^{ab}	0.43± 0.18 ^{ab}	0.55± 0.28 ^{b}	0.21± 0.07 ^{ab}	0.24± 0.05 ^{ab}	***	ns	***
E4	carveol acetate	1343	B [20]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr± 0.02 ^{cd}	0.12± 0.05 ^{bcd}	0.20± 0.04 ^{d}	0.10 ± 0.01 ^{ab}	0.18± 0.05 ^{cd}	0.10 ± 0.02 ^{bc}	tr± 0.01 ^{ab}	0.10± 0.02 ^{abc}	***	***	***
E5	hexy isobutanoate	1378	B [21]	0.10± 0.03 ^{a}	0.10± 0.04 ^{a}	0.14± 0.02 ^{ab}	tr± 0.03 ^{a}	tr± 0.05 ^{a}	0.16± 0.04 ^{ab}	0.32± 0.06 ^{b}	0.12± 0.03 ^{ab}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***
	Total Alkanes			0.14	0.1	0.2	0.07	0.11	0.19	0.36	0.14	0.44	0.52	0.74	0.27	0.65	0.72	0.26	0.4			
ALK1	nonane	900	A	0.41± 0.15 ^{ab}	0.32± 0.11 ^{ab}	0.43± 0.19 ^{ab}	0.14± 0.18 ^{ab}	0.13± 0.10 ^{ab}	0.28± 0.11 ^{ab}	nd ^{a}	0.17± 0.02 ^{ab}	0.20± 0.11 ^{ab}	0.38± 0.14 ^{ab}	0.71± 0.29 ^{b}	0.36± 0.11 ^{ab}	0.51± 0.07 ^{ab}	0.39± 0.22 ^{ab}	0.29± 0.05 ^{ab}	0.27± 0.04 ^{ab}	*	*	*
ALK2	decane	1000	A	0.80± 0.24 ^{bcd}	0.49± 0.13 ^{abcd}	nd ^{a}	0.37± 0.11 ^{abc}	0.60± 0.26 ^{abcd}	1.1± 0.21 ^{de}	1.7± 0.29 ^{e}	0.83± 0.33 ^{cd}	0.14± 0.02 ^{ab}	0.13± 0.02 ^{ab}	0.10 ± 0.11 ^{a}	tr± 0.08 ^{a}	0.18± 0.02 ^{a}	0.31± 0.01 ^{abc}	0.19± 0.02 ^{abc}	0.14± 0.01 ^{ab}	***	***	***
ALK3	undecane	1100	A	0.26± 0.15	0.14± 0.09	0.19± 0.11	tr± 0.05	0.24± 0.06	0.14± 0.1	tr± 0.08	0.11 ± 0.06	nd	nd	nd	nd	nd	nd	nd	nd	**	ns	ns
ALK4	dodecane	1199	A	0.48± 0.08	0.37± 0.03	0.46± 0.05	0.31± 0.1	0.33± 0.1	0.44± 0.13	0.46± 0.1	0.44± 0.12	0.39± 0.36	0.38± 0.34	0.18± 0.11	0.10± 0.08	0.11± 0.1	0.11± 0.04	0.10± 0.09	0.08± 0.04	ns	ns	ns
ALK5	tridecane	1299	A	nd	nd	nd	nd	nd	nd	nd	nd	0.61± 0.67	0.58± 0.68	0.23± 0.17	0.14± 0.11	0.13± 0.08	0.11 ± 0.06	0.10 ± 0.06	tr± 0.04	ns	ns	ns
ALK6	tetradecane	1399	A	0.11 ± 0.02	tr± 0.03	tr± 0.02	tr± 0.03	0.10± 0.06	0.10± 0.03	tr± 0.03	0.10 ± 0.02	0.50± 0.48	0.49± 0.21	0.28± 0.23	0.22± 0.1	tr± 0.03	0.14± 0.05	0.14± 0.07	0.11± 0.06	ns	ns	ns
ALK7	pentadecane	1499	A	nd	nd	nd	nd	nd	nd	nd	nd	0.25± 0.19	0.27± 0.19	0.18± 0.08	0.15± 0.08	0.17± 0.04	0.12± 0..02	0.14± 0.04	0.12± 0.03	**	ns	ns
ALK8	hexadecane	1600	A	nd	nd	nd	nd	nd	nd	nd	nd	0.10± 0.06	0.10 ± 0.06	0.10 ± 0.03	tr± 0..03	tr± 0.02	tr± 0.01	tr± 0.01	tr± 0.01	**	ns	ns
ALK9	heptadecane	1700	A	nd	nd	nd	nd	nd	nd	nd	nd	tr± 0.01	tr± 0.02	tr± 0.02	tr± <0.01	0.72± 0.12	0.69± 0.39	tr± 0.01	tr± 0.01	ns	ns	ns
ALK10	octadecane			nd	nd	nd	nd	nd	nd	nd	nd	tr± 0.01	tr± 0.01	tr± 0.01	nd	nd	nd	nd	nd	ns	ns	ns
	Total			2.1	1.4	1.1	0.94	1.4	2.1	2.3	1.6	2.2	2.5	1.8	1.1	1.9	1.9	0.95	0.86			

Table 1. *Cont.*

Code	Compound	LRI_{expt} [a]	ID [b]	Percentage Composition (%) [c]																p [d]		
				2018								2020										
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	E [e]	G [f]	GxE [g]
	Monoterpenes																					
M1	α-thujene	933	B [22]	0.27± 0.09	0.24± 0.08	0.29± 0.13	0.30± 0.11	0.22± 0.1	0.41± 0.19	0.32± 0.14	0.22± 0.13	0.11± 0.02	0.10 ± 0.02	0.10± 0.02	0.14± 0.02	0.11± 0.01	0.24± 0.02	0.15± 0.02	0.14± 0.02	ns	ns	ns
M2	α-pinene	943	A	0.62± 0.05 abcd	0.85± 0.22 bcd	0.52± 0.19 abcd	0.62± 0.18 abcd	1.0± 0.42 d	0.89± 0.20 cd	0.43± 0.20 ab	0.62± 0.31 abcd	0.26± 0.04 abcd	0.14± 0.11 abc	0.20± 0.09 abc	tr± 0.01 a	0.10± 0.01 ab	0.15± 0.01 abc	0.12± 0.01 a	0.40± 0.09 abcd	***	ns	***
M3	camphene	960	A	2.5± 0.5	0.33± 0.07	0.29± 0.12	0.21± 0.08	0.35± 0.1	0.48± 0.05	0.66± 0.26	0.22± 0.08	0.11± 0.01	0.13± 0.04	0.17± 0.02	0.16± 0.06	0.22± 0.07	0.45± 0.03	0.28± 0.09	0.10 ± 0.03	ns	ns	ns
M4	sabinene	981	A	0.44± 0.13	0.33± 0.04	0.66± 0.39	0.27± 0.04	0.28± 0.05	0.45± 0.03	0.53± 0.13	0.36± 0.06	0.27± 0.02	0.25± 0.01	0.32± 0.04	0.39± 0.03	0.22± 0.08	0.49± 0.05	0.29± 0.05	0.23± 0.04	ns	ns	ns
M5	β-pinene	989	A	3.0± 0.64 ab	5.2± 1.6 b	0.96± 0.36 ab	5.4± 1.6 b	3.8± 1.6 ab	2.7± 0.99 ab	0.79± 0.24 ab	4.5± 1.1 ab	2.8± 0.8 ab	3.9± 1.1 ab	1.7± 0.39 ab	5.5± 0.69 b	3.8± 0.84 ab	0.13± 0.02 a	3.1± 0.17 ab	4.8± 1.1 ab	**	**	**
M6	myrcene	992	A	1.1± 0.26 a	1.9± 0.64 a	2.1± 0.74 a	2.6± 0.22 a	1.6± 0.37 a	2.1± 0.61 a	0.84± 0.34 a	1.1± 0.45 a	1.9± 0.11 a	2.6± 0.48 a	7.3± 0.65 b	7.9± 0.53 b	2.0± 0.76 a	1.9± 0.08 a	1.7± 0.27 a	2.1± 0.26 a	***	***	***
M7	α-phellandrene	1013	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.33± 0.02 bc	0.31± 0.03 b	0.39± 0.03 cd	0.30± 0.01 b	0.40± 0.03 cd	0.53± 0.03 e	0.53± 0.02 e	0.43± 0.03 d	***	***	***
M8	delta-3-carene	1019	A	0.24± 0.10 ab	0.23± 0.18 ab	0.25± 0.04 ab	0.25± 0.12 ab	0.22± 0.11 ab	0.21± 0.10 ab	0.32± 0.09 b	0.23± 0.05 ab	tr± 0.01 ab	tr± 0.01 ab	tr± 0.01 a	tr± 0.01 ab	nd a	0.13± 0.10 ab	nd a	tr± 0.02 ab	**	ns	**
M9	α -terpinene	1025	A	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	0.46± 0.08 b	0.42± 0.11 b	0.37± 0.06 b	0.35± 0.02 b	0.32± 0.03 b	0.37± 0.15 b	0.30± 0.02 b	0.48± 0.07 b	***	ns	***
M10	m-cymene	1032	A	4.3± 0.61 abcd	3.6± 0.41 abc	3.5± 0.69 ab	3.8± 0.43 abc	3.4± 0.78 ab	5.0± 0.71 abcde	2.8± 0.61 a	3.7± 0.55 abc	8.9± 1.4 f	6.6± 2.0 cdef	5.4± 0.28 abcde	7.9± 0.27 ef	4.2± 0.24 abcd	7.3± 0.20 def	5.8± 0.68 abcdef	6.0± 0.47 bcdef	***	***	***
M11	limonene	1034	A	39± 8.2 ab	43± 0.56 abc	33± 5.1 a	32± 2.3 a	39± 3.1 ab	32± 4.5 a	29± 3.9 cd	33± 3.1 a	54± 2.9 bcd	58± 4.5 bcd	59± 2.1 cd	46± 0.27 abc	65± 2.7 d	59± 2.1 cd	61± 1.6 cd	59± 1.9 cd	***	***	***
M12	β-(E)-ocimene	1049	B [23]	0.19± 0.03 a	0.18± 0.07 a	0.17± 0.05 a	0.24± 0.03 a	0.17± 0.02 a	0.16± 0.02 a	0.42± 0.08 a	0.18± 0.02 a	0.39± 0.04 a	0.25± 0.06 a	0.32± 0.11 a	0.46± 0.05 a	0.34± 0.08 a	0.28± 0.04 a	1.2± 0.22 b	0.42± 0.09 a	***	***	***
M13	γ-terpinene	1066	A	4.2± 1.2 ab	4.3± 1.2 ab	3.6± 0.60 a	5.9± 0.28 abcd	5.6± 0.27 abc	5.5± 1.4 abc	2.1± 0.90 a	5.6± 1.4 abc	17± 0.86 f	16± 1.6 f	10± 1.5 de	15± 0.67 f	8.0± 0.36 bcd	13± 1.3 ef	9.3± 0.60 ef	14± 0.27 f	***	***	***
M14	terpinolene	1097	A	0.62± 0.19	0.89± 0.07	0.53± 0.09	0.43± <0.01	0.36± 0.22	0.73± 0.2	0.57± 0.14	0.9± 0.31	0.75± 0.08	0.73± 0.11	0.76± 0.05	0.69± 0.06	0.79± 0.11	0.82± 0.04	0.84± 0.16	0.86± 0.12	*	ns	ns
M15	allo-ocimene	1132	B [24]	0.11± 0.06 a	0.10 ± 0.01 a	0.10 ± 0.05 a	0.31± 0.03 ab	0.24± 0.01 ab	0.13± 0.04 ab	0.31± 0.27 ab	0.13± 0.08 ab	0.33± 0.12 ab	0.14± 0.07 ab	0.23± 0.03 ab	0.57± 0.03 b	0.29± 0.01 ab	0.27± 0.05 ab	1.7± 0.36 c	0.41± 0.04 ab	***	***	***
M16	*p*-mentha-1,5,8-triene	1135	B [22]	0.26± 0.05 abc	0.10 ± 0.01 ab	0.22± 0.02 abc	0.56± 0.09 d	0.26± 0.07 abc	0.13± 0.09 ab	0.49± 0.17 cd	0.19± 0.08 ab	0.10± 0.02 ab	tr± 0.02 a	tr± 0.01 ab	0.12± 0.01 ab	0.10 ± <0.01 ab	0.10 ± <0.01 ab	0.34± 0.11 bcd	0.10 ± <0.01 ab	***	***	***
M17	pentylcyclohexa-1,3-diene	1166	B [19]	0.21± 0.05 ab	0.23± 0.08 ab	0.25± 0.03 ab	0.46± 0.11 b	0.31± 0.03 ab	0.06 ± 0.04 a	0.26± 0.16 ab	0.20± 0.01 ab	0.36± 0.09 b	0.34± 0.12 ab	0.23± 0.01 ab	0.34± 0.10 ab	0.27± 0.02 ab	0.18± 0.02 ab	0.22± 0.02 ab	0.25± 0.02 ab	*	*	*
M18	dihydrocarvone trans	1208	A	0.39± 0.09 e	0.36± 0.05 de	0.35± 0.08 de	0.19± 0.06 abcde	0.27± 0.05 cde	0.18± 0.04 abcd	0.20± 0.08 abcde	0.26± 0.02 bcde	tr± 0.02 ab	0.10± 0.01 abc	0.10 ± 0.02 abc	tr± 0.01 a	0.10 ± 0.03 abc	tr± 0.01 a	0.10 ± 0.02 abc	tr± 0.01 a	***	*	***
M19	carveol trans	1217	B [19]	0.23± 0.05	nd	0.10 ± 0.06	nd	0.10 ± 0.05	0.10 ± 0.06	0.16± 0.06	0.13± 0.08	0.10± 0.01	0.13± 0.03	0.19± 0.06	0.10 ± 0.01	0.15± 0.01	0.10± 0.02	0.10 ± 0.01	0.10 ± <0.01	*	ns	ns
M20	(E)-dihydrocarvone	1240	B [25]	0.79± 0.12 d	0.79± 0.14 d	0.67± 0.10 cd	0.41± 0.08 bc	0.57± 0.09 bcd	0.43± 0.05 bc	0.38± 0.06 b	0.59± 0.03 bcd	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
M21	L-carvone	1248	A	0.43± 0.19	0.36± 0.1	0.24± 0.02	0.18± 0.03	0.23± 0.08	0.34± 0.15	0.44± 0.07	0.29± 0.06	0.22± 0.03	0.14± 0.04	0.10 ± 0.01	tr± 0.02	tr± 0.01	nd	tr± 0.03	nd	**	ns	ns
M22	D-carvone	1262	A	0.96± 0.19 cd	0.57± 0.11 abc	1.5± 0.05 d	0.71± 0.06 abc	0.81± 0.13 bcd	0.61± 0.14 abc	0.75± 0.17 abc	1.1± 0.12 cd	0.20± 0.01 ab	0.12± 0.02 ab	tr± 0.02 a	0.10 ± 0.01 abc	0.10 ± 0.01 a	0.21± 0.01 ab	0.15± 0.02 ab	0.10 ± 0.01 abc	***	***	***
M23	thymol	1290	A	0.17± 0.05 c	0.11± 0.14 bc	0.12± 0.04 bc	0.15± 0.09 c	0.10± 0.08 ab	0.10± 0.03 bc	nd a	0.14± 0.11 bc	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a	***	***	***
M24	carvacrol	1317	A	0.54± 0.08 e	0.42± 0.09 cde	0.45± 0.03 de	0.60 ± 0.02 e	0.29± 0.03 bcd	0.39 ± 0.03 cde	0.18± 0.04 abc	0.52± 0.04 de	nd a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 a	tr± 0.01 ab	tr± 0.01 a	***	***	***
	Total			61	64	50	56	59	53	42	54	89	90	87	86	87	86	87	90			

Table 1. *Cont.*

Code	Compound	LRI_{expt} [a]	ID [b]	Percentage Composition (%) [c]																p [d]		
				2018								2020										
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	E [e]	G [f]	GxE [g]
	Monoterpenoid Alcohols																					
MA1	(+)-cis-p-mentha-2,8-dien-1-ol	1122	A	0.10± 0.03	0.15± 0.01	tr± 0.03	0.28 ± 0.03	0.10± 0.02	0.10± 0.04	tr± 0.03	0.14 ± 0.01	tr± 0.01	tr± 0.01	tr± 0.02	tr± 0.01	tr± 0.01	nd	tr± 0.01	tr± 0.01	ns	ns	ns
MA2	dihydrolinalool	1142	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	tr± 0.01 [a]	tr± 0.01 [b]	nd [a]	nd [a]	tr± 0.01 [a]	nd [a]	***	***	***
MA3	trans-pinocarveol	1147	B [26]	0.59± 0.13 [c]	0.63± 0.17 [c]	0.30± 0.08 [abc]	0.20± 0.08 [ab]	0.28± 0.02 [abc]	0.35± 0.21 [abc]	tr± 0.03 [a]	0.45± 0.10 [bc]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
MA4	terpinen-4-ol	1184	A	0.10± 0.01 [bc]	nd [a]	tr± 0.03 [ab]	tr± 0.03 [abc]	tr± 0.03 [ab]	tr± 0.07 [abc]	nd [a]	0.13± 0.03 [c]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
MA5	(E)-8-hydroxylinalool	1349	B [19]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	tr± 0.01 [ab]	0.10± 0.03 [bc]	0.10± 0.01 [c]	tr± 0.01 [ab]	0.10± 0.01 [c]	tr± 0.01 [ab]	tr± 0.01 [a]	tr± 0.01 [ab]	***	***	***
	Total			0.79	0.78	0.38	0.53	0.39	0.48	0.06	0.72	0.05	0.13	0.16	0.09	0.09	0.03	0.05	0.05			
	Sesquiterpenes																					
S1	α-ylangene	1384	B [22]	0.26± 0.11	0.24± 0.07	0.17± 0.11	tr± 0.01	0.16± 0.05	0.19± 0.1	0.20± 0.26	0.20± 0.14	0.10± 0.03	0.32± 0.25	0.27± 0.07	0.26± 0.1	0.16± 0.07	0.23± 0.06	0.16± 0.06	0.27± 0.08	ns	ns	ns
S2	α-copaene	1390	A	1.1 ± 0.02 [e]	0.86 ± 0.01 [de]	0.62 ± 0.03 [bcde]	0.10 ± 0.02 [a]	0.15 ± 0.05 [ab]	0.49 ± 0.03 [abcd]	0.78 ± 0.04 [cde]	0.77 ± 0.05 [cde]	tr± <0.01 [a]	0.39± 0.31 [abcd]	0.30± 0.05 [abc]	tr± 0.01 [a]	tr± 0.01 [ab]	0.17± 0.03 [ab]	0.30± 0.10 [abc]	0.42± 0.09 [abcd]	***	***	***
S3	(E)-β-caryophyllene	1430	B [27]	tr± 0.03	tr± 0.02	nd	nd	tr± 0.04	nd	nd	nd	tr± 0.01	tr± 0.01	tr± 0.01	tr± 0.01	nd	nd	nd	nd	ns	ns	ns
S4	β-caryophyllene	1445	A	4.4± 0.61 [cd]	5.5± 0.32 [d]	4.1± 0.43 [bcd]	2.5± 0.39 [abc]	4.3± 1.3 [cd]	4.1± 1.2 [bcd]	2.4± 0.29 [abc]	2.2± 0.50 [abc]	2.3± 0.37 [abc]	2.9± 0.66 [abc]	2.4± 0.22 [abc]	1.3± 0.52 [a]	1.7± 0.29 [ab]	2.0± 0.45 [abc]	0.89± 0.06 [a]	0.97± 0.19 [a]	***	***	***
S5	(+)-aromadendrene	1452	A	0.17± 0.04 [de]	0.21± 0.01 [e]	0.15± 0.04 [cde]	tr± 0.07 [abc]	0.13± 0.03 [abcde]	0.15± 0.08 [bcde]	tr± 0.06 [abc]	0.10± 0.01 [abcd]	0.10 ± 0.02 [abc]	0.10 ± 0.02 [abcd]	0.10± 0.02 [abcd]	tr± 0.01 [a]	tr± 0.01 [a]	tr± 0.01 [abc]	tr± <0.01 [a]	tr± 0.01 [ab]	***	***	***
S6	curcumene	1472	B [28]	0.18± 0.09 [cde]	0.23± 0.11 [e]	0.19± 0.06 [de]	tr± 0.05 [abcde]	0.15± 0.22 [bcde]	0.22± 0.19 [e]	tr± 0.03 [abcde]	0.12± 0.05 [abcde]	tr± 0.01 [abc]	0.10 ± 0.01 [abcd]	tr± 0.01 [abc]	tr± 0.01 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	***	**	***
S7	α-humulene	1479	A	0.42± 0.16 [abc]	0.70± 0.58 [c]	0.38± 0.29 [abc]	0.49± 1.1 [abc]	0.51± 0.76 [bc]	0.40± 0.65 [abc]	0.18± 1.2 [ab]	0.26± 0.9 [ab]	0.30± 0.14 [abc]	0.51± 0.04 [abc]	0.24± 0.06 [ab]	0.30± 0.09 [ab]	0.40± 0.06 [abc]	0.14± 0.03 [ab]	0.12± 0.01 [a]	0.14± 0.01 [ab]	***	***	***
S8	α-gurjunene	1495	B [29]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.10 ± 0.02 [bc]	0.10 ± 0.01 [bc]	0.10± <0.01 [bc]	0.10 ± 0.01 [ab]	0.10 ± 0.01 [bc]	0.10± 0.02 [bc]	0.10± 0.03 [c]	0.10± 0.01 [bc]	***	ns	***
S9	β-selinene	1508	B [30]	3.0± 0.05 [ab]	2.7± 0.06 [ab]	1.5± 0.02 [a]	4.6± 0.15 [b]	2.2± 0.19 [ab]	1.9± 0.12 [a]	3.3± 0.26 [ab]	3.0± 0.14 [ab]	2.5± 0.62 [ab]	1.6± 0.12 [a]	0.96± 0.16 [a]	1.4± 0.28 [a]	1.2± 0.32 [a]	0.85± 0.16 [a]	1.1± 0.23 [a]	1.7± 0.33 [a]	***	***	***
S10	valencene	1514	A	nd [a]	nd [a]	nd [a]	2.9± 0.44 [b]	nd [a]	nd [a]	nd [a]	0.20± 0.07 [a]	0.15± 0.21 [a]	0.15± 0.19 [a]	0.10± 0.01 [a]	2.6± 0.40 [b]	0.10± 0.05 [a]	0.10 ± 0.07 [a]	0.12± 0.04 [a]	0.18± 0.08 [a]	***	***	***
S11	α-selinene	1515	B [31]	0.61 ± 0.02 [bc]	0.60 ± 0.06 [bc]	0.43 ± 0.05 [abc]	0.63± 0.44 [bc]	0.54 ± 0.04 [abc]	0.44± 0.03 [abc]	0.71 ± 0.02 [c]	0.59± 0.01 [abc]	0.28± 0.06 [abc]	0.31± 0.09 [abc]	0.29± 0.04 [abc]	0.23± 0.05 [ab]	0.22± 0.05 [ab]	0.13± 0.08 [a]	0.23± 0.06 [ab]	0.33± 0.03 [abc]	***	ns	***
S12	kessane	1557	B [19]	nd [a]	0.12± 0.02 [a]	nd [a]	2.8± 0.05 [c]	nd [a]	nd [a]	nd [a]	nd [a]	0.26± 0.03 [a]	0.12± 0.09 [ab]	tr± 0.01 [a]	1.7± 0.21 [b]	0.10 ± 0.01 [a]	tr± 0.01 [ab]	tr± 0.01 [b]	tr± 0.01 [a]	***	***	***
S13	β-gurjuene [$]	1560	B [29]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	tr± 0.01 [b]	tr± 0.01 [ab]	nd [a]	tr± 0.03 [c]	tr± 0.01 [ab]	tr± 0.01 [ab]	nd [a]	nd [a]	***	***	***
	Total			10	11	7.5	14	8.2	7.9	7.7	7.4	6.1	6.6	4.8	8	3.9	3.8	3	4.2			
	Phthalides																					
P1	3-butylhexahydrophthalide	1662	B [19]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	tr± 0.01 [abc]	tr± 0.01 [ab]	tr± 0.01 [abc]	tr± 0.01 [ab]	tr± 0.01 [ab]	tr± 0.01 [bc]	tr± 0.01 [bc]	tr± 0.01 [ab]	***	ns	***
P2	butylphthalide	1676	A	5.0± 0.01 [b]	5.2± 0.03 [b]	9.4± 0.05 [c]	6.6± 0.01 [bc]	7.1± 0.03 [bc]	6.7± 0.01 [bc]	9.8± 0.06 [c]	7.0± 0.03 [bc]	0.73± 0.39 [a]	0.52± 0.28 [a]	0.93± 0.30 [a]	0.88± 0.28 [a]	0.67± 0.43 [a]	0.93± 0.60 [a]	1.6± 0.40 [a]	1.0± 0.30 [a]	***	*	***
P3	(Z)-3-butylidenephthalide	1685	B [19]	0.15± 0.06 [b]	0.18± 0.05 [b]	0.36± 0.09 [c]	0.15± 0.02 [bc]	0.23± 0.02 [b]	0.17± 0.07 [b]	0.25± 0.34 [bc]	0.18± 0.25 [b]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
P4	sedanenolide	1748	A	4.8± 0.30 [abcde]	9.7± 2.3 [cdef]	15± 1.9 [f]	16± 1.6 [f]	14± 3.0 [f]	9.5± 2.9 [bcdef]	11± 3.0 [def]	13± 2.2 [ef]	1.3± 0.49 [ab]	0.78± 0.18 [a]	2.3± 0.47 [abc]	1.9± 0.32 [abc]	1.4± 0.83 [ab]	3.1± 0.72 [abcd]	2.6± 0.28 [abcd]	1.4± 0.36 [ab]	***	***	***
P5	trans-neocnidilide	1755	B [19]	0.26± 0.03	0.24± 0.03	1.8± 0.02	0.16± 0.04	0.30± 0.06	0.78± 0.06	0.99± 0.04	0.94± 0.04	0.34± 0.1	0.13± 0.05	0.19± 0.22	0.08± 0.02	1.7± 0.88	0.59± 0.22	0.50± 0.06	0.24± 0.06	ns	ns	ns
P6	(E)-ligustilide	1764	B [32]	0.12± 0.02 [abc]	0.14± 0.10 [abc]	0.24± 0.01 [c]	0.23± 0.03 [c]	0.25± 0.05 [c]	0.14± 0.01 [abc]	0.18± 0.09 [ab]	0.18± 0.05 [ab]	tr± 0.01 [b]	tr± 0.01 [b]	tr± 0.02 [b]	tr± 0.01 [b]	0.10± 0.01 [ab]	tr± 0.01 [b]	tr± 0.01 [b]	tr± 0.01 [b]	***	ns	***
	Total			10	16	27	23	22	17	22	21	2.4	1.5	3.5	2.9	3.9	4.7	4.7	2.7			

Table 1. *Cont.*

Code	Compound	LRI_{expt} [a]	ID [b]	Percentage Composition (%) [c]														p [d]		
				2018								2020								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	E [e]	G [f]	GxE [g]
	Oxides																					
O1	(Z)-limonene oxide	1147	A	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.49± 0.37 [ab]	0.87± 0.11 [bc]	0.66± 0.04 [bc]	1.1± 0.15 [c]	0.66± 0.05 [bc]	1.7± 0.26 [d]	0.73± 0.07 [bc]	***	***	***
O2	caryophyllene oxide	1610	A	tr± 0.01 [ab]	0.13± 0.04 [b]	0.25± 0.05 [c]	0.10± 0.02 [ab]	010± 0.07 [ab]	0.10± 0.02 [ab]	tr± 0.01 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	***	***
	Total Unknowns			0.04	0.13	0.25	0.05	0.08	0.09	0.02	0	0	0.49	0.87	0.66	1.1	0.66	1.7	0.73			
U1	unknown 1	n/a		0.57± 0.09 [abc]	0.31± 0.03 [ab]	0.43± 0.06 [ab]	0.19± 0.02 [ab]	0.27± 0.01 [ab]	0.71± 0.20 [bc]	1.2± 0.47 [c]	0.51± 0.29 [abc]	0.10 ± 0.02 [ab]	tr± 0.02 [a]	tr± 0.04 [a]	tr± 0.01 [a]	0.11± 0.02 [ab]	0.18± 0.02 [ab]	0.13± 0.01 [ab]	0.10± 0.01 [ab]	***	**	***
U2	unknown 2	n/a		2.3± 0.63 [abc]	1.7± 0.03 [abc]	2.1± 0.06 [abc]	0.84± 0.02 [ab]	1.0± 0.01 [ab]	2.7± 0.20 [bc]	3.4± 0.47 [c]	1.5± 0.29 [abc]	0.28± 0.01 [a]	0.22± 0.05 [a]	0.47± 0.10 [a]	0.14± 0.04 [a]	0.63± 0.14 [ab]	0.65± 0.27 [ab]	0.44± 0.08 [a]	0.24± 0.05 [a]	***	*	***
U3	unknown 3	753		nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.14± 0.04 [ab]	tr± 0.01 [ab]	tr± 0.01 [ab]	nd [a]	tr± 0.01 [b]	tr± 0.01 [ab]	tr± 0.01 [a]	tr± 0.01 [a]	***	ns	***
U4	unknown 4	1081		nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.07 ± 0.02 [b]	tr± 0.02 [b]	0.10 ± 0.01 [b]	0.10 ± 0.02 [b]	0.10 ± 0.02 [bc]	0.11± 0.02 [cd]	0.15± 0.01 [d]	0.10 ± 0.01 [bc]	***	***	***
U5	unknown 5	1279		0.16± 0.06 [ab]	0.10± 0.01 [ab]	0.10± 0.01 [ab]	0.13± 0.03 [ab]	0.24 ± 0.01 [b]	0.11 ± 0.01 [ab]	0.17 ± 0.03 [ab]	0.10 ± 0.04 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	**	ns	**
U6	unknown 6	1362		0.10± 0.02 [ab]	0.10± 0.04 [ab]	nd [a]	0.16± 0.01 [b]	tr± 0.04 [a]	0.10± 0.01 [ab]	0.10± 0.01 [ab]	0.10± 0.04 [ab]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	*	***
U7	unknown 7	1539		0.25± 0.05 [cd]	0.33± 0.01 [d]	0.19± 0.02 [bcd]	0.10 ± 0.01 [ab]	0.15 ± 0.06 [abc]	0.10± 0.08 [abc]	0.18± 0.15 [bcd]	0.15± 0.06 [abc]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	***	*	***
U8	unknown 8	1542		tr± 0.01 [a]	nd [a]	0.10± 0.03 [ab]	nd [a]	0.10 ± 0.04 [ab]	0.10± 0.04 [ab]	0.10 ± 0.01 [ab]	0.10 ± 0.03 [ab]	nd [a]	0.10± 0.05 [b]	0.10± 0.02 [b]	nd [a]	0.10± 0.02 [b]	0.10± 0.02 [ab]	tr± 0.01 [ab]	0.11± 0.01 [b]	***	**	***
U9	unknown 9	1653		nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.10± 0.05 [ab]	tr± 0.02 [a]	tr± 0.02 [a]	tr± 0.01 [ab]	tr± 0.01 [ab]	tr± 0.03 [a]	tr± 0.01 [ab]	0.16± 0.08 [b]	**	**	**
U10	unknown 10	1776		nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	nd [a]	0.04 ± 0.02 [ab]	tr± 0.01 [ab]	tr± 0.01 [ab]	nd [a]	tr± 0.02 [ab]	tr± 0.03 [ab]	tr± 0.01 [ab]	tr± 0.01 [ab]	***	ns	**
	Total			3.4	2.5	2.9	1.4	1.8	3.8	5.1	2.4	0.7	0.44	0.67	0.29	1	1.1	0.81	0.72			

[a] Linear retention index on a HP-5MS column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; [$] tentatively identified, spectral quality value of 70 was used for this compound. [c] Percentage composition of total peak area divided by compound peak area; means labelled with letters are significantly different ($p < 0.05$) according to the GxE interaction; means of three replicate samples; tr, trace amounts <0.10%; nd, not detected. [d] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [e] Harvest year. [f] Genotype. [g] Harvest year × genotype interaction. Cells have been colour coded; red expresses the genotype with the higher value compared to harvest year; green expresses the genotype with the lower value compared to harvest year; no colour expresses no difference in percentage composition for both years.

Previous research has shown that monoterpenes comprise the majority of the aroma profile of celery. In this study and for both years, monoterpenes comprised the majority of the aroma composition of the eight celery genotypes, making up an average of 55% of the aroma composition in 2018 and 88% in 2020, which is a significantly higher proportion of the total profile and confirms previous research. Orav, Kailas and Jegorova [33] reported similar results in Estonian grown celery, where monoterpenes content comprised 85.3% of total flavour profile. In particular, limonene was one of the most abundant compounds with an average percentage composition of 31% in 2018 and 58% in 2020. Limonene odour has been described as citrusy, pine and minty [5,16]. These are not typical descriptors used to describe celery odour and although its prominence is dominant in celery, its contribution to the aroma profile is minimal. Other terpenoid compounds including camphene, α-pinene and β-pinene, γ-terpinene, β-caryophyllene, α-humulene and kessane identified in this study were also detected in many other studies in varying proportions [8–10,12,14,33,34].

Phthalide compounds are known as odour active compounds and main contributors to the characteristic odour of celery [2,15,33–36]. These compounds impart a "herbal" and "celery-like" aroma [5,16]. The proportion of the aroma profile comprised of phthalide compounds varied between years and genotype, with 2018 exhibiting a higher proportion composition compared to 2020. Lund, Wagner and Bryan [15] identified sedanenolide, 3-n-butylphthalide, hexahy-dro-3-n-butylphthalide and β-selinene to exhibit a celery-like odour. Three of these compounds were identified in all eight genotypes in both harvest years but their contribution to the composition varied. Sedanenolide and β-selinene had a higher proportion of the 2018 grown celery and are observed in the highest proportion in genotype 12. van Wassenhove, Dirinck, Vulsteke and Schamp [14] observed slight differences in the concentration of these compounds between years, however, unlike this study, no significant differences were reported. Furthermore, they presented a similar phthalide content, ranging from 6–11%, while in this study 19% and 3% was comprised of phthalides. The variation in the prominence of sedanenolide found in celery is very apparent not only in this study but in a plethora of studies where the percentage composition ranges from 0.2–39.5% [5]. Genotype 12 exhibited a high proportion of monoterpenes and the highest proportion of sesquiterpenes for both harvest years. In 2018, genotype 10 expressed the highest proportion of phthalides compared to other genotypes, exhibiting a high percentage of 3-n-butylphthalide (9.4%) and sedanenolide (15%) and genotype 12 had the highest proportion of sedanenolide (16%). On the other hand, genotypes 18 and 22 in 2020 exhibited the highest proportion of these compounds including 3-n-butylphthalide (3.1 and 2.6%, respectively). Turner et al. [5] identified 3-n-butylphthalide to be the most commonly reported phthalide [2,3,11,13,16,33,35,36]. Based on this observation, genotypes 10 and 12 in 2018 and genotype 22 in 2020 could be perceived as the genotypes with the strongest celery odour.

In terms of other compounds, smaller differences in the average composition between the years were observed: alcohols 1.3% and 0.15%, esters 0.16% and 0.5% and finally alkanes 1.6% for both 2018 and 2020 harvests, respectively. Limited research has been published about these types of compounds and their contribution to the celery aroma profile. By combining GC/MS and gas chromatography/olfactometry (GC/O), Turner et al. [16] identified compounds that contribute to the distinct celery aroma and how the aroma changed and developed throughout maturity. Using two of the same genotypes also used in this study (12 and 22), the aroma development over three time-points was studied: two-weeks before commercial maturity, at commercial maturity and two-weeks after commercial maturity. Monoterpene, sesquiterpene and phthalide compounds identified in the present study reflect those compounds observed by Turner et al. [16] and demonstrate that they are strongly influenced by maturity. Once commercial maturity was reached, the relative abundance of these compounds in the overall profile decreased, while alcohol and ester compounds became more abundant. Esters also identified by Turner et al. [16], including carveol acetate and hexyl hexanoate, were reported to contribute to green, herbal and damp odours in overmature celery according to GC/O analysis. The ester composition

in the present study also varied as a consequence of both genotype and harvest year (Table 1) and a higher ester composition was observed from the 2020 harvest; however, methyl butanoate and (E)-pinocarvyl acetate were not significantly influenced by the genotype, only harvest year.

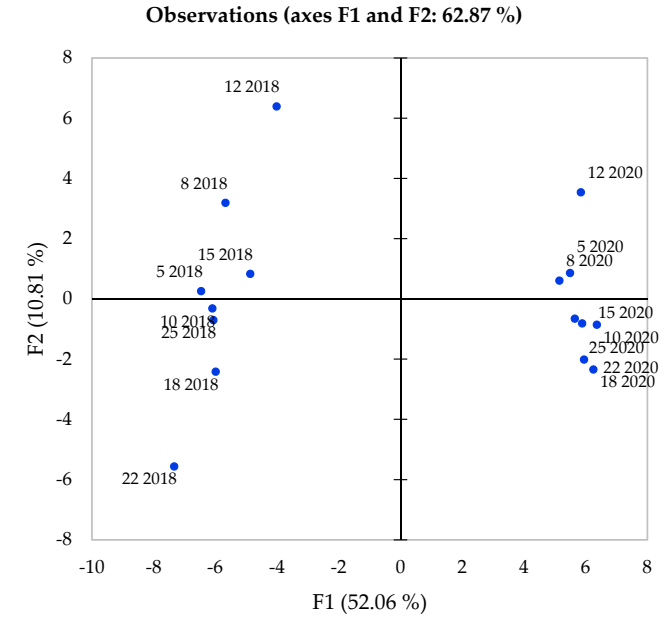
Principal component analysis (PCA) allowed for the visual comparison of the volatile composition of the eight celery genotypes in 2018 and 2020 (Figure 1) and the examination of any correlations occurring between genotype, harvest year and chemical compounds. Using only the significant compounds for harvest year, genotype and their interaction, a clear divide between the compounds associated with each year was observed. Principal component one (F1) and two (F2) explained 62.78% in total of the variation present in the data and it can be observed that the first axis separated samples from the two harvest years (2018 and 2020), while the second axis separated the various genotypes within a harvest year. Differences between the harvest years were apparent as is exhibited by the separation along the F1 component, which accounts for 52.06% of the variation. Genotypes were consistently separated across the F2 component for both years, which explains 10.81% of the variation. Metabolic pathways are genetically regulated, leading to the hypothesis that compounds that are important to a particular cultivar should remain fairly constant in their relative abundance between seasons and any deviations in these compounds are most likely due to external factors rather than genotype [37]. Genotypes 12, 8 and 5 for both years along with genotype 15 from 2018 were positively correlated with F2. Conversely, genotypes 10, 18, 22 and 25 for both years were negatively associated with F2.

Predominantly, monoterpenes and phthalides were separated across F2 and influenced by genotype, while sesquiterpenes, aldehydes and esters were separated across F1, respectively. Strong significant relationships were also observed between the compound groups, such as with alcohols and aldehydes expressing strong and positive correlations together, while low boiling monoterpenes including delta-3-carene and limonene expressed strong negative correlations with alcohols and aldehydes. Conversely, sesquiterpenes and phthalides had a negative correlation with the above monoterpenes and, instead, expressed a positive correlation with higher boiling monoterpenes including L-carvone, thymol and carvacrol.

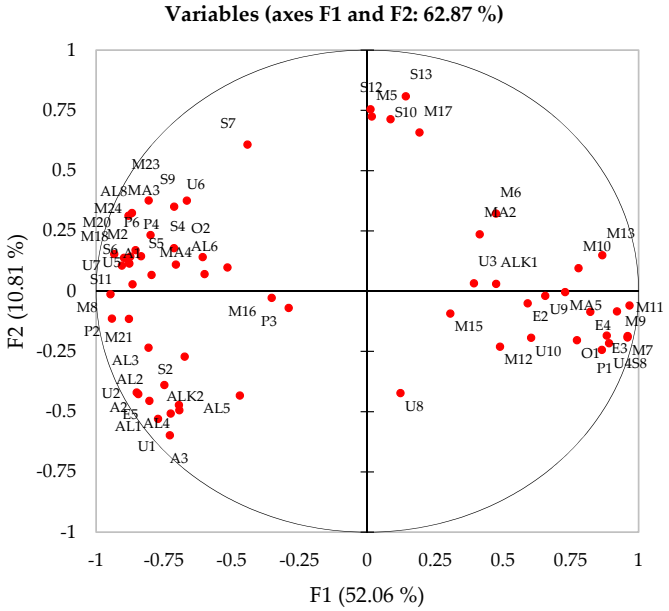
In 2018, the genotype had a stronger influence over the volatile composition and this is reflected through the more noticeable separation between the eight genotypes and a stronger association with aroma compounds. However, genotypes 12, 18, 22 and 25 exhibited similar placement on the observation plot between the two years, albeit on opposing sides of F2. Monoterpenes (M2, 8, 16, 18, 21, 22, 23, 24), monoterpenoid alcohols (MA3, 4), sesquiterpenes (S2, 4, 5, 6, 9) and phthalides (P2, 3, 4,6) were positively correlated with celery samples grown in 2018. Conversely, monoterpenes (M6, 7, 9, 10, 11, 12, 13, 15), sesquiterpenes (S8, 10, 12, 13), monoterpenoid alcohols (MA2, 5) were positively correlated with celery samples grown in 2020. The spread of monoterpene and sesquiterpene compounds across the plot and presence within all genotypes across both years (Table 1) proves these are fundamental compounds to celery. As it can be observed from Figure 1, the aroma profile in 2018 consisted of a higher proportion of phthalide compounds than in 2020, where all phthalides, apart from 3-butylhexahydro phthalide (P1), appeared closely associated with the 2018 samples. Due to the odour active nature of sedanenolide and other phthalides and the strong celery odours that these compounds impart, celery genotypes exhibiting a high proportion of these compounds are more likely to possess a strong characteristic celery odour.

The harvest year and genotype both had an influence on the volatile content of celery samples, however, a much stronger influence over the percentage composition for all genotypes and the majority of volatile compounds was observed by harvest year. Genotypes exhibited fewer significant differences over the majority of monoterpenes, aldehydes, sesquiterpenes and phthalides. Although the genotype is known to play a role in predetermining the aroma composition [37], the variation caused by harvest year and, therefore, the growing environment possessed a more significant role in determining

the aroma composition (Table 1, Figure 1). Differences in climate during growth are most likely the cause of these compositional changes and will be discussed further in Section 3.3. The aroma and flavour quality of certain genotypes such as 12, 18 and 25 were consistent across the two years demonstrating that these genotypes may provide consistent quality crop for celery growers and breeders irrespective of the environmental changes. Carrying out sensory profiling on these cultivars will permit the examination of the impact of the different compositions caused by genotype and harvest year on flavour perception.



(**A**)



(**B**)

Figure 1. *Cont.*

A1	3-methyl-3-buten-1-ol	M24	carvacrol
A2	(E)-2-penten-1-ol	MA2	dihydrolinalool
A3	1-pentanol	MA3	trans-pinocarveol
AL1	hexanal	MA4	terpinen-4-ol
AL2	(E)-2-hexenal	MA5	(E)-8-hydroxylinalool
AL3	heptanal	S2	α-copaene
AL4	(E)-2-heptenal	S4	β-caryophyllene
AL5	n-octanal	S5	(+)-aromadendrene
AL6	m-tolualdehyde	S6	curcumene
AL8	(E,E)-2,6-nonadienal	S7	α-humulene
E2	1-octen-3-yl-acetate	S8	α-gurjunene
E3	(E)-pinocarvyl acetate	S9	β-selinene
E4	carveol acetate	S10	valencene
E5	hexyl hexanoate	S11	α-selinene
ALK1	nonane	S12	kessane
ALK2	decane	S13	β-gurjuene
M2	α-pinene	P1	3-butylhexahydro phthalide
M5	β-pinene	P2	3-n-butylphthalide
M6	myrcene	P3	(Z)-3-butylidenephthalide
M7	α -phellandrene	P4	sedanenolide
M8	delta-3-carene	P6	(cis)-ligustilide
M9	α -terpinene	O1	(Z)-limonene oxide
M10	m-cymene	O2	caryophyllene oxide
M11	limonene	U1	unknown 1
M12	β-(E)-ocimene	U2	unknown 2
M13	γ-terpinene	U3	unknown 3
M15	allo-ocimene	U4	unknown 4
M16	p-mentha-1,5,8-triene	U5	unknown 5
M17	pentylcyclohexa-1,3-diene	U6	unknown 6
M18	dihydrocarvone trans	U7	unknown 7
M20	(E)-dihydrocarvone	U8	unknown 8
M21	L-carvone	U9	unknown 9
M23	thymol	U10	unknown 10
		(**C**)	

Figure 1. Principal component analysis of eight celery samples harvested in 2018 and 2020 showing correlations with volatile compounds. (**A**) Projection of the samples; (**B**) Distribution of variables; (**C**) Compound codes as appear in plot (**B**).

3.2. Sensory Evaluation of Fresh Celery Samples

The sensory profile of the eight celery samples was generated by a trained panel who came to the consensus of 22 and 24 terms for the quantitative assessment of samples in the 2018 and 2020 samples, respectively. The two additional attributes in 2020 were that of "fresh parsley flavour" and "celery residue in mouth" as an aftereffect. Table 2 shows the mean panel scores for these attributes. Out of the 22 attributes that were profiled in 2018, 14 of these were found to be significantly different between the genotypes and in 2020, 18 out of the 24 attributes were found to be significantly different. There were few significant assessor × sample interactions identified for both the 2018 and 2020 harvests, which suggests that the panelists scored samples in a consistent manner [38].

Statistical comparison of sensory differences between years could not be completed due to the two-year difference between harvests, however, general trends will be discussed. All appearance attributes showed a strong significant difference for both years between

genotypes and this is due to the fact that the genotypes selected for the study included genotypes that were white, green or pink and with varying heights. The scoring for these attributes remained consistent between years for each genotype. Similarly, mouthfeel attributes of crunchiness and moistness scored consistently between the years for each genotype. A relationship between the ribbed appearance of the petiole with the stringiness mouthfeel was observed and it changed significantly between the years for individual genotypes more than any other attribute. Lignin, a key component in providing mechanical structure in higher plants, such as celery, has been shown to be influenced by abiotic and biotic stresses. Low temperatures have been observed [39,40] to influence the synthesis of lignin and its precursors. Li et al. [41] identified all microRNAs two celery varieties to be sensitive to temperature stress and a stronger response was observed towards cold stress, suggesting that cooler temperatures are optimal for celery growth. The structural differences observed in the genotypes in 2018 could be a response to stress and the cooler temperatures of 2020 provided optimal temperatures for lignin synthesis, which causes these genotypes to be perceived as more crunchy, stringy and firm.

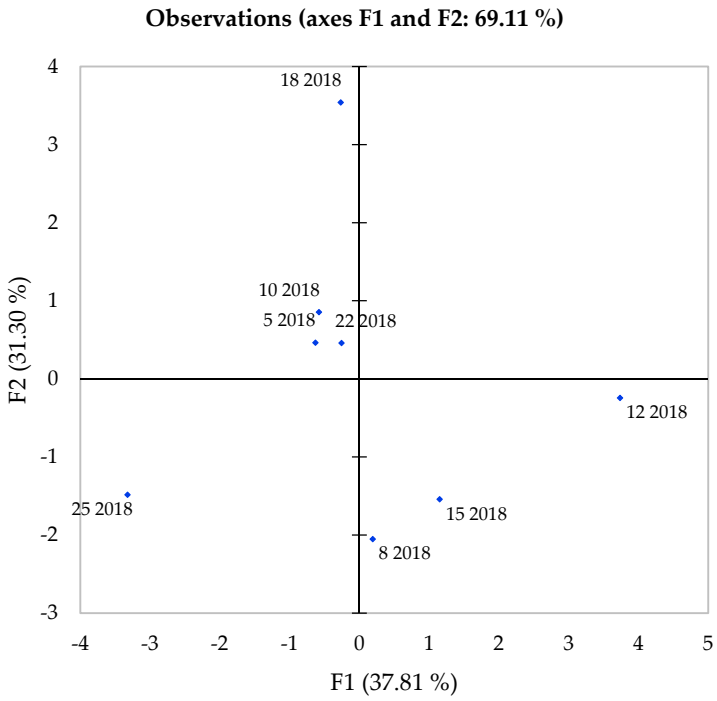
The odour and flavour attributes evaluated displayed clear significant differences between both genotypes and harvest year. The attributes "watery/cucumber" and "rocket" flavour along with "grass/green" odour were scored highly in the 2018 harvest, while "fresh fennel and parsley" flavour were scored highly in the 2020 harvest. "Fresh coriander" aroma and flavour along with "soapy" flavour were scored similarly for both years. Genotype 25 was scored low for both years for flavour and aroma attributes apart from the "watery/cucumber" flavour, while genotype 12 was scored as the most bitter for both years. Combining these attributes with the volatile compounds identified through GC/MS (Table 1) provided a deeper understanding in the differences within the aroma composition and its impact on flavour perception. Principal component analysis was used to visualise the sensory and chemical differences across the eight genotypes and the volatile compounds identified (Table 1) and the attributes related to odour and flavour were used as variables (Figures 2 and 3).

Firstly, a clear variation between the genotype was observed in 2018 (Figure 2) whereby principal component one (F1) and two (F2) explained 69.11% of the total variation within the data. The first axis separates genotypes 5, 10, 18 and 22 from other genotypes, whereas the second axis separates genotypes 8, 15 and 12. Genotype 25 had low scores for most of the flavour attributes and only scored high in the watery and cucumber flavour. On the other hand, genotype 12 negatively correlated with genotype 25 and was associated with a parsley and grass-like odour with a rocket aftertaste. Genotype 18 was positively correlated to the fresh fennel flavour with the soapy characteristics that accompany many members of the Apiaceae family, such as coriander. A grouping of aroma compounds in the centre of the PCA was observed, whereas the sensory characteristics were positioned in the outer rim of the biplot with genotypes 5, 10 and 22 grouped in the middle of the observation plot. Apart from genotype 10, these exhibited an average volatile content (Table 1) compared to genotype 12 along with no strong association with sensory attributes (Figure 2). Many of the phthalides were associated with genotypes 12 and 10.

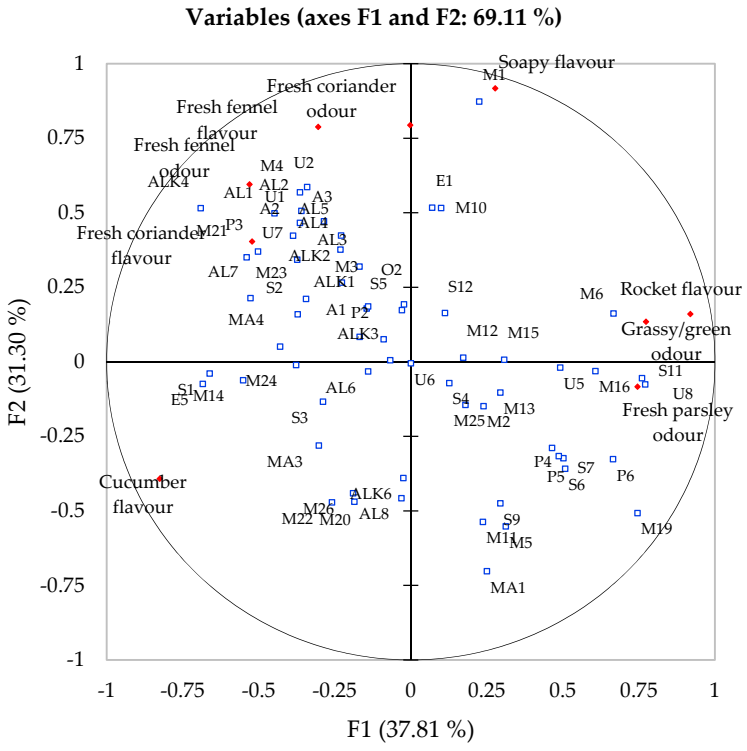
Table 2. Mean panel scores for sensory attributes of the eight celery samples harvested in 2018 and 2020.

Attribute	Score [A]																	
	2018									2020								
	5	8	10	12	15	18	22	25	p [b]	5	8	10	12	15	18	22	25	p [B]
Appearance																		
Colour	56.4 ^{b}	63.6 ^{ab}	62.6 ^{ab}	72.9 ^{a}	72.1 ^{a}	65.6 ^{ab}	70.5 ^{a}	26.8 ^{c}	***	46.3 ^{cd}	53.0 ^{bcd}	44.6 ^{d}	67.5 ^{ab}	61.0 ^{abc}	55.6 ^{abcd}	70.5 ^{a}	14.7 ^{e}	***
Stalk thickness	49.8 ^{ab}	49.5 ^{ab}	55.8 ^{a}	20.9 ^{b}	58.7 ^{a}	62.5 ^{a}	61.3 ^{a}	55.0 ^{a}	***	60.6 ^{abc}	47.7 ^{cde}	36.2 ^{def}	20.7 ^{ee}	51.1 ^{cd}	74.1 ^{a}	72.0 ^{ab}	59.8 ^{abc}	***
Ribbed	46.6 ^{bc}	61.0 ^{ab}	61.7 ^{a}	65.9 ^{a}	35.5 ^{cd}	25.4 ^{d}	34.2 ^{cd}	37.4 ^{cd}	***	60.3 ^{ab}	65.8 ^{a}	66.6 ^{a}	68.5 ^{a}	45.9 ^{b}	50.7 ^{b}	56.4 ^{ab}	55.6 ^{ab}	***
Aroma																		
Fresh fennel	16.5	14.2	18.9	15.5	15.3	18.6	15.4	18.2	ns	32.1	22.1	22.8	21.1	23.6	19.8	30.8	20.3	*
Grassy/green	32.6 ^{a}	31.0 ^{ab}	32.1 ^{ab}	36.3 ^{a}	30.7 ^{ab}	28.3 ^{ab}	35.3 ^{a}	21.1 ^{b}	***	27.1 ^{ab}	33.8 ^{a}	25.9 ^{ab}	32.8 ^{a}	34.5 ^{a}	34.6 ^{a}	28.5 ^{ab}	18.2 ^{b}	***
Fresh parsley	14.1	19.7	19.0	19.1	20.6	16.7	16.7	10.8	ns	18.0	19.2	20.8	16.8	20.6	19.4	17.3	16.4	ns
Fresh coriander	12.8	12.1	14.2	11.7	14.2	17.5	15.4	11.1	ns	15.4	13.0	14.8	12.0	14.2	16.6	16.3	7.7	ns
Taste/flavour																		
Bitter	23.1 ^{abc}	24.0 ^{abc}	24.7 ^{abc}	35.9 ^{a}	28.2 ^{abc}	31.3 ^{ab}	24.4 ^{abc}	15.5 ^{c}	**	33.2 ^{abc}	20.6 ^{abc}	35.0 ^{ab}	38.4 ^{a}	35.2 ^{a}	34.4 ^{ab}	33.0 ^{abc}	19.6 ^{c}	***
Sweet	15.2 ^{bcd}	20.3 ^{ab}	21.6 ^{ab}	10.6 ^{d}	15.6 ^{bcd}	12.2 ^{cd}	20.0 ^{ab}	24.6 ^{a}	***	17.3 ^{abc}	25.0 ^{abc}	20.0 ^{abc}	17.1 ^{abc}	13.1 ^{c}	14.8 ^{bc}	18.1 ^{abc}	23.7 ^{ab}	**
Fresh fennel	11.9	10.3	12.6	11.0	7.7	13.6	11.6	11.3	ns	27.5 ^{a}	23.5 ^{ab}	23.3 ^{ab}	16.9 ^{ab}	21.1 ^{ab}	13.7 ^{b}	23.3 ^{ab}	21.3 ^{ab}	**
Rocket	11.3 ^{bc}	13.4 ^{bc}	12.4 ^{bc}	23.8 ^{a}	16.6 ^{abc}	16.9 ^{abc}	10.4 ^{bc}	7.7 ^{c}	***	1.1	1.8	2.7	3.8	4.2	0.7	3.4	1.3	ns
Fresh coriander	17.5	16.3	16.0	9.6	15.0	18.1	18.9	14.1	ns	17.2	18.2	21.2	19.1	16.7	18.2	17.9	11.6	ns
Soapy	18.2 ^{ab}	12.4 ^{b}	16.4 ^{ab}	18.4 ^{ab}	15.4 ^{ab}	23.7 ^{a}	16.3 ^{ab}	13.0 ^{ab}	*	14.9 ^{ab}	14.2 ^{ab}	19.1 ^{ab}	20.0 ^{a}	17.4 ^{ab}	22.9 ^{a}	14.1 ^{ab}	9.3 ^{b}	***
Watery/cucumber	25.7 ^{ab}	33.2 ^{ab}	30.4 ^{ab}	9.1 ^{c}	30.0 ^{ab}	22.4 ^{b}	27.9 ^{ab}	37.7 ^{a}	***	19.8 ^{ab}	15.7 ^{ab}	12.1 ^{b}	10.8 ^{b}	16.2 ^{ab}	20.5 ^{ab}	23.2 ^{ab}	27.0 ^{a}	**
Fresh parsley	nd	nd	nd	nd	nd	nd	nd	nd		15.5	14.7	13.8	16.7	15.2	13.0	11.0	9.7	ns
Mouthfeel																		
Crunchy	65.4 ^{abc}	62.6 ^{bc}	64.9 ^{abc}	56.7 ^{c}	70.2 ^{ab}	66.4 ^{abc}	73.7 ^{a}	62.5 ^{bc}	***	70.6 ^{ab}	65.8 ^{ab}	72.9 ^{a}	66.7 ^{ab}	74.2 ^{a}	58.5 ^{b}	74.7 ^{a}	67.6 ^{ab}	**
Stringy	40.8 ^{b}	46.6 ^{b}	40.1 ^{b}	64.1 ^{a}	33.2 ^{b}	40.6 ^{b}	35.1 ^{b}	35.2 ^{b}	***	53.2 ^{bc}	62.8 ^{ab}	61.8 ^{ab}	74.2 ^{a}	54.4 ^{bc}	45.7 ^{c}	51.1 ^{bc}	45.1 ^{c}	***
Moist	50.6 ^{a}	47.2 ^{a}	50.0 ^{a}	29.7 ^{b}	53.1 ^{a}	44.3 ^{a}	51.4 ^{a}	54.8 ^{a}	***	55.0 ^{abc}	51.0 ^{bc}	44.8 ^{c}	28.3 ^{d}	49.3 ^{bc}	50.3 ^{bc}	54.8 ^{bc}	57.6 ^{ab}	***
Firmness of first bite	63.7	59.9	63.3	59.2	68.9	65.7	67.6	58.6	ns	69.3 ^{ab}	65.2 ^{ab}	68.1 ^{ab}	66.2 ^{ab}	72.4 ^{ab}	60.6 ^{b}	74.9 ^{a}	65.1 ^{ab}	*
After effects																		
Celery residue in mouth	nd	nd	nd	nd	nd	nd	nd	nd		51.4 ^{ab}	51.1 ^{ab}	52.5 ^{ab}	64.0 ^{a}	48.3 ^{b}	45.8 ^{b}	48.8 ^{ab}	39.4 ^{b}	***
Soapy	16.9 ^{ab}	15.7 ^{ab}	16.7 ^{ab}	21.2 ^{ab}	19.9 ^{ab}	24.8 ^{a}	18.6 ^{ab}	12.9 ^{b}	*	15.4 ^{b}	14.4 ^{b}	21.1 ^{b}	23.2 ^{a}	18.0 ^{b}	21.2 ^{b}	14.4 ^{b}	14.6 ^{b}	**
Grassy/green	27.7	27.0	27.9	27.6	28.4	26.4	31.4	19.0	ns	14.8	20.6	19.0	18.4	21.3	20.1	21.7	15.3	ns
Numbness	13.1	8.6	9.6	11.5	10.0	14.0	9.8	9.0	ns	11.4 ^{a}	12.1 ^{a}	11.5 ^{a}	11.7 ^{a}	12.6 ^{a}	13.2 ^{a}	9.8 ^{b}	7.3 ^{b}	**
Bitter	17.4 ^{bc}	18.4 ^{bc}	18.3 ^{bc}	29.0 ^{a}	19.1 ^{bc}	25.7 ^{ab}	16.0 ^{bc}	12.0 ^{c}	***	18.0 ^{bc}	20.9 ^{abc}	28.5 ^{a}	27.5 ^{ab}	25.5 ^{ab}	23.0 ^{abc}	19.6 ^{abc}	13.5 ^{c}	***

[A] Means are from two replicate samples; differing small letters (a, b, c, d, e, f) represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$); nd, not detected. [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.



(**A**)



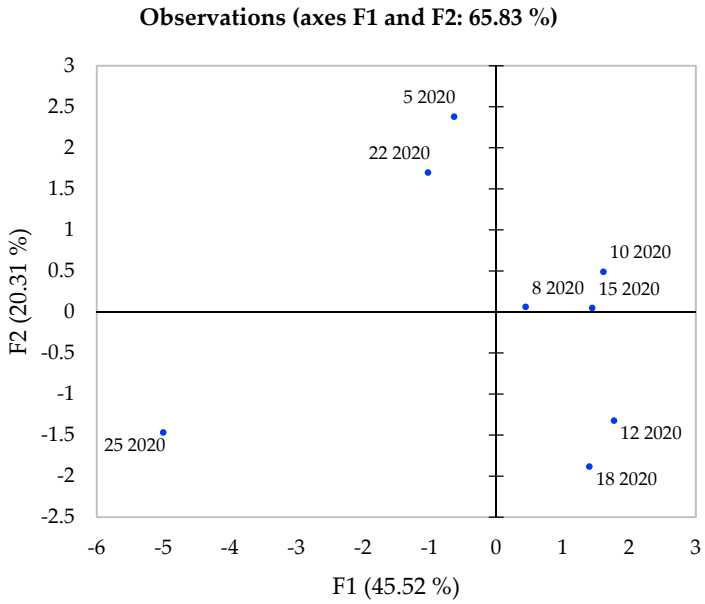
(**B**)

Figure 2. *Cont.*

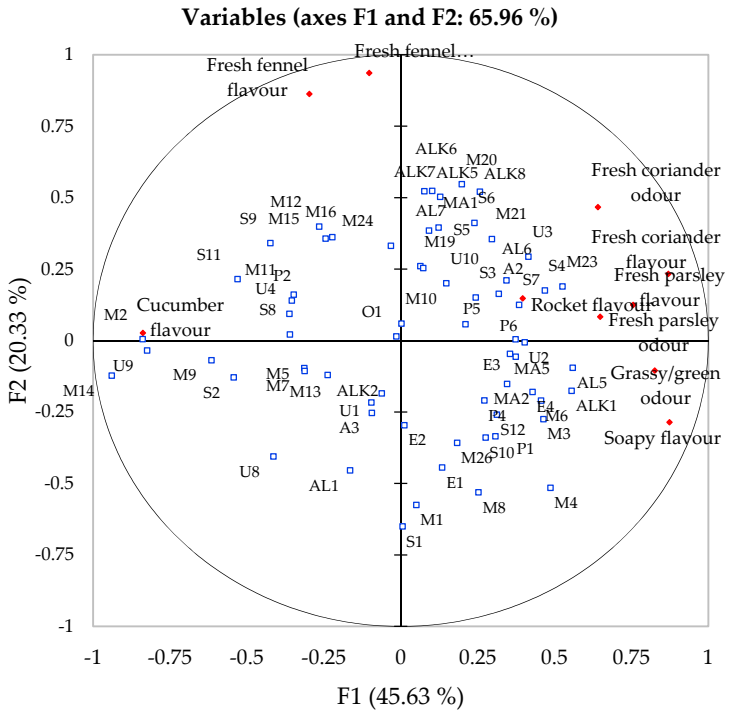
A1	3-methyl-3-buten-1-ol	M20	dihydrocarvone trans
A2	(E)-2-penten-1-ol	M21	carveol trans
A3	1-pentanol	M22	(E)-dihydrocarvone
AL1	hexanal	M23	L-carvone
AL2	(E)-2-hexenal	M24	D-carvone
AL3	heptanal	M25	thymol
AL4	(E)-2-heptenal	M26	carvacrol
AL5	n-octanal	MA1	(+)-cis-p-mentha-2,8-dien-1-ol
AL6	m-tolualdehyde	MA3	trans-pinocarveol
AL7	nonanal	MA4	terpinen-4-ol
AL8	(E,E)-2,6-nonadienal	S1	α-ylangene
E1	methyl butanoate	S2	α-copaene
E5	hexyl hexanoate	S3	(E)-β-caryophyllene
ALK1	nonane	S4	β-caryophyllene
ALK2	decane	S5	(+)-aromadendrene
ALK3	undecane	S6	curcumene
ALK4	dodecane	S7	α-humulene
ALK6	tetradecane	S9	β-selinene
M1	α-thujene	S11	α-selinene
M2	α-pinene	S12	kessane
M3	camphene	P2	3-n-butylphthalide
M4	sabinene	P3	(Z)-3-butylidenephthalide
M5	β-pinene	P4	sedanenolide
M6	myrcene	P5	trans-neocnidilide
M10	m-cymene	P6	(cis)-ligustilide
M11	limonene	O2	caryophyllene oxide
M12	β-(E)-ocimene	U1	unknown 1
M13	γ-terpinene	U2	unknown 2
M14	terpinolene	U5	unknown 5
M15	allo-ocimene	U6	unknown 6
M16	*p*-mentha-1,5,8-triene	U7	unknown 7
M19	pentylcyclohexa-1,3-diene	U8	unknown 8

(**C**)

Figure 2. Principal component analysis of eight celery samples harvested in 2018 showing correlations with volatile compounds and sensory attributes. (**A**) Projection of the samples; (**B**) Distribution of variables; (**C**) Compound codes as they appear in plot (**B**).



(**A**)



(**B**)

Figure 3. *Cont.*

A2	(E)-2-penten-1-ol	M19	pentylcyclohexa-1,3-diene
A3	1-pentanol	M20	dihydrocarvone trans
AL1	hexanal	M21	carveol trans
AL5	n-octanal	M23	L-carvone
AL6	m-tolualdehyde	M24	D-carvone
AL7	nonanal	M26	carvacrol
E1	methyl butanoate	MA1	(+)-cis-p-mentha-2,8-dien-1-ol
E2	1-octen-3-yl-acetate	MA2	dihydrolinalool
E3	(E)-pinocarvyl acetate	MA5	(E)-8-hydroxylinalool
E4	carveol acetate	S1	α-ylangene
ALK1	nonane	S2	α-copaene
ALK2	decane	S3	(E)-β-caryophyllene
ALK5	tridecane	S4	β-caryophyllene
ALK6	tetradecane	S5	(+)-aromadendrene
ALK7	pentadecane	S6	curcumene
ALK8	hexadecane	S7	α-humulene
M1	α-thujene	S8	α-gurjunene
M2	α-pinene	S9	β-selinene
M3	camphene	S10	valencene
M4	sabinene	S11	α-selinene
M5	β-pinene	S12	kessane
M6	myrcene	P1	3-butylhexahydro phthalide
M7	α -phellandrene	P2	3-n-butylphthalide
M8	delta-3-carene	P4	sedanenolide
M9	α -terpinene	P5	trans-neocnidilide
M10	m-cymene	P6	(cis)-ligustilide
M11	limonene	O1	(Z)-limonene oxide
M12	β-(E)-ocimene	U1	unknown 1
M13	γ-terpinene	U2	unknown 2
M14	terpinolene	U3	unknown 3
M15	allo-ocimene	U4	unknown 4
M16	*p*-mentha-1,5,8-triene	U8	unknown 8
		U9	unknown 9

(**C**)

Figure 3. Principal component analysis of eight celery samples harvested in 2020 showing correlations with volatile compounds and sensory attributes. (**A**) Projection of the samples; (**B**) Distribution of the variables; (**C**) Compound codes as they appear in plot (**B**).

Overall, it seems that the majority of monoterpenes were negatively correlated with the first principal component (F1) and compounds belonging to classes such as alcohols, sesquiterpenes and phthalides were positively associated with F1 along with the majority of the flavour attributes. Samples harvested in 2018 exhibited a lower proportion of monoterpenes but a higher proportion of alcohols and aldehydes, thus, explaining the low association with many of the flavour and aroma attributes from the sensory analysis.

In 2020, principal component one (F1) and two (F2) explained 65.96% of the total variation present and it can be observed that the first axis separates genotypes 5, 8, 10, 15 and 22, whereas the second axis separates genotypes 12, 18 and 25. According to the data presented in Figure 3, the genotype appears to express a weaker influence over the volatile composition than in 2018, which explains 20.31% of the variation present within the data. Differences in the volatile composition for the celery samples harvested in 2020 resulted in differences in the flavour perception. Compared to 2018 where genotypes 12, 18 and 25 were reported as the most distinctive, genotypes 5, 10, 12, 18, 22 and 25 became

more distinguished from the remainder genotypes and displayed close associations with individual attributes. "Fresh fennel" was shown to be closely associated with genotype 18 in 2018, but became more strongly associated with genotypes 5 and 22 in 2020. In 2020, "fresh coriander", "parsley" and "grass green" positively correlated with F1 were associated with genotypes 8, 10, 12, 15 and 18, while the "fresh fennel" odour and flavour attributes in the top left quadrant (Figure 3) were associated with genotypes 5 and 22. The cucumber flavour remained in a similar position for both years, showing a close association to genotype 25. The most consistent genotype out of the eight was genotype 25 in terms of sensory and volatile profile; in both harvests, it appeared to be the least aromatic reflected by its close association to the cucumber flavour. Celery samples harvested in 2020 exhibited a higher proportion of monoterpenes which contribute to the herbal sensory attributes. Within the correlation matrix, fresh fennel exhibited many positive correlations with compounds that contribute to warm, herbal, sweet and spearmint odours such as (E)-dihydrocarvone (M20), L-carvone (M24), (E)-β-caryophyllene (S3) and α-humulene (S7) as well as sedanenolide (P4) and (cis)-ligustilide (P6). Afifi, El-Mahis, Heiss and Farag [42] classified 12 fennel varieties based on their aroma profile and similarities can be observed when comparing the monoterpene profile of celery in this study with the aroma profiles of the fresh fennel used by Afifi et al. [42].

According to the results presented so far, samples harvested in 2020 had a more complex aroma profile leading to more flavourful genotypes compared to those harvested in 2018. Genotypes such as 10, 12 and 15 had a strong association with odour active compounds such as phthalides and, thus, associated with herbal flavour attributes such as fennel, coriander and parsley. However, genotypes grown in 2018 expressed a higher proportion of phthalides, which suggests that the typical celery odour would be more noticeable in these celery genotypes. Thappa et al. [43] investigated the variation of major components of genetically improved celery and reported that celery with a high phthalide content, such as those harvested in 2018, led to higher quality celery. The confirmation of whether this statement remains true for the celery used in this study requires the completion of consumer acceptability and preference trials.

3.3. Environmental Differences between Harvest Years and Influence on the Aroma Profile

In this study, clear differences in the volatile and sensory profile of the same genotypes grown in the same region of the United Kingdom across two different years were observed. Environmental data including climatic variances in temperature, rainfall and relative humidity were collected at the nearest weather station to the farm of growth and provided by G's Fresh (Table 3). These environmental differences were hypothesised to influence the chemical composition within the crop. The daily air temperatures in 2018 (average 18 °C) were much higher than those in 2020 (average 14 °C). This change in temperature may have led to a warmer soil temperature in 2018, with a daily average presented to be over 7 °C warmer than in 2020. Although no differences in the volume of precipitation between years were observed, a large difference can be seen between the relative humidity. The impact of different growing conditions, such as temperature, on the flavour composition in celery is inadequately investigated and, within this experiment, only two growing seasons have been used; therefore, any conclusions that are drawn here can only be hypothesised. The utilisation of multiple years would generate more data and information about how celery responds to different climates and environments, which would produce a robust and vast dataset that will indicate more significant relationships between the plant's response towards the environment and confirm or disprove any of the theories discussed in this section.

Table 3. Environmental data recorded at the nearest weather station to the farm of celery growth and provided by G's Fresh.

Weeks after Field Transplant	2018				2020			
	Air Temp (°C)	Soil Temp (°C)	Rainfall (mm)	Relative Humidity (%)	Air Temp (°C)	Soil Temp (°C)	Rainfall (mm)	Relative Humidity (%)
1	17.0	17.1	0.0	73.0	9.8	9.6	0.1	82.0
2	14.7	17.3	0.0	81.3	11.4	10.7	0.0	74.6
3	16.4	18.1	0.1	66.1	9.4	9.9	0.0	67.9
4	17.0	24.4	0.0	94.8	16.7	16.9	0.0	63.3
5	18.9	27.9	0.0	98.5	15.7	17.3	0.0	62.3
6	19.8	28.6	0.0	99.7	14.4	16.1	0.0	71.1
7	18.2	25.5	0.0	99.4	12.0	12.6	0.0	86.4
8	20.4	29.0	0.0	99.0	17.2	18.3	0.2	80.7
9	21.4	26.7	0.1	70.5	19.6	21.5	0.0	69.1
10	20.9	27.7	0.0	71.8	16.0	18.6	0.0	78.9
11	17.3	20.7	0.2	99.9	16.0	17.6	0.2	86.6
12	18.4	28.6	0.0	98.6				
13	15.8	17.5	0.0	93.9				
Average	18.2	23.8	0.2	88.1	14.3	15.4	0.05	74.8

Being such a widely grown and consumed crop, it was expected that certain celery cultivars have been developed to grow under a range of temperatures. For example, cultivars EC 99249-1, RRL 85-1 and NRCSS-A have been identified as suitable for growth under the Indian climate, producing excellent essential oil content and high yield [44,45]. However, climates with long growing seasons with temperatures between 16 °C and 21 °C, with light rainfall and suitable irrigation, are thought to be optimal growing conditions for celery [6]. Kader [46] identified that preharvest factors including environmental conditions (temperatures, rainfall and wind speed) and agricultural techniques (planting density, irrigation and pesticide regimes) could often result in a decline in flavour quality. For other crops, such as apples, that are dependent on ester formation for flavour, Fellman, Miller and Mattinson [37] stressed the importance of genotype along with abiotic factors such as growing temperatures and cultural practices and they stated that these are "critical factors" involved in the synthesis of precursors involved in ester formation. Esters comprised a higher proportion of the aroma profile of celery grown in 2020 than celery grown in 2018 (Table 1), contributing to aroma such as fruity, apple and green and are shown to be associated with a grassy/green odour (Figure 3). With respect to celery, perhaps the lower temperatures exhibited in 2020 were more preferable for ester formation.

The influence of temperature on isoprene formation, the smallest terpene unit and building block for more complex monoterpenes, has been discussed by Sharkey, Wiberley and Donohue [47], whereby isoprene expresses a relationship with temperature and light and provides plant protection in the form of thermotolerance. Light and temperature have an influence in controlling the monoterpene and sesquiterpene plant emission as reported by Ibrahim et al. [48], where the total monoterpene and sesquiterpene emissions in silver birch (*Betula pendula*) and European aspen (*Populus tremula*) trees increased at higher temperatures and peaked at 18 °C. Sesquiterpene content was positively correlated to temperature whilst monoterpenes expressed the opposite and was identified at higher abundances at lower temperatures. These findings support the volatile results from celery presented in Table 1, where the total sesquiterpene content was higher in 2018 when higher temperatures were recorded and, by contrast, monoterpenes comprised the majority of the aroma profile in 2020 when lower temperatures were observed. From these findings it can be hypothesised that sesquiterpenes act as a protective mechanism from heat stress within celery.

How phthalide compounds, the characteristic compounds imparting celery odour, react to different environmental stimuli have not previously been studied. Although existing research discusses the importance of their presence in celery samples, there is a poor understanding on how they are synthesised and what the factors that influence the abundance of these compounds are [5]. Sedanenolide made up the highest proportion of

the phthalide profile in both 2018 and 2020, albeit much higher in 2018. Overall, samples harvested in 2018 had a higher total phthalide content than celery grown in 2020, which mimicks a similar pattern to sesquiterpenoid compounds (Table 1) and, thus, possibly acts as a protective mechanism in response to the heat stress. Synthesising aromatic compounds is a standard response to abiotic stresses, such as temperature, in order to protect the crop [49]. Possessing a lower total phthalide content in 2020 explained why aromas and flavours such as fresh coriander and parsley were revealed and are becoming more apparent to human assessors (Table 2).

4. Conclusions

Harvest year showed a stronger influence over the aroma composition of eight celery genotypes compared to genotypes, leading to differences in the aroma profile and, thus, creating sensory differences between two different years. Completing volatile analysis and sensory evaluation of the eight genotypes of celery demonstrated that the celery genotypes harvested in 2018 were perceived as being less herbal and associated with green aroma and cucumber flavour compared to the samples harvested in 2020. Samples harvested in 2020 imparted herbal flavour notes such as parsley, fennel and coriander, which are all members of the Apiaceace family potentially because these flavour notes were revealed when dominant aromas derived from pthalides were less abundant.

Although the genotypes were observed to play less of a role than the harvest year, the genetic make-up of the crop undoubtedly plays a role in predetermining the flavour profile as well as the capacity to synthesise aroma compounds in response to stress [37,46–48], as shown by a high proportion of compounds expressing significant differences according to genotype, the variation caused by genotype and the variation in genotype perception from sensory evaluation. The eight genotypes used in this study all exhibited clear differences within the aroma composition; however, less variation between years was apparent for genotype 25, which imparted a cucumber flavour and was less associated with aromatic compounds. Similarly genotype 12, with a strong fresh parsley odour, had a constant aroma profile over the two harvest years and expressed a high proportion of sesquiterpenes and phthalide compounds according to the volatile composition.

The influence of the environment on the aroma composition was also evident in this study (Figure 1) with the majority of the compounds identified as significantly different between the two harvest years. The chemical composition was different in each year, with alcohol (including monoterpenoid alcohols), aldehyde, sesquiterpene and phthalide content all being in higher proportions in 2018. The warmer and dryer climates experienced in 2018 could explain these compositional differences, particularly with sesquiterpene and phthalide compounds, which have been previously observed to act as a crop protective mechanism in response to heat stress. Taking into consideration these observations, the celery grown in 2018 could be the preferred flavour, but this hypothesis would require consumer acceptability and preference trials to confirm this.

There is currently limited research to support the impact of the environment on the volatile composition and sensory profile of celery and, in order to confirm the environmental role, further work using controlled growth combined with sensory and chemical analysis needs to be carried out to provide a deeper understanding of the environmental relationship and how it affects volatile composition. Additionally, growing celery in alternative geographical locations could elucidate this relationship and provide more evidence as to how different environments affect the volatile composition. Providing explanations concerning the causes of aroma composition variation within celery, as well as other Apiaceae crops, will aid breeders to focus breeding programs on temperature resistant crops or steer fresh produce growers to utilise crops that are more resilient to the geographical climate of growth. These considerations, combined with regular inhouse taste panels and quality testing, will ultimately lead to better tasting crops with more stable flavour qualities.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods10061335/s1, Table S1: Origin and images of the eight celery samples used in this study and harvested in 2018 and 2020.

Author Contributions: Conceptualization, L.T., C.W., F.G. and S.L.; methodology, L.T. and S.L.; software, L.T. and S.L.; validation, L.T. and S.L.; formal analysis, L.T.; investigation, L.T.; resources, L.T.; data curation, L.T.; writing—original draft preparation, L.T.; writing—review and editing, L.T., F.G., C.W. and S.L.; supervision, S.L., F.G. and C.W.; funding acquisition: F.G. and C.W. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The funders discussed the design of the study with the research team but they were not involved in the collection or analysis of data. Frances Gawthrop is a co-author on this paper; he reviewed the manuscript and agreed to publish the results. The other authors declare no conflicts of interest.

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5808 Appendix VII - Origin and images of the eight celery samples used in this study and harvested in
5809 2018 and 2020.
5810

Line	Origin	Harvest 2018	Harvest 2020
5	USA		
8	AUS		
10	UK		
12	UK		

15	USA		
18	EU		
22	USA		
25	EU		

5811

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Investigating the Relationship of Genotype and Geographical Location on Volatile Composition and Sensory Profile of Celery (*Apium graveolens*)

Lucy Turner [1], Stella Lignou [1,*], Frances Gawthrop [2] and Carol Wagstaff [1]

[1] Department of Food and Nutritional Sciences, University of Reading, Harry Nursten Building, Whiteknights, Reading RG6 6DZ, UK; L.Turner@pgr.reading.ac.uk (L.T.); c.wagstaff@reading.ac.uk (C.W.)
[2] A.L. Tozer Ltd., Pyports, Downside Bridge Road, Cobham KT11 3EH, UK; frances.gawthrop@tozerseeds.com
* Correspondence: s.lignou@reading.ac.uk; Tel.: +44-(0)118-378-8717

Abstract: Numerous varieties of celery are grown in multiple countries to maintain supply, demand and availability for all seasons; thus, there is an expectation for a consistent product in terms of taste, flavour, and overall quality. Differences in climate, agronomy and soil composition will all contribute to inconsistencies. This study investigated the volatile and sensory profile of eight celery genotypes grown in the UK (2018) and Spain (2019). Headspace analysis determined the volatile composition of eight genotypes, followed by assessment of the sensory profile using a trained panel. Significant differences in the volatile composition and sensory profile were observed; genotype and geographical location both exerted influences. Two genotypes exhibited similar aroma composition and sensory profile in both locations, making them good candidates to drive breeding programmes aimed at producing varieties that consistently display these distinctive sensory properties. Celery samples harvested in the UK exhibited a higher proportion of sesquiterpenes and phthalides, whereas samples harvested in Spain expressed a higher aldehyde and ketone content. Studying the relationship between growing environment and genotype will provide information to guide growers in how to consistently produce a high-quality crop.

Keywords: celery; aroma; volatile compounds; SPME GCMS; phthalides; terpenes; harvest

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1. Introduction

Apium graveolens, commonly known as celery, is a vegetable with long fibrous stalks, belonging to the Apiaceae or Umbelliferae family, characterised by its discoid or 'umbrella'-shaped flowers, known as umbels. Similar to other members of the Apiaceae family, including carrots, coriander and parsley, celery possesses a strong, distinct flavour profile, placing it as a key component in soups, stocks and sauces [1,2]. Compounds that constitute the aroma profile include a range of monoterpenes (myrcene, limonene, β-pinene and γ-terpinene), sesquiterpenes (β-caryophyllene, α-humulene, α- and β- selinene) and phthalides (sedanenolide, neocnidilide and 3-n-butylphthalide) [2–7]. The latter compounds have been reported throughout the literature to be the characteristic odour compounds of celery [7], with odour characteristics identified by Turner, Dawda, Gawthrop, Wagstaff and Lignou [8] of 'celery', 'cooked celery' and 'herbal'. Celery has long been grown and consumed globally and, for this reason, the aroma profile has been studied using a range of cultivars, grown in a variety of years and geographical locations, and analysed using extraction methods including solvent assisted flavour extraction (SAFE) and solid phase microextraction (SPME) which are, most typically, followed by gas chromatography/mass spectrometry (GCMS) [3–6,8]. Possibly the earliest investigation, completed by Gold and Wilson [9], determined the volatile composition of celery juice using distillation followed by gas chromatography. This identified a collection of compounds ranging from aldehydes, esters, alcohols and, most importantly, phthalides. More recently completed work not only

confirms the compounds identified by Gold and Wilson [9] but displays the complex aroma profile of celery and the variety of compound groups that comprise the aroma profile [7].

As a commonly used vegetable, there is an expectation for celery to be available continuously for consumers; however, in countries such as the United Kingdom, this is not possible due to the unfavourable winter conditions. During the summer months, celery can be grown in the UK as the environment is suitable for growth and, often, celery can continue to be grown on the east coast through autumn. Nevertheless, the annual consumer demand for celery is not met. To combat this issue, celery is grown in warmer locations, such as southern Spain, where they are packaged and processed and then transported to UK retailers. Although offering a solution to meet the demand, utilising seasons in Spain means growing in arid and semi-arid conditions, requiring different agronomy compared to that needed for the UK's growing environment, and thus creating inconsistencies within the aroma quality of the celery produce available. While not thoroughly understood within celery, the influence of abiotic and biotic factors upon the aroma of crops in general has been investigated by others, and differences have been observed [7,10–13]. Exposure to different stresses such as temperature, relative humidity, soil and water compositions have been shown to influence the production of primary and secondary metabolites, ultimately leading to variation within the volatile composition [7,10]. Previously, Turner, Lignou, Gawthrop and Wagstaff [10] observed significant differences in the volatile composition and sensory profile of eight celery genotypes grown in the same geographical location in 2018 and 2020. Despite the genotypes displaying significant interactions, it was the differences in environment over the two seasons that had a stronger influence over the volatile composition of celery. The review recently completed by the authors [7] combined data from previously published experiments that investigated the aroma profile of celery, identifying missing data through the exclusion of information, including cultivar name, origin, location of growth, harvest year and conditions of growth. Exposing variation in the presence or absence of compounds and their composition within celery, the authors concluded that without stating all experimental information, the data became unrepeatable. To overcome this, the authors put forward the Minimum Information About a Plant Aroma Experiment (MIAPAE), inviting authors to include parameters used during preharvest, harvest and postharvest as well as extraction and analysis methods, allowing for the building of a repository whereby aroma data for plants can repeated and interpreted correctly [7].

Albeit limited, investigations exploring the impact of geographical locations on celery have been completed; Marongiu et al. [11] compared the volatile composition of wild celery grown and collected in Portugal and Italy as well as using different extraction methods (super critical fluid extraction and hydrodistillation). Differences in the composition caused by both the geographical location and extraction method were observed. Phthalide compounds including sedanenolide and neocnidilide expressed significant differences according to these factors, ultimately concluding that environmental differences between Portugal and Italy were the main cause of observed compositional differences. The cultivar of the wildtype celery used in this study was not included, nor were differences in agricultural techniques and growing environments. However, observed variances in the aroma composition in celery caused by these factors have previously been displayed. Rożek, Nurzyńska-Wierda and Kosior [12] identified that drought stress led to an increase in essential oil due to an increase in the production of secondary metabolites, whereas van Wassenhove, Dirinck, Schamp and Vulsteke [13] observed changes in the phthalide and terpene content when nitrogenous fertiliser (organic and/or inorganic) was applied to celery.

This study aims to investigate the relationship between genotype and geographical location of cultivation upon the volatile composition of eight celery varieties grown in Ely, UK in 2018 and Aguilas, Spain in 2019. Sensory evaluation using a trained panel was completed to understand how chemical and physiological changes lead to differences in the organoleptic perception and to identify interactions between compound groups

and geographical location. Ultimately, this information can be used to assist breeders and growers to develop and select cultivars that are optimal for specific growing environments, to produce a consistently flavoured product. Although factors such as temperature and relative humidity are uncontrollable, growers can apply organic/inorganic fertilisers, herbicides/fungicides and supplementary irrigation to aid optimal conditions for celery growth.

2. Materials and Methods

2.1. Celery Material and MIAPAE Standard

2.1.1. Sample Information

The eight parental celery genotypes used in these field trials were chosen due to their differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each line used in this paper, the origins of these parental breeding lines and their image postharvest can be found in Supplementary Material (Table S1). Prior to GC/MS analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis. As expected, volatile loss was observed between fresh and freeze-dried samples, however, consistency in relative amount was observed throughout repetitions and the most reported compounds were also identified. Freeze-drying is a method that has been used previously to preserve the volatile content of herbs [14–16], and, furthermore, Hoffman [17] identified freeze-drying as a preservation method that best retains a typical aroma at a strong intensity.

2.1.2. Timing, Location and Environment

Celery seed (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd. (Cobham, United Kingdom) were grown in commercial conditions and harvested in Cambridgeshire (United Kingdom) by G's Fresh Ltd. (Ely, United Kingdom (52°21′12.9″ N 0°17′15.6″ E)) during spring/summer 2018. In 2019, the same eight parental varieties of celery were grown and harvested in Aguilas, Spain by G's España Ltd. (37°25′43.2″ N 1°39′56.2″ W).

Celery grown in the UK was grown on sandy loam soils with naturally high groundwater and a peaty surface, whereas celery grown in Spain was grown on Calcisol soils. Both harvests were grown in a randomised block design, using commercial celery products as border plants to remove edge effects and subjected to the same commercial conditions including application of agronomic techniques, fertilizer and irrigation as commercial celery. For both years, 20–25 mm of overhead irrigation was used every four days, and standard commercial fertiliser, pest and disease control regimes were applied. In 2018, plugs were transplanted mid-June after 22 days' growth in the nursery, then harvested 91 days later. The average daily air temperature was 18.2 °C, with 0.2 mm of rainfall daily and an average relative humidity of 88.1%. Average wind speed was 1.9 ms and the dew point was 15.5 °C. In 2019, plugs were transplanted in early January after growing for 20 days in the nursery, then harvested in late March, 87 days later. The average daily air temperature was 17.6 °C, with 0.4 mm of average rainfall and an average relative humidity of 77.3%. Average wind speed was 1.7 ms and dew point was 6.0 °C. Prior to harvest, the celery was subject to regular in-field assessment to ensure standards for commercial quality were met, including visual and taste tests. These celeries were harvested within a close timeframe of the commercial produce also being grown in the field, which acted as an indicator for the appropriate commercial harvest maturity.

2.1.3. Raw Material Collection, Processing Storage

The celery was grown at a density of 10 plants m^{-2}, and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles, and sealed in labelled bags for transportation to the University of Reading (United Kingdom). Harvesting in Spain followed the same procedure; however, celery was packed into cool boxes and transported to the UK

in refrigerated conditions using G's Fresh Ltd. courier. Transportation took two days and samples were collected from G's Fresh (Ely, Cambridgeshire, UK) before transportation back to the University of Reading.

Celery samples used for sensory evaluation were refrigerated for one day before presenting to the trained panel, whereas samples for aroma analysis were immediately frozen at −80 °C for one week and subsequently freeze-dried for five days. Samples were then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ, USA) and stored in an airtight container for a maximum of two weeks before analysis with gas chromatography/mass spectrometry (GC/MS).

2.2. Chemicals Reagents

For GC/MS analysis, calcium chloride and the alkane standard C_6–C_{25} (100 µg mL^{-1}) in diethyl ether were obtained from Merck (Poole, UK).

2.3. Volatile Analysis Using SPME GCMS

For headspace sampling, the celery sample (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and filled to 5 mL using HPLC-grade water in a 15 mL SPME vial fitted with a screw cap. Samples were analysed by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA) according to Turner et al. [8,10].

2.4. Sensory Evaluation of Fresh Celery Samples

Sensory evaluation was carried out using quantitative descriptive analysis (QDA™) to determine the sensory characteristics of the eight celery samples, and the characteristics were estimated quantitatively. The trained sensory panel at the Sensory Science Centre (University of Reading, n = 12; 11 female and 1 male) was used to develop a consensus vocabulary to describe the sensory characteristics of the eight celery genotypes. The terms were discussed by the panellists as a group, facilitated by a panel leader, and this led to a consensus of 22 and 23 attributes for the UK and Spanish harvest, respectively. The sensory assessment of the samples was carried out according to Turner et al. [8] at the Sensory Science Centre (University of Reading) using Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Guelph, ON, Canada) to acquire the data.

2.5. Statistical Analysis

The percentage composition was calculated from the peak area data collected by SPME GC/MS analysis, and quantitative data for each compound identified in the SPME GC/MS analysis were analysed by both one- and two-way analysis of variance (ANOVA) and principal component analysis (PCA) using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's honest significant difference post hoc test was applied to determine which sample means differed significantly ($p < 0.05$) between geographical location and the celery genotypes. Only those compounds exhibiting significant differences between geographical location (G), genotype (*E*) and their interaction (GxE) were included in the PCA.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out the ANOVA of sensory panel data. The means from sensory data were taken over two sessions for all assessors and correlated with the percentage composition means from the instrumental data via PCA using XLSTAT.

3. Results and Discussion

3.1. Volatile Composition

In total, 118 compounds were detected in the headspace of the eight celery genotypes in both geographical locations (UK and Spain) (Table 1). Sixty-five compounds were identified in 2018 across eight genotypes, including: 22 monoterpenes, ten sesquiterpenes, eight aldehydes, five alcohols (three of which are classified as monoterpenoid

alcohols) and five phthalides. Additional compounds were identified in the headspace of the same genotypes from the Spanish harvest including: 27 monoterpenes, 17 aldehydes, 11 sesquiterpenes and alcohols (six of which are classified as monoterpenoid alcohols), nine ketones and six phthalides. Quantitative differences were observed between the two geographical locations as well as the eight genotypes in this study, and two-way ANOVA revealed significant differences in aroma difference caused by both factors. Where Spanish grown celery displayed higher alcohol, aldehyde and ketone content, UK grown celery expressed a much higher monoterpene, sesquiterpene and phthalide content. Seventeen compounds expressed no significant difference in relative amount by these factors and seven of these came from lower boiling compounds, including camphene, sabinene and β-pinene, along with D-carvone and carvacrol. These low boiling monoterpenes were not observed to differ significantly when harvested in 2018 and 2020 in the UK [10], suggesting that monoterpenes are fundamental to the crop and factors including genotype and climate hold limited influence over the abundance of these compounds.

Table 1. Percentage composition of volatile compounds identified in the headspace of eight celery genotypes using SPME GC/MS and harvested in UK 2018 and Spain 2019.

Code	Compound	LRI_{exp} [A]	ID [B]	Percentage Composition (%) [C]														*p*-Value [D]		
				UK								Spain								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G [E]	E [F]	GxE [G]
	Alcohols																					
A1	3-methyl-3-buten-1-ol	730	A	0.42 ± 0.08 ^{abc}	0.31 ± 0.04 ^{ab}	0.94 ± 0.27 ^{c}	0.35 ± 0.14 ^{abc}	0.22 ± 0.07 ^{a}	0.23 ± 0.06 ^{a}	0.30 ± 0.12 ^{ab}	0.39 ± 0.06 ^{abc}	0.60 ± 0.35 ^{abc}	0.40 ± 0.06 ^{abc}	0.91 ± 0.27 ^{bc}	0.59 ± 0.13 ^{abc}	0.36 ± 0.05 ^{abc}	0.57 ± 0.22 ^{abc}	0.54 ± 0.02 ^{abc}	0.49 ± 0.13 ^{abc}	**	**	**
A2	2-methyl-1-butanol	742	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.01 ^{ab}	0.10 ± 0.03 ^{ab}	0.12 ± 0.02 ^{b}	0.11 ± 0.01 ^{ab}	nd ^{a}	0.10 ± 0.04 ^{ab}	0.10 ± 0.05 ^{ab}	0.10 ± 0.02 ^{ab}	***	***	***
A3	(*E*)-2-penten-1-ol	758	A	0.73 ± 0.28 ^{ab}	0.42 ± 0.16 ^{ab}	0.64 ± 0.04 ^{ab}	0.23 ± 0.08 ^{a}	0.32 ± 0.09 ^{ab}	0.65 ± 0.23 ^{ab}	1.2 ± 0.54 ^{ab}	0.50 ± 0.22 ^{ab}	0.72 ± 0.34 ^{ab}	1.3 ± 0.25 ^{b}	1.1 ± 0.18 ^{ab}	0.71 ± 0.09 ^{ab}	0.60 ± 0.09 ^{ab}	0.81 ± 0.31 ^{ab}	0.87 ± 0.24 ^{ab}	0.52 ± 0.06 ^{ab}	**	*	*
A4	1-pentanol	763	A	0.21 ± 0.06 ^{a}	0.11 ± 0.04 ^{a}	0.31 ± 0.20 ^{a}	0.13 ± 0.10 ^{a}	0.23 ± 0.15 ^{a}	0.39 ± 0.14 ^{ab}	0.63 ± 0.25 ^{ab}	0.28 ± 0.08 ^{a}	1.6 ± 0.27 ^{b}	0.50 ± 0.11 ^{a}	0.76 ± 0.28 ^{ab}	0.49 ± 0.06 ^{a}	1.1 ± 0.13 ^{ab}	0.87 ± 0.34 ^{ab}	1.5 ± 0.51 ^{b}	0.88 ± 0.22 ^{ab}	***	***	***
A5	1-hexanol	862	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.53 ± 0.19 ^{ab}	0.44 ± 0.27 ^{ab}	0.79 ± 0.44 ^{b}	0.40 ± 0.21 ^{ab}	0.33 ± 0.08 ^{ab}	0.40 ± 0.10 ^{ab}	0.48 ± 0.14 ^{ab}	0.47 ± 0.23 ^{ab}	***	***	***
	Total Aldehydes			1.4	0.84	1.9	0.71	0.77	1.3	2.1	1.2	3.5	2.7	3.7	2.3	2.4	2.7	3.5	2.5			
AH1	2-methyl-2-butenal	739	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.16 ± 0.07 ^{bc}	0.15 ± 0.08 ^{bc}	0.14 ± 0.06 ^{bc}	0.13 ± 0.02 ^{abc}	0.23 ± 0.03 ^{c}	0.19 ± 0.04 $^{b\,c}$	0.19 ± 0.05 ^{bc}	0.10 ± 0.03 ^{ab}	***	***	***
AH2	(*E*)-2-pentenal	753	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.78 ± 0.04 ^{c}	0.13 ± 0.08 ^{a}	0.34 ± 0.14 ^{ab}	nd ^{a}	0.78 ± 0.08 ^{c}	0.80 ± 0.36 ^{c}	0.77 ± 0.09 ^{bc}	0.38 ± 0.11 ^{abc}	***	***	***
AH3	hexanal	800	A	9.7 ± 0.8 ^{a}	1.3 ± 0.46 ^{a}	2.6 ± 0.32 ^{a}	0.65 ± 0.29 ^{a}	2.0 ± 0.39 ^{a}	8.9 ± 2.7 ^{a}	13 ± 5.5 ^{a}	6.3 ± 1.2 ^{a}	25 ± 7.8 ^{a}	24 ± 6.2 ^{a}	14 ± 5.2 ^{a}	8.6 ± 3.6 ^{a}	22 ± 7.5 ^{a}	24 ± 4.9 ^{a}	25 ± 7.0 ^{a}	22 ± 6.3 ^{a}	**	**	**
AH4	(*E*)-2-hexenal	849	A	0.18 ± 0.11 ^{abc}	tr ± 0.02 ^{a}	tr ± 0.02 ^{a}	0.04 ± 0.01 ^{ab}	0.03 ± 0.03 ^{a}	0.15 ± 0.11 ^{abc}	0.20 ± 0.08 ^{abc}	0.11 ± 0.05 ^{abc}	0.56 ± 0.13 ^{c}	0.57 ± 0.24 ^{c}	0.30 ± 0.10 ^{abc}	0.30 ± 0.07 ^{abc}	0.55 ± 0.11 ^{c}	0.54 ± 0.19 ^{c}	0.57 ± 0.15 ^{c}	0.51 ± 0.20 ^{bc}	***	***	***
AH5	heptanal	901	A	tr ± 0.03 ^{ab}	nd ^{a}	0.28 ± 0.15 ^{ab}	0.16 ± 0.13 ^{ab}	0.25 ± 0.16 ^{ab}	0.23 ± 0.14 ^{ab}	0.29 ± 0.08 ^{ab}	0.25 ± 0.15 ^{ab}	0.68 ± 0.18 ^{b}	0.58 ± 0.18 ^{ab}	0.51 ± 0.13 ^{ab}	0.48 ± 0.10 ^{ab}	0.49 ± 0.35 ^{ab}	0.57 ± 0.13 ^{ab}	0.61 ± 0.20 ^{ab}	0.72 ± 0.12 ^{b}	**	**	**
AH6	(*E*)-2-heptenal	954	A	0.19 ± 0.22 ^{a}	1.6 ± 0.55 ^{ab}	1.6 ± 0.23 ^{ab}	0.52 ± 0.04 ^{a}	1.5 ± 0.10 ^{ab}	3.2 ± 1.5 ^{abc}	4.2 ± 1.3 ^{abc}	1.8 ± 0.97 ^{ab}	6.4 ± 0.75 ^{bcd}	8.1 ± 0.23 ^{cd}	6.0 ± 0.36 ^{bcd}	6.1 ± 0.64 ^{bcd}	11 ± 0.55 ^{d}	7.8 ± 0.33 ^{cd}	7.3 ± 0.45 ^{cd}	7.5 ± 0.40 ^{cd}	***	***	***
AH7	benzaldehyde	969	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	3.3 ± 1.8 ^{b}	1.7 ± 0.50 ^{ab}	1.9 ± 0.14 ^{b}	1.9 ± 0.26 ^{b}	1.7 ± 0.10 ^{ab}	1.6 ± 0.48 ^{ab}	1.7 ± 0.22 ^{ab}	1.9 ± 0.22 ^{b}	***	***	***
AH8	*n*-octanal	1007	A	0.10 ± 0.10 ^{ab}	nd ^{a}	0.49 ± 0.06 ^{abcd}	0.27 ± 0.06 ^{abc}	0.39 ± 0.19 ^{abcd}	0.51 ± 0.26 ^{abcd}	0.51 ± 0.17 ^{abcd}	0.51 ± 0.23 ^{abcd}	0.86 ± 0.19 ^{cd}	0.95 ± 0.22 ^{cde}	0.56 ± 0.10 ^{abcd}	0.63 ± 0.13 ^{abcd}	1.6 ± 0.35 ^{e}	0.78 ± 0.21 ^{bcd}	0.54 ± 0.04 ^{abcd}	1.0 ± 0.22 ^{de}	***	***	***
AH9	phenacetaldehyde	1049	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.31 ± 0.13 ^{bc}	0.24 ± 0.04 ^{bc}	0.26 ± 0.06 ^{bc}	0.42 ± 0.06 ^{c}	0.26 ± 0.02 ^{bc}	0.24 ± 0.06 ^{bc}	0.23 ± 0.98 ^{b}	0.29 ± 0.05 ^{bc}	***	***	***
AH10	(*E*)-2-octenal	1057	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	3.3 ± 1.3 ^{b}	2.2 ± 1.5 ^{ab}	1.5 ± 0.39 ^{ab}	1.4 ± 0.39 ^{ab}	3.4 ± 0.89 ^{b}	3.5 ± 1.2 ^{b}	2.8 ± 0.96 ^{b}	3.5 ± 1.0 ^{b}	***	***	***
AH11	*m*-tolualdehyde	1086	B [18]	0.33 ± 0.07 ^{a}	0.24 ± 0.02 ^{a}	4.0 ± 0.28 ^{c}	1.1 ± 0.28 ^{ab}	0.95 ± 0.02 ^{ab}	0.19 ± 0.02 ^{a}	0.26 ± 0.05 ^{a}	1.6 ± 0.29 ^{b}	0.72 ± 0.57 ^{ab}	0.66 ± 0.26 ^{ab}	0.71 ± 0.17 ^{ab}	0.91 ± 0.19 ^{ab}	0.64 ± 0.06 ^{ab}	0.68 ± 0.32 ^{ab}	0.57 ± 0.10 ^{a}	0.97 ± 0.08 ^{ab}	***	***	***
AH12	nonanal	1105	A	0.33 ± 0.14 ^{abc}	0.12 ± 0.02 ^{ab}	0.20 ± 0.03 ^{abc}	0.10 ± 0.01 ^{a}	0.17 ± 0.03 ^{abc}	0.16 ± 0.10 ^{abc}	0.22 ± 0.17 ^{abc}	0.19 ± 0.09 ^{abc}	0.68 ± 0.11 ^{c}	0.59 ± 0.18 ^{abc}	0.39 ± 0.10 ^{b}	0.35 ± 0.13 ^{abc}	0.57 ± 0.16 ^{abc}	0.64 ± 0.35 ^{bc}	0.61 ± 0.08 ^{abc}	0.59 ± 0.11 ^{abc}	***	***	***
AH13	(*E*,*E*)-2,4-octadienal	1110	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.15 ± 0.05 ^{b}	0.13 ± 0.04 ^{b}	0.11 ± 0.01 ^{b}	0.13 ± 0.03 ^{b}	0.16 ± 0.02 ^{b}	0.15 ± 0.03 ^{b}	0.14 ± 0.05 ^{b}	0.20 ± 0.02 ^{b}	***	***	***
AH14	(*E*,*Z*)-2,6-nonadienal	1162	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.06 ^{ab}	0.15 ± 0.03 ^{abc}	0.11 ± 0.02 ^{abc}	0.12 ± 0.02 ^{abc}	0.29 ± 0.10 ^{c}	0.23 ± 0.02 ^{bc}	0.23 ± 0.16 ^{bc}	0.28 ± 0.05 ^{c}	***	***	***
AH15	(*E*)-2-nonenal	1165	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.03 ^{ab}	0.10 ± 0.02 ^{ab}	tr ± 0.03 ^{ab}	0.14 ± 0.02 ^{b}	0.10 ± 0.01 ^{ab}	0.10 ± 0.01 ^{ab}	tr ± 0.05 ^{ab}	0.12 ± 0.10 ^{b}	***	***	***
AH16	myrtenal	1207	B [19]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.19 ± 0.02 ^{ab}	0.14 ± 0.02 ^{a}	0.10 ± 0.03 ^{a}	0.11 ± 0.01 ^{a}	0.16 ± 0.04 ^{ab}	0.15 ± 0.04 ^{ab}	0.10 ± 0.06 ^{a}	0.37 ± 0.21 ^{b}	***	***	***
AH17	(*E*,*E*)-2,6-nonadienal	1156	A	0.21 ± 0.04 ^{ab}	0.30 ± 0.03 ^{ab}	0.18 ± 0.02 ^{ab}	0.18 ± 0.04 ^{ab}	0.17 ± 0.03 ^{ab}	0.16 ± 0.08 ^{ab}	tr ± 0.03 ^{a}	0.22 ± 0.08 ^{ab}	0.36 ± 0.11 ^{ab}	0.48 ± 0.24 ^{b}	0.20 ± 0.03 ^{ab}	0.16 ± 0.05 ^{ab}	0.41 ± 0.11 ^{ab}	0.35 ± 0.11 ^{ab}	0.46 ± 0.22 ^{ab}	0.20 ± 0.17 ^{ab}	*	*	*
	Total Esters			11	3.6	9.4	3.0	5.5	14	19	11	44	41	28	23	44	44	43	41			
E1	methyl butanoate	717	A	tr ± 0.03 ^{abc}	tr ± 0.01 ^{a}	tr ± 0.02 ^{abc}	tr ± 0.01 ^{ab}	tr ± 0.02 ^{ab}	tr ± 0.04 ^{ab}	tr ± 0.05 ^{ab}	tr ± 0.01 ^{ab}	0.22 ± 0.14 ^{cd}	0.18 ± 0.01 ^{abcd}	0.25 ± 0.04 ^{d}	0.17 ± 0.01 ^{abcd}	0.18 ± 0.04 ^{abcd}	0.18 ± 0.04 ^{abcd}	0.16 ± 0.02 ^{abcd}	0.19 ± 0.03 ^{bcd}	***	***	***
E2	methyl pentanoate	837	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.34 ± 0.23 ^{b}	0.24 ± 0.02 ^{ab}	0.37 ± 0.13 ^{b}	0.40 ± 0.09 ^{b}	0.23 ± 0.07 ^{ab}	0.39 ± 0.18 ^{b}	0.27 ± 0.05 ^{ab}	0.30 ± 0.05 ^{ab}	***	***	***
E3	methyl hexanoate	921	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.25 ± 0.12 ^{ab}	0.29 ± 0.16 ^{ab}	0.12 ± 0.01 ^{ab}	0.10 ± 0.03 ^{ab}	0.25 ± 0.09 ^{ab}	0.38 ± 0.10 ^{b}	0.28 ± 0.10 ^{bc}	0.24 ± 0.11 ^{ab}	***	***	***
E4	carveol acetate	1343	B [20]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.21 ± 0.05 ^{bc}	0.14 ± 0.02 ^{ab}	0.22 ± 0.04 ^{bc}	0.17 ± 0.04 ^{bc}	0.20 ± 0.04 ^{bc}	0.27 ± 0.08 ^{bc}	0.20 ± 0.05 ^{a}	0.29 ± 0.10 ^{c}	***	***	***
E5	hexyl isobutanoate	1378	B [21]	0.10 ± 0.03 0.14	0.10 ± 0.04 0.10	0.14 ± 0.02 0.20	tr ± 0.03 0.07	0.10 ± 0.05 0.11	0.16 ± 0.04 0.19	0.32 ± 0.06 0.36	0.12 ± 0.03 0.14	0.15 ± 0.12 1.2	0.15 ± 0.12 1.0	0.40 ± 0.04 1.4	0.22 ± 0.11 1.0	0.18 ± 0.13 1.0	0.11 ± 0.16 1.3	0.36 ± 0.23 1.3	0.13 ± 0.11 1.2	ns	ns	ns
	Total Ketones																					

Table 1. *Cont.*

Code	Compound	LRI$_{exp}$ [A]	ID [B]	Percentage Composition (%) [C]														*p*-Value [D]		
				UK								Spain								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G [E]	E [F]	GxE [G]
K1	2-methyl-3-pentanone	746	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.05 ^{ab}	0.10 ± 0.02 ^{ab}	0.19 ± 0.02 ^{b}	0.10 ± 0.01 ^{ab}	0.10 ± 0.01 ^{a}	0.10 ± 0.02 ^{ab}	0.10 ± 0.01 ^{ab}	0.10 ± 0.02 ^{ab}	***	***	***
K2	3-heptanone	884	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.14 ± 0.05 ^{a}	0.13 ± 0.08 ^{a}	0.12 ± 0.08 ^{a}	tr ± 0.02 ^{a}	0.10 ± 0.03 ^{a}	0.13 ± 0.01 ^{a}	0.13 ± 0.03 ^{a}	0.13 ± 0.04 ^{a}	***	***	**
K3	2-heptanone	889	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.49 ± 0.14 ^{b}	0.48 ± 0.15 ^{b}	0.31 ± 0.08 ^{ab}	0.17 ± 0.12 ^{ab}	0.39 ± 0.08 ^{ab}	0.49 ± 0.12 ^{b}	0.44 ± 0.16 ^{b}	0.56 ± 0.18 ^{b}	***	***	**
K4	1-octen-3-one	976	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	3.0 ± 0.55 ^{b}	3.9 ± 1.7 ^{b}	2.9 ± 0.17 ^{b}	2.3 ± 0.35 ^{ab}	4.4 ± 0.61 ^{b}	3.3 ± 0.73 ^{b}	3.5 ± 1.3 ^{b}	3.9 ± 0.95 ^{b}	***	***	**
K5	(*E*,*E*)-3,5-octadien-2-one	1070	B [22]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.79 ± 0.14 ^{b}	1.1 ± 0.29 ^{b}	0.60 ± 0.14 ^{ab}	0.81 ± 0.23 ^{b}	1.3 ± 0.15 ^{b}	0.82 ± 0.19 ^{b}	1.3 ± 0.41 ^{b}	0.63 ± 0.45 ^{ab}	***	***	***
K6	acetophenone	1073	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.30 ± 0.16 ^{b}	0.25 ± 0.16 ^{b}	0.27 ± 0.05 ^{b}	0.31 ± 0.04 ^{b}	0.25 ± 0.01 ^{b}	0.26 ± 0.07 ^{b}	0.28 ± 0.07 ^{b}	0.29 ± 0.02 ^{b}	***	***	***
K7	3,5-octadien-2-one	1092	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	2.2 ± 0.65 ^{b}	2.4 ± 1.1 ^{b}	0.92 ± 0.38 ^{ab}	0.81 ± 0.32 ^{ab}	2.1 ± 0.77 ^{b}	2.2 ± 1.0 ^{b}	2.2 ± 0.81 ^{b}	2.1 ± 0.91 ^{ab}	***	***	***
K8	*p*-methyl-acetophenone	1179	B [23]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.11 ± 0.04 ^{ab}	0.10 ± 0.01 ^{a}	tr ± 0.03 ^{a}	0.10 ± 0.04 ^{a}	0.10 ± 0.04 ^{ab}	nd ^{a}	0.10 ± 0.05	0.22 ± 0.10 ^{b}	***	***	*
K9	dihydrojasmone	1378	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.62 ± 0.33 ^{ab}	0.69 ± 0.38 ^{b}	0.06 ± 0.04 ^{ab}	0.17 ± 0.13 ^{ab}	0.71 ± 0.36 ^{b}	0.63 ± 0.26 ^{ab}	0.30 ± 0.21 ^{ab}	0.57 ± 0.15 ^{ab}	***	***	***
	Total Alkanes			0	0	0	0	0	0	0	0	7.8	9.1	5.4	4.8	9.4	7.9	8.3	8.5			
ALK1	nonane	900	A	0.41 ± 0.15 ^{ab}	0.32 ± 0.11 ^{ab}	0.43 ± 0.19 ^{ab}	0.14 ± 0.18 ^{a}	0.13 ± 0.10 ^{a}	0.28 ± 0.11 ^{ab}	nd ^{a}	0.17 ± 0.02 ^{a}	0.84 ± 0.44 ^{ab}	0.62 ± 0.36 ^{ab}	0.69 ± 0.21 ^{ab}	0.27 ± 0.14 ^{a}	1.7 ± 0.34 ^{b}	0.41 ± 0.06 ^{ab}	0.36 ± 0.16 ^{ab}	0.90 ± 0.35 ^{ab}	*	*	*
ALK2	decane	1000	A	0.80 ± 0.24 ^{abcd}	0.49 ± 0.13 ^{ab}	nd ^{a}	0.37 ± 0.11 ^{ab}	0.60 ± 0.26 ^{abc}	1.1 ± 0.21 ^{bcde}	1.7 ± 0.29 ^{ef}	0.83 ± 0.33 ^{abcd}	1.6 ± 0.18 ^{def}	1.7 ± 0.33 ^{ef}	1.5 ± 0.36 ^{cdef}	1.6 ± 0.05 ^{def}	2.2 ± 0.21 ^{f}	1.9 ± 0.05 ^{ef}	1.9 ± 0.18 ^{ef}	1.6 ± 0.19 ^{def}	***	***	***
ALK3	undecane	1100	A	0.26 ± 0.15 ^{abcd}	0.14 ± 0.09	0.19 ± 0.11 ^{abcd}	0.04 ± 0.05 ^{a}	0.24 ± 0.06 ^{abc}	0.14 ± 0.10 ^{abc}	0.07 ± 0.08 ^{a}	0.11 ± 0.06 ^{ab}	0.60 ± 0.31 ^{cd}	0.27 ± 0.10 ^{abcd}	0.57 ± 0.04 ^{bcd}	0.63 ± 0.02 ^{f}	0.55 ± 0.03 ^{bcd}	0.33 ± 0.03 ^{abcd}	0.43 ± 0.12 ^{abcd}	0.52 ± 0.05 ^{abcd}	***	***	***
ALK4	dodecane	1199	A	0.48 ± 0.08	0.37 ± 0.03	0.46 ± 0.05	0.31 ± 0.10	0.33 ± 0.10	0.44 ± 0.13	0.46 ± 0.10	0.44 ± 0.12	0.48 ± 0.23	0.20 ± 0.03	0.37 ± 0.10	0.31 ± 0.05	0.26 ± 0.03	0.29 ± 0.03	0.27 ± 0.04	0.34 ± 0.08	ns	ns	ns
ALK5	tridecane	1299	A	nd	nd	nd	nd	nd	nd	nd	nd	0.16 ± 0.03	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
ALK6	tetradecane	1399	A	0.11 ± 0.02	tr ± 0.03	tr ± 0.02	tr ± 0.03	0.10 ± 0.06	0.10 ± 0.03	tr ± 0.03	0.10 ± 0.02	0.16 ± 0.12	tr ± 0.03	tr ± 0.01	tr ± 0.01	tr ± 0.01	tr ± 0.03	tr ± 0.02	0.10 ± 0.06	ns	ns	ns
ALK7	pentadecane	1499	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.15 ± 0.02 ^{a}	nd ^{a}	tr ± 0.05 ^{a}	nd ^{a}	0.18 ± 0.02 ^{a}	0.14 ± 0.01 ^{a}	0.14 ± 0.02 ^{a}	nd ^{a}	**	**	**
	Total Monoterpenes			2.1	1.4	1.1	0.94	1.4	2.1	2.3	1.6	4.0	2.8	3.2	2.8	4.9	3.1	3.1	3.4			
M1	α-thujene	933	B [24]	0.27 ± 0.09	0.24 ± 0.08	0.29 ± 0.13	0.30 ± 0.11	0.22 ± 0.10	0.41 ± 0.19	0.32 ± 0.14	0.22 ± 0.13	0.64 ± 0.31	0.52 ± 0.19	1.1 ± 0.17	0.78 ± 0.20	0.42 ± 0.02	0.58 ± 0.14	0.64 ± 0.06	0.72 ± 0.22	ns	ns	ns
M2	α-pinene	943	A	0.62 ± 0.05	0.85 ± 0.22	0.52 ± 0.19	0.62 ± 0.18	1.0 ± 0.42	0.89 ± 0.20	0.43 ± 0.20	0.62 ± 0.31	0.83 ± 0.14	0.49 ± 0.26	1.0 ± 0.30	0.81 ± 0.16	0.77 ± 0.33	0.69 ± 0.10	1.1 ± 0.58	0.75 ± 0.46	ns	ns	ns
M3	camphene	960	A	2.5 ± 0.5	0.33 ± 0.07	0.29 ± 0.12	0.21 ± 0.08	0.35 ± 0.10	0.48 ± 0.05	0.66 ± 0.26	0.22 ± 0.08	0.73 ± 0.21	0.57 ± 0.05	0.93 ± 0.05	0.94 ± 0.13	0.73 ± 0.12	0.45 ± 0.32	0.96 ± 0.11	0.68 ± 0.14	ns	ns	ns
M4	sabinene	981	A	0.44 ± 0.13	0.33 ± 0.04	0.66 ± 0.39	0.27 ± 0.04	0.28 ± 0.05	0.45 ± 0.03	0.53 ± 0.13	0.36 ± 0.06	0.37 ± 0.25	0.29 ± 0.08	0.34 ± 0.19	0.32 ± 0.09	0.31 ± 0.08	0.38 ± 0.15	0.30 ± 0.07	0.34 ± 0.07	ns	ns	ns
M5	β-pinene	989	A	3.0 ± 0.64	5.2 ± 1.6	0.96 ± 0.36	5.4 ± 1.6	3.8 ± 1.6	2.7 ± 0.99	0.79 ± 0.24	4.5 ± 1.1	2.3 ± 0.63	2.1 ± 1.1	1.5 ± 0.38	2.6 ± 0.65	3.5 ± 1.4	1.1 ± 0.18	2.5 ± 1.3	2.9 ± 1.9	ns	ns	ns
M6	myrcene	992	A	1.1 ± 0.26 ^{abc}	1.9 ± 0.64 ^{abc}	2.6 ± 0.74 ^{bc}	2.6 ± 0.22 ^{bc}	1.6 ± 0.37 ^{abc}	2.1 ± 0.61 ^{abc}	0.84± 0.34 ^{ab}	1.1 ± 0.45 ^{abc}	0.51 ± 0.03 ^{a}	0.54± 0.19 ^{ab}	1.8 ± 0.46 ^{abc}	1.4 ± 0.06 ^{abc}	0.48 ± 0.10 ^{a}	1.1 ± 0.25 ^{abc}	0.56 ± 0.18 ^{ab}	0.51 ± 0.05 ^{a}	***	***	***
M7	α-phellandrene	1013	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.37 ± 0.16 ^{bc}	0.31 ± 0.03 ^{b}	0.52 ± 0.06 ^{c}	0.40 ± 0.06 ^{bc}	0.33 ± 0.04 ^{b}	0.39 ± 0.03 ^{bc}	0.39 ± 0.07 ^{bc}	0.37 ± 0.03 ^{bc}	***	***	***
M8	Δ -3-carene	1019	A	0.24 ± 0.10	0.23 ± 0.18	0.25 ± 0.04	0.25 ± 0.12	0.22 ± 0.11	0.21 ± 0.10	0.32 ± 0.09	0.23 ± 0.05	0.72 ± 0.33	0.69 ± 0.39	0.94 ± 0.74	0.63 ± 0.44	0.54 ± 0.30	0.58 ± 0.30	0.77 ± 0.38	0.77 ± 0.46	ns	ns	ns
M9	*m*-cymene	1032	A	4.3 ± 0.61	3.6 ± 0.41	3.5 ± 0.69	3.8 ± 0.43	3.4 ± 0.78 ^{a}	5.0 ± 0.71	2.8 ± 0.61	3.7 ± 0.55	3.8 ± 0.94	3.7 ± 1.1	4.6 ± 1.3	3.4 ± 0.67	2.3 ± 0.94	3.9 ± 0.82	3.4 ± 1.5	3.3 ± 1.1	ns	ns	ns
M10	limonene	1034	A	39 ± 8.2 ^{bc}	43 ± 0.56 ^{c}	33 ± 5.1 ^{abc}	32 ± 2.3 ^{abc}	39 ± 3.1 ^{bc}	32 ± 4.5 ^{abc}	29 ± 3.9 ^{abc}	33 ± 3.1 ^{abc}	11 ± 4.9 ^{a}	19 ± 1.9 ^{abc}	24 ± 7.6 ^{abc}	21 ± 2.1 ^{abc}	11 ± 6.1 ^{a}	12 ± 5.1 ^{a}	15 ± 5.3 ^{ab}	11 ± 5.3 ^{a}	***	***	***
M11	β-(*E*)-ocimene	1049	B [25]	0.19 ± 0.01 ^{a}	0.18 ± 0.07 ^{a}	0.17 ± 0.02 ^{a}	0.24 ± 0.03 ^{a}	0.17 ± 0.02 ^{a}	0.16 ± 0.02 ^{a}	0.42 ± 0.08 ^{a}	0.18 ± 0.02 ^{a}	1.3 ± 0.91 ^{ab}	0.71 ± 0.32 ^{a}	nd ^{a}	nd ^{a}	1.7 ± 0.29 ^{ab}	1.1 ± 0.28 ^{a}	nd ^{a}	3.1 ± 0.43 ^{b}	***	***	***

Table 1. *Cont.*

Code	Compound	LRI$_{exp}$ [A]	ID [B]	Percentage Composition (%) [C]														*p*-Value [D]		
				UK								Spain								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G [E]	E [F]	GxE [G]
M12	γ-terpinene	1066	A	4.2 ± 1.2^{bcd}	4.3 ± 1.2 ^{bcd}	3.6 ± 0.60 ^{abcd}	5.9 ± 0.28 ^{d}	5.6 ± 0.27 ^{cd}	5.5 ± 1.4 ^{cd}	2.1 ± 0.90 ^{ab}	5.6 ± 1.4 ^{d}	0.72 ± 0.12 ^{a}	2.6 ± 1.4 ^{abcd}	2.2 ± 0.36 ^{abc}	2.0 ± 0.35 ^{ab}	1.2 ± 0.24 ^{ab}	1.1 ± 0.24 ^{ab}	1.1 ± 0.20 ^{ab}	1.1 ± 0.36 ^{ab}	***	***	***
M13	terpinolene	1097	A	0.62 ± 0.19 ^{abc}	0.89 ± 0.07 ^{c}	0.53 ± 0.09 ^{abc}	0.43 ± 0.01 ^{abc}	0.36 ± 0.22 ^{abc}	0.73 ± 0.20 ^{bc}	0.57 ± 0.14 ^{abc}	0.90 ± 0.31 ^{c}	0.35 ± 0.08 ^{abc}	0.25 ± 0.18 ^{abc}	0.13 ± 0.08 ^{ab}	0.20 ± 0.14 ^{ab}	0.38 ± 0.14 ^{abc}	0.34 ± 0.14 ^{abc}	nd ^{a}	0.25 ± 0.18 ^{abc}	***	***	**
M14	*allo*-ocimene	1132	B [26]	0.11 ± 0.06 ^{ab}	0.10 ± 0.01 ^{ab}	0.10 ± 0.05 ^{ab}	0.31 ± 0.03 ^{b}	0.24 ± <0.01 ^{ab}	0.13 ± 0.04 ^{ab}	0.31 ± 0.27 ^{b}	0.13 ± 0.08 ^{ab}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	**
M15	β-thujone	1124	B [23]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.02 ^{ab}	tr ± 0.02 ^{a}	0.10 ± 0.01 ^{abc}	0.20 ± 0.04 ^{c}	tr ± 0.02 ^{ab}	0.10 ± 0.02 ^{ab}	0.17 ± 0.12^{bc}	0.10 ± 0.02 ^{ab}	***	***	***
M16	*p*-mentha-1,5,8-triene	1135	B [27]	0.26 ± 0.05 ^{ab}	0.10 ± 0.01 ^{ab}	0.22 ± 0.02 ^{ab}	0.56 ± 0.09 ^{b}	0.26 ± 0.07 ^{ab}	0.13 ± 0.09 ^{ab}	0.49 ± 0.17 ^{ab}	0.19 ± 0.08 ^{ab}	0.10 ± 0.02 ^{ab}	tr ± 0.02 ^{a}	0.16 ± 0.04 ^{ab}	0.55 ± 0.15 ^{ab}	0.10 ± 0.01 ^{ab}	0.17 ± 0.05 ^{ab}	0.50 ± 0.27 ^{ab}	0.10 ± 0.06 ^{ab}	**	**	**
M17	(Z)-carveol	1147	B [19]	0.48 ± 0.13 ^{bcd}	0.57 ± 0.17 ^{cd}	0.23 ± 0.08 ^{abc}	0.18 ± 0.08 ^{ab}	0.24 ± 0.02 ^{ab}	0.31 ± 0.21 ^{abc}	tr ± 0.03 ^{a}	0.13 ± 0.10 ^{ab}	0.51 ± 0.07 ^{cd}	0.45 ± 0.21 ^{bcd}	0.65 ± 0.09^{d}	0.44 ± 0.02 ^{bcd}	0.34 ± 0.07 ^{abcd}	0.51 ± 0.14 ^{cd}	0.26 ± 0.09 ^{abcd}	0.60 ± 0.23 ^{d}	***	***	***
M18	pentylcyclohexa-1,3-diene	1166	B [19]	0.20 ± 0.05 ^{ab}	0.23 ± 0.08 ^{ab}	0.25 ± 0.03 ^{ab}	0.46 ± 0.11 ^{abc}	0.31 ± 0.03 ^{ab}	0.10 ± 0.04 ^{a}	0.26 ± 0.16 ^{ab}	0.20 ± 0.01 ^{ab}	0.20 ± 0.06 ^{ab}	0.13 ± 0.09 ^{a}	0.19 ± 0.08 ^{ab}	0.20 ± 0.02 ^{ab}	0.16 ± 0.05 ^{ab}	0.19 ± 0.02 ^{ab}	0.12 ± 0.09 ^{a}	0.30 ± 0.14 ^{ab}	*	*	*
M19	(Z)-dihydrocarvone	1208	A	0.39 ± 0.09 ^{b}	0.36 ± 0.05 ^{b}	0.35 ± 0.08 ^{b}	0.19 ± 0.06 ^{ab}	0.27 ± 0.05 ^{ab}	0.18 ± 0.04 ^{ab}	0.20 ± 0.08 ^{ab}	0.26 ± 0.02 ^{ab}	0.35 ± 0.03 ^{b}	0.28 ± 0.02 ^{ab}	0.30 ± 0.05 ^{b}	0.25 ± 0.06 ^{ab}	0.23 ± 0.12 ^{ab}	0.20 ± 0.14 ^{ab}	nd ^{a}	0.39 ± 0.06 ^{b}	**	**	**
M20	camphor	1157	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.27 ± 0.15 ^{bc}	0.17 ± 0.04 ^{abc}	0.22 ± 0.06 ^{abc}	0.17 ± 0.05 ^{abc}	0.18 ± 0.08 ^{abc}	0.23 ± 0.06 ^{bc}	0.15 ± 0.03 ^{ab}	0.38 ± 0.13 ^{c}	***	***	***
M21	isoborneol	1173	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.25 ± 0.14 ^{b}	0.17 ± 0.03 ^{ab}	0.16 ± 0.06 ^{ab}	0.17 ± 0.04 ^{ab}	0.19 ± 0.04 ^{ab}	0.25 ± 0.04 ^{b}	0.18 ± 0.05 ^{ab}	0.23 ± 0.12 ^{b}	***	***	***
M22	(E)-dihydrocarvone	1240	B [27]	0.79 ± 0.12 ^{f}	0.79 ± 0.14 ^{f}	0.67 ± 0.10 ^{ef}	0.41 ± 0.08 ^{cde}	0.57 ± 0.09 ^{ef}	0.43 ± 0.05 ^{de}	0.38 ± 0.06 ^{bcde}	0.59 ± 0.03 ^{ef}	0.10 ± 0.03 ^{ab}	0.10 ± 0.04 ^{a}	0.10 ± 0.02 ^{ab}	0.10 ± 0.01 ^{ab}	0.10 ± 0.02 ^{a}	0.11 ± 0.03 ^{abc}	tr ± 0.04 ^{a}	0.14 ± 0.09 ^{abcd}	***	***	***
M23	β-cyclocitral	1230	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.04 ^{b}	0.12 ± 0.02 ^{b}	0.11 ± 0.03 ^{b}	0.18 ± 0.02 ^{b}	0.15 ± 0.01 ^{b}	0.12 ± 0.02 ^{b}	0.10 ± 0.01 ^{b}	0.14 ± 0.06 ^{b}	***	***	***
M24	L-carvone	1248	A	0.96 ± 0.19 ^{bcd}	0.57 ± 0.11 ^{abc}	1.5 ± 0.05 ^{d}	0.71 ± 0.06 ^{abc}	0.81 ± 0.13 ^{abcd}	0.61 ± 0.14 ^{abc}	0.75 ± 0.17 ^{abcd}	1.1 ± 0.12 ^{cd}	0.38 ± 0.22 ^{abc}	0.26 ± 0.11 ^{ab}	0.18 ± 0.06 ^{ab}	0.14 ± 0.02 ^{a}	0.23 ± 0.08 ^{ab}	0.36 ± 0.03 ^{abc}	0.17 ± 0.08 ^{ab}	0.45 ± 0.23 ^{abc}	***	***	***
M25	D-carvone	1262	A	0.43 ± 0.19	0.36 ± 0.10	0.24 ± 0.02	0.18 ± 0.03	0.23 ± 0.08	0.34 ± 0.15	0.44 ± 0.07	0.29 ± 0.06	0.33 ± 0.13	0.27 ± 0.06	0.60 ± 0.13	0.36 ± 0.17	0.30 ± 0.10	0.48 ± 0.11	0.52 ± 0.11	0.47 ± 0.18	ns	ns	ns
M26	thymol	1290	A	0.17 ± 0.05 ^{b}	0.11 ± 0.14 ^{ab}	0.12 ± 0.04 ^{ab}	0.15 ± 0.09 ^{ab}	0.11 ± 0.08 ^{ab}	0.10 ± 0.03 ^{ab}	nd ^{a}	0.14 ± 0.11 ^{ab}	0.15 ± 0.09 ^{ab}	0.12 ± 0.07 ^{ab}	0.15 ± 0.01 ^{ab}	0.16 ± 0.01 ^{ab}	0.12 ± 0.01 ^{ab}	0.19 ± 0.08^{b}	0.10 ± 0.03 ^{ab}	0.16 ± 0.05 ^{ab}	*	*	*
M27	carvacrol	1317	A	0.54 ± 0.08	0.42 ± 0.09	0.45 ± 0.03	0.60 ± 0.02	0.29 ± 0.03	0.39 ± 0.03	0.18 ± 0.04	0.52 ± 0.04	0.44 ± 0.21	0.36 ± 0.27	0.45 ± 0.05 ^{a}	0.53 ± 0.08	0.31 ± 0.12	0.56 ± 0.23	0.19 ± 0.07	0.39 ± 0.14	ns	ns	ns
	Total Monoterpenoid Alcohols			61	64	50	56	59	53	42	54	27	34	42	38	26	27	29	30			
MA1	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	1122	A	0.10 ± 0.03	0.15 ± 0.01	tr ± 0.03	0.28 ± 0.03	0.10 ± 0.02	0.10 ± 0.03	tr ± 0.03	0.14 ± 0.01	0.15 ± 0.03	0.16 ± 0.01	0.15 ± 0.03	0.13 ± 0.02	0.12 ± 0.07	0.13 ± 0.02	0.12 ± 0.03	0.19 ± 0.13	ns	ns	ns
MA2	dihydrolinalool	1142	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.75 ± 0.31 ^{abc}	0.33 ± 0.26 ^{abc}	0.93 ± 0.08^{bc}	1.2 ± 0.06^{c}	0.78 ± 0.18 ^{abc}	0.64 ± 0.30 ^{abc}	0.29 ± 0.11 ^{ab}	0.48 ± 0.24 ^{abc}	***	***	***
MA3	(Z)-pinocarveol	1147	B [28]	0.59 ± 0.13 ^{a}	0.63 ± 0.17 ^{a}	0.30 ± 0.08 ^{a}	0.20 ± 0.08 ^{a}	0.28 ± 0.02 ^{a}	0.35 ± 0.21 ^{a}	tr ± 0.06 ^{a}	0.45 ± 0.10 ^{a}	0.29 ± 0.09 ^{a}	0.21 ± 0.10 ^{a}	0.11 ± 0.06 ^{a}	0.10 ± 0.01 ^{a}	0.20 ± 0.10 ^{a}	0.47 ± 0.32 ^{a}	0.15 ± 0.03 ^{a}	0.57 ± 0.42 ^{a}	*	*	*
MA4	terpinen-4-ol	1184	A	0.10 ± 0.01 ^{ab}	nd ^{a}	tr ± 0.03 ^{a}	tr ± 0.03 ^{ab}	tr ± 0.03 ^{a}	0.10 ± 0.07 ^{ab}	nd ^{a}	0.13 ± 0.03 ^{ab}	0.10 ± 0.09 ^{ab}	0.15 ± 0.04 ^{ab}	0.13 ± 0.03 ^{ab}	0.18 ± 0.02 ^{b}	0.10 ± 0.04 ^{ab}	0.15 ± 0.06 ^{ab}	nd ^{a}	0.20 ± 0.04 ^{b}	***	***	***
MA5	α-terpineol	1211	A	nd	nd	nd	nd	nd	nd	nd	nd	0.10 ± 0.04	nd	0.10 ± 0.01	0.10 ± 0.01	tr ± 0.03	0.10 ± 0.01	tr ± 0.03	0.13 ± 0.09	ns	ns	ns
MA6	(E)-8-hydroxylinalool	1349	B [19]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.19 ± 0.05 ^{b}	0.15 ± 0.06 ^{b}	0.10 ± 0.04 ^{ab}	0.10 ± 0.01 ^{ab}	0.10 ± 0.02 ^{ab}	0.18 ± 0.03 ^{b}	0.10 ± 0.06 ^{ab}	0.18 ± 0.05 ^{b}	***	***	***
MA7	caryophylladienol II	1665	B [19]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.1± 0.05 ^{b}	nd ^{a}	0.10± 0.01 ^{b}	0.10± 0.02 ^{b}	0.10± 0.01 ^{b}	0.11± 0.03 ^{b}	0.10 ± 0.02 ^{b}	0.10 ± 0.03 ^{b}	***	***	***
	Total Sesquiterpenes			0.79	0.78	0.38	0.53	0.39	0.48	0.06	0.72	1.6	1.0	1.6	1.9	1.4	1.8	0.77	1.7			
S1	α-ylangene	1384	B [27]	0.26 ± 0.11 ^{c}	0.24 ± 0.07 ^{c}	0.17 ± 0.11 ^{c}	tr ± 0.01 ^{ab}	0.16 ± 0.05 ^{bc}	0.19 ± 0.10 ^{c}	0.20 ± 0.26 ^{c}	0.20 ± 0.14 ^{c}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***
S2	α-copaene	1390	A	1.1 ± 0.02^{e}	0.86 ± 0.01 ^{de}	0.62 ± 0.03 ^{cde}	0.10 ± 0.02 ^{ab}	0.15 ± 0.05 ^{ab}	0.49 ± 0.03 ^{bcd}	0.78 ± 0.04 ^{de}	0.77 ± 0.05 ^{de}	0.14 ± 0.04 ^{ab}	0.09 ± 0.06 ^{ab}	0.06 ± 0.02 ^{ab}	nd ^{a}	nd ^{a}	0.12 ± 0.05 ^{ab}	0.24 ± 0.07 ^{abc}	0.22 ± 0.18 ^{abc}	***	***	***
S3	(E)-β-caryophyllene	1430	B [29]	tr ± 0.03	tr ± 0.02	nd	nd	tr ± 0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	ns	ns	ns
S4	β-caryophyllene	1445	A	4.4 ± 0.61 ^{bc}	5.5 ± 0.32 ^{c}	4.1 ± 0.43 ^{bc}	2.5 ± 0.39 ^{ab}	4.3 ± 1.3 ^{bc}	4.1 ± 1.2 ^{bc}	2.4 ± 0.29 ^{ab}	2.2 ± 0.50 ^{ab}	0.67 ± 0.52 ^{a}	0.60 ± 0.40 ^{a}	1.4 ± 0.73 ^{a}	1.0 ± 0.15 ^{a}	0.46 ± 0.17 ^{a}	1.2 ± 0.13 ^{a}	0.55 ± 0.28 ^{a}	0.69 ± 0.28 ^{a}	***	***	***
S5	(+)-aromadendrene	1452	A	0.17 ± 0.04 ^{de}	0.21 ± 0.01 ^{e}	0.15 ± 0.04 ^{cde}	tr ± 0.07 ^{abc}	0.13 ± 0.03 ^{cde}	0.15 ± 0.08 ^{cde}	0.10 ± 0.06 ^{abc}	0.10 ± 0.01^{bcd}	tr ± 0.01 ^{ab}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***

Table 1. *Cont.*

Code	Compound	LRI_{exp} [A]	ID [B]	Percentage Composition (%) [C]														*p*-Value [D]		
				UK								Spain								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G [E]	E [F]	GxE [G]
S6	curcumene	1472	B [30]	0.18 ± 0.09 ^{abcd}	0.23 ± 0.11 ^{b}	0.19 ± 0.06 ^{b}	0.09 ± 0.05 ^{a}	0.15 ± 0.22 ^{b}	0.22 ± 0.19 ^{b}	tr ± 0.03 ^{bcde}	0.12 ± 0.05 ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	***	ns	***
S7	α-humulene	1479	A	0.42 ± 0.16 ^{abcd}	0.70 ± 0.58 ^{d}	0.38 ± 0.29 ^{abcd}	0.49 ± 0.10 ^{bcd}	0.51 ± 0.76 ^{cd}	0.40 ± 0.65 ^{abcd}	0.18 ± 0.01 ^{abc}	0.26 ± 0.91 ^{abcd}	0.11 ± 0.02 ^{ab}	0.10 ± 0.06 ^{a}	0.10 ± 0.05 ^{a}	0.10 ± 0.02 ^{a}	0.19 ± 0.04 ^{abc}	0.10 ± 0.06 ^{a}	tr ± 0.03 ^{a}	0.13 ± 0.05 ^{abc}	***	***	***
S8	β-selinene	1508	B [31]	3.0 ± 0.05 ^{cd}	2.7 ± 0.06 ^{bcd}	1.5 ± 0.02 ^{abc}	4.6 ± 0.15 ^{d}	2.2 ± 0.19 ^{abcd}	1.9 ± 0.12 ^{abc}	3.3 ± 0.26 ^{cd}	3.0 ± 0.14 ^{bcd}	0.35 ± 0.25 ^{ab}	0.31 ± 0.16 ^{ab}	0.31 ± 0.17 ^{ab}	1.3 ± 0.29 ^{abc}	0.17 ± 0.06 ^{a}	0.40 ± 0.26 ^{ab}	0.36 ± 0.15 ^{ab}	0.50 ± 0.12 ^{ab}	***	***	***
S9	valencene	1514	A	nd ^{a}	nd ^{a}	nd ^{a}	2.9 ± 0.44 ^{c}	nd ^{a}	nd ^{a}	nd ^{a}	0.20 ± 0.07 ^{a}	nd ^{a}	nd ^{a}	tr ± 0.02 ^{a}	2.1 ± 0.16 ^{b}	tr ± 0.02 ^{a}	tr ± 0.01 ^{a}	tr ± 0.02 ^{a}	0.36 ± 0.05 ^{a}	***	***	***
S10	α-selinene	1515	B [32]	0.61 ± 0.02 ^{c}	0.60 ± 0.02 ^{c}	0.43 ± 0.05 ^{abc}	0.63 ± 0.44 ^{c}	0.54 ± 0.04 ^{bc}	0.44 ± 0.03 ^{abc}	0.71 ± 0.02 ^{c}	0.59 ± 0.07 ^{c}	0.10 ± 0.04 ^{a}	tr ± 0.03 ^{a}	tr ± 0.03 ^{a}	0.14 ± 0.03 ^{ab}	tr ± 0.02 ^{a}	tr ± 0.05 ^{a}	tr ± 0.04 ^{a}	0.10 ± 0.02 ^{a}	***	***	***
S11	kessane	1557	B [19]	nd ^{a}	0.12 ± 0.02 ^{a}	nd ^{a}	2.8 ± 0.05^{c}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr ± 0.03 ^{a}	tr ± 0.01 ^{a}	nd ^{a}	2.0 ± 0.13^{b}	nd ^{a}	tr ± 0.02 ^{a}	nd ^{a}	0.36 ± 0.05 ^{a}	***	***	***
S12	cuparene [$]	1530	B [33]	nd	nd	nd	nd	nd	nd	nd	nd	tr ± 0.02	nd	nd	nd	tr ± 0.01	tr ± 0.01	nd	tr ± 0.04	ns	ns	ns
S13	(*E*)-nerolidol	1540	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr ± 0.02 ^{a}	tr ± 0.02 ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.02 ^{a}	tr ± 0.04 ^{a}	tr ± 0.03 ^{a}	tr ± 0.03 ^{a}	**	**	**
S14	liguloxide [$]	1560	B [34]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr ± 0.01 ^{a}	nd ^{a}	tr ± 0.05 ^{a}	nd ^{a}	tr ± 0.01 ^{a}	**	*	*
	Total Phthalides			10	11	7.5	14	8.2	7.9	7.7	7.4	1.4	1.2	1.9	6.7	0.95	2.0	1.3	2.4			
P1	3-butylhexahydro phthalide	1662	B [19]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr ± 0.04 ^{abc}	tr ± 0.02 ^{ab}	tr ± 0.01 ^{abc}	nd ^{a}	0.10 ± 0.01^{bc}	0.10 ± 0.02^{c}	tr ± 0.01 ^{abc}	0.10 ± 0.01^{bc}	***	***	***
P2	3-n-butylphthalide	1676	B [8,10]	5.0 ± 0.01 ^{abc}	5.2 ± 0.03 ^{abc}	9.4 ± 0.05 ^{cd}	6.6 ± 0.01 ^{abcd}	7.1 ± 0.03 ^{abcd}	6.7 ± 0.01 ^{abcd}	9.8 ± 0.06 ^{d}	7.0 ± 0.03 ^{abcd}	4.2 ± 1.1 ^{ab}	3.6 ± 0.81 ^{a}	5.6 ± 1.1 ^{abcd}	8.5 ± 0.86 ^{bcd}	4.9 ± 0.93 ^{ab}	5.6 ± 1.4 ^{abcd}	5.2 ± 1.3 ^{abc}	4.6 ± 0.87 ^{ab}	***	***	***
P3	(*Z*)-3-butylidenephthalide	1685	B [19]	0.15 ± 0.06 ^{ab}	0.22 ± 0.05 ^{abc}	0.36 ± 0.09^{b}	0.16 ± 0.02 ^{ab}	0.25 ± 0.02 ^{ab}	0.17 ± 0.07 ^{ab}	0.25 ± 0.34 ^{ab}	0.18 ± 0.25 ^{ab}	0.22 ± 0.20 ^{ab}	0.10 ± 0.04 ^{a}	0.13 ± 0.01 ^{ab}	0.13 ± 0.01 ^{ab}	0.25 ± 0.06 ^{ab}	0.17 ± 0.06 ^{ab}	0.10 ± 0.01 ^{a}	0.14 ± 0.04 ^{ab}	*	*	*
P4	sedanenolide	1748	B [8,10]	4.8 ± 0.30 ^{abcd}	9.7 ± 2.3 ^{bcde}	15 ± 1.9 ^{e}	16 ± 1.6 ^{e}	14 ± 3.0 ^{e}	9.5 ± 2.9 ^{abcde}	11 ± 3.0 ^{cde}	13 ± 2.2 ^{de}	1.1 ± 0.30 ^{ab}	0.96 ± 0.03 ^{a}	3.7 ± 1.1 ^{abc}	9.2 ± 1.1 ^{abcde}	1.5 ± 0.49 ^{ab}	2.0 ± 0.89 ^{ab}	0.92 ± 0.52 ^{a}	1.3 ± 1.1 ^{ab}	***	***	***
P5	(*Z*)-neocnidilide	1755	B [19]	0.26 ± 0.03 ^{a}	0.13 ± 0.03 ^{a}	1.8 ± 0.02 ^{c}	0.16 ± 0.04 ^{a}	0.30 ± 0.06 ^{ab}	0.78 ± 0.06 ^{abc}	0.99 ± 0.04 ^{abc}	0.94 ± 0.04 ^{abc}	1.4 ± 1.1 ^{abc}	0.45 ± 0.24 ^{abc}	1.2 ± 0.24 ^{abc}	0.14 ± 0.01 ^{a}	0.37 ± 0.15 ^{ab}	1.7 ± 0.55 ^{bc}	1.0 ± 0.23 ^{abc}	1.1 ± 0.19 ^{abc}	***	***	***
P6	(*E*)-ligustilide	1764	B [8,10]	0.12 ± 0.02 ^{a}	0.15 ± 0.10 ^{a}	0.24 ± 0.01 ^{a}	0.23 ± 0.03 ^{a}	0.25 ± 0.05 ^{a}	0.14 ± 0.01 ^{a}	0.18 ± 0.09 ^{a}	0.18 ± 0.05 ^{a}	tr ± 0.02 ^{a}	tr ± 0.02 ^{a}	0.10 ± 0.03 ^{a}	0.11 ± 0.03 ^{a}	0.25 ± 0.04 ^{a}	tr ± 0.02 ^{a}	tr ± 0.01 ^{a}	tr ± 0.02 ^{a}	*	*	*
	Total Aromatic Hydrocarbons			10	16	27	23	22	17	22	21	7.0	5.1	11	18	7.3	9.6	7.3	7.2			
AHC1	toluene	769	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.24 ± 0.11 ^{bc}	0.23 ± 0.11 ^{bc}	0.38 ± 0.10 ^{c}	0.25 ± 0.07 ^{bc}	0.17 ± 0.01 ^{ab}	0.19 ± 0.04 ^{abc}	0.29 ± 0.06 ^{bc}	0.27 ± 0.08 ^{bc}	***	***	***
AHC2	*p*-xylene	876	B [19]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.11 ± 0.08 ^{ab}	0.12 ± 0.06 ^{b}	0.14 ± 0.05 ^{b}	0.09 ± 0.01 ^{ab}	0.11 ± 0.01 ^{ab}	0.17 ± 0.05 ^{b}	0.15 ± 0.03 ^{b}	0.15 ± 0.03 ^{b}	***	***	***
	Total Oxides			0	0	0	0	0	0	0	0	0.35	0.35	0.52	0.34	0.28	0.36	0.44	0.42			
O1	caryophyllene oxide	1610	A	tr ± 0.01 ^{abc}	0.13 ± 0.04 ^{abcdef}	0.25 ± 0.05 ^{cdef}	tr ± 0.02 ^{abcd}	0.10 ± 0.07 ^{abcde}	0.10 ± 0.02 ^{abcde}	tr ± 0.01 ^{ab}	nd ^{a}	0.25 ± 0.06 ^{cdef}	0.27 ± 0.08 ^{cdef}	0.28 ± 0.04 ^{ef}	0.24 ± 0.09 ^{bcdef}	0.26 ± 0.03 ^{cdef}	0.33 ± 0.11 ^{f}	0.22 ± 0.03 ^{abcdef}	0.27 ± 0.11 ^{def}	***	***	***
	Lactone																					
L1	γ-nonalactone	1372	A	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.01 ^{bcd}	0.10 ± 0.02 ^{bcd}	tr ± 0.01 ^{abc}	tr ± 0.01 ^{ab}	0.10 ± 0.01 ^{bcde}	0.10 ± 0.01 ^{cde}	0.10 ± 0.03 ^{de}	0.10 ± 0.01^{e}	***	***	***
L2	dihydroactinolide	1557	B [35]	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr ± 0.06 ^{ab}	0.10 ± 0.05 ^{abc}	0.10 ± 0.02 ^{abc}	n.d. ^{a}	0.16 ± 0.01 ^{c}	0.10 ± 0.06 ^{abc}	0.10 ± 0.03 ^{bc}	tr ± 0.02 ^{ab}	***	***	***
	Total Unknowns			0	0	0	0	0	0	0	0	0.10	0.13	0.11	0.03	0.32	0.15	0.19	0.13			
U1	unknown 1	n/a		0.57 ± 0.09 ^{abc}	0.31 ± 0.03 ^{ab}	0.43 ± 0.06 ^{ab}	0.19 ± 0.02 ^{ab}	0.27 ± 0.01 ^{ab}	0.71 ± 0.20 ^{bc}	1.2 ± 0.47^{c}	0.51 ± 0.29 ^{abc}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***
U2	unknown 2	n/a		2.3 ± 0.63 ^{bc}	1.7 ± 0.03 ^{abc}	2.1 ± 0.06 ^{abc}	0.84 ± 0.02 ^{ab}	1.0 ± 0.01 ^{ab}	2.7 ± 0.20 ^{bc}	3.4 ± 0.47 ^{c}	1.5 ± 0.29 ^{abc}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	***	***
U3	unknown 3	735		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.19 ± 0.08 ^{b}	0.17 ± 0.05 ^{b}	0.25 ± 0.01 ^{b}	0.25 ± 0.05 ^{b}	0.14 ± 0.01 ^{b}	0.16 ± 0.04 ^{b}	0.23 ± 0.02 ^{b}	0.18 ± 0.03 ^{b}	***	***	***
U4	unknown 4	766		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	Nd ^{a}	0.17 ± 0.08 ^{b}	0.15 ± 0.03 ^{b}	0.23 ± 0.03 ^{b}	0.17 ± 0.01 ^{b}	0.12 ± 0.02 ^{ab}	0.11 ± 0.09 ^{ab}	0.15 ± 0.01 ^{b}	0.19 ± 0.02 ^{b}	***	***	***
U5	unknown 5	787		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.23 ± 0.11 ^{b}	0.20 ± 0.07 ^{b}	0.23 ± 0.09 ^{b}	0.23 ± 0.05 ^{b}	0.16 ± 0.02 ^{ab}	0.18 ± 0.06 ^{ab}	0.28 ± 0.06 ^{b}	0.22 ± 0.05 ^{b}	***	***	***

Table 1. *Cont.*

Code	Compound	LRI_{exp} [A]	ID [B]	Percentage Composition (%) [C]														*p*-Value [D]		
				UK								Spain								
				5	8	10	12	15	18	22	25	5	8	10	12	15	18	22	25	G [E]	E [F]	GxE [G]
U6	unknown 6	896		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.22 ± 0.09 ^{b}	0.16 ± 0.04 ^{b}	0.25 ± 0.07 ^{b}	0.22 ± 0.05 ^{b}	0.17 ± 0.01 ^{b}	0.22 ± 0.03 ^{b}	0.22 ± 0.05 ^{b}	0.16 ± 0.06 ^{b}	***	***	***
U7	unknown 7	971		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.64 ± 0.04 ^{bc}	0.52 ± 0.06 ^{ab}	1.1 ± 0.01 ^{c}	0.78 ± 0.17 ^{bc}	0.42 ± 0.04 ^{ab}	0.58 ± 0.02 ^{bc}	0.64 ± 0.05 ^{bc}	0.73 ± 0.03 ^{b}	***	***	***
U8	unknown 8	1249		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.54 ± 0.18 ^{b}	0.46 ± 0.06 ^{b}	0.65 ± 0.06 ^{b}	0.59 ± 0.02 ^{b}	0.55 ± 0.03 ^{b}	0.56 ± 0.13 ^{b}	0.52± 0.05 ^{b}	0.49± 0.02 ^{b}	***	***	***
U9	unknown 9	1279		0.16 ± 0.06 ^{ab}	0.08 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	0.13 ± 0.03 ^{a}	0.24 ± 0.01 ^{ab}	0.11 ± 0.01 ^{a}	0.17 ± 0.03 ^{ab}	0.10 ± 0.04 ^{ab}	0.29 ± 0.12 ^{ab}	0.18 ± 0.06 ^{ab}	0.19 ± 0.07 ^{ab}	0.18 ± 0.02 ^{ab}	0.17 ± 0.05 ^{ab}	0.22 ± 0.05 ^{ab}	0.14 ± 0.04 ^{ab}	0.50 ± 0.19 ^{bc}	*	*	*
U10	unknown 10	1362		0.10 ± 0.02 ^{ab}	0.09 ± 0.03 ^{ab}	nd ^{a}	0.16 ± 0.01 ^{b}	0.03 ± 0.04 ^{a}	0.10 ± 0.01 ^{ab}	0.08 ± 0.01 ^{ab}	0.07 ± 0.4 ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	***	**	***
U11	unknown 11	1506		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.05 ^{ab}	0.10 ± 0.01 ^{ab}	0.13 ± 0.04^{b}	0.10 ± 0.05 ^{ab}	0.10 ± 0.03 ^{a}	0.13 ± 0.05 ^{b}	0.13 ± 0.03 ^{b}	0.13 ± 0.06 ^{b}	**	***	***
U12	unknown 12	1539		0.25 ± 0.02 ^{ab}	0.33 ± 0.04 ^{b}	0.19 ± 0.02 ^{ab}	0.13 ± 0.01 ^{a}	0.10 ± 0.04 ^{ab}	0.10 ± 0.01 ^{a}	0.18 ± 0.01 ^{ab}	0.12 ± 0.04 ^{ab}	0.10 ± 0.04 ^{a}	0.10 ± 0.07 ^{a}	0.17 ± 0.04 ^{ab}	0.20 ± 0.02 ^{ab}	0.11 ± 0.02 ^{a}	0.17 ± 0.07 ^{ab}	0.10 ± 0.01 ^{a}	0.13 ± 0.06 ^{ab}	**	**	**
U13	unknown 13	1684		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	tr ± 0.06 ^{a}	tr ± 0.02 ^{a}	tr ± 0.02 ^{a}	tr ± 0.03 ^{a}	tr ± 0.02 ^{a}	0.10 ± 0.01 ^{a}	tr ± 0.02 ^{a}	tr ± 0.01 ^{a}	*	**	*
U14	unknown 14	1706		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.10 ± 0.09 ^{ab}	tr ± 0.02 ^{ab}	0.10 ± 0.02 ^{ab}	0.11 ± 0.01 ^{b}	0.10 ± 0.04 ^{ab}	0.13 ± 0.02^{b}	0.10 ± 0.03 ^{ab}	0.10 ± 0.05 ^{ab}	***	***	***
U15	unknown 15	1799		nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	nd ^{a}	0.13 ± 0.03 ^{b}	0.13 ± 0.05 ^{b}	0.18 ± 0.01 ^{b}	0.13 ± 0.04 ^{b}	0.10 ± 0.01 ^{b}	0.18 ± 0.04 ^{b}	0.12 ± 0.02 ^{b}	0.13 ± 0.05 ^{b}	***	***	***
	Total			3.4	2.5	2.9	1.4	1.8	3.8	5.1	2.4	2.7	2.2	3.5	3.0	2.2	2.7	2.6	3.0			

[A] Linear retention index on a H*P*-5MS column. [B] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value >80 was used); LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agrees with those in the literature cited; $ tentatively identified, spectral quality value of 70 was used for this compound. [C] Percentage composition of total peak area divided by compound peak area; means labelled with letters are significantly different ($p < 0.05$) according to the GxE interaction; means of three replicate samples; tr, trace amounts <0.10%; nd, not detected. [D] Probability, obtained by ANOVA, that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level. [E] Geographical location. [F] Genotype. [G] Geographical location x genotype interaction. Cells are colour coded; orange expresses the location giving the higher value for each compound for each genotype; green expresses the location giving the lower value of each compound for each genotype; no colour expresses no difference in percentage composition for both locations.

As observed in various studies, monoterpenes, sesquiterpenes and phthalides are the most reported compound groups to contribute to celery's aroma profile [4–6,8,36,37]. The composition of celery grown in UK expressed an average of 55% monoterpenes, 20% phthalides and 9.2% sesquiterpenes, whereas genotypes grown in Spain had an average of 32%, 2.2% and 9%, respectively. Monoterpenes comprised most of the composition of the aroma profile of all celery genotypes grown in the UK, with limonene, γ-terpinene, β-pinene and *m*-cymene exhibiting the highest proportion of monoterpenes [4,7]. A lower proportion of monoterpenes comprised Spanish-grown celery, however, genotypes 10 and 12 displayed over 10% more than the other genotypes (Table 1). The authors previously carried out gas chromatography–olfactometry (GC/O) on two celery genotypes (12 and 25) and reported that these compounds contribute citrus, fresh, pine, and mint odours to celery [8]. Although these compounds comprised much of the aroma profile, their odour activity remains low and, therefore, they would not be considered characteristic compounds to celery. By completing aroma extraction dilution analysis (AEDA), Kurobayashi, Kouno, Fujita, Morimitsu and Kubota [38] identified the flavour dilution (FD) factor of volatile compounds of raw and boiled celery. Phthalides including 3-n-butylphthalide and ligustilide were found to have the highest FD factor of 3125, whereas myrcene, a monoterpene also identified within the current study, had a FD value of 625. Uhlig, Chang and Jen [3] investigated the effect of phthalides on celery flavour using eight celery cultivars of varying origins, observing a positive correlation with total phthalide content and the intensity of the 'celery flavour' attribute. Significant variation between celery cultivars and phthalide content was also observed, most obviously in the concentration of sedanenolide. This is reflected in the current study.

The prominence of phthalides and their contribution to celery aroma is apparent throughout literature. A review completed by the authors [7] identified 3-n-butylphthalide and sedanenolide to be the most reported phthalides in celery, with odour descriptors such as celery, herbal and cooked celery. These compounds have been identified as characteristic compounds to celery aroma, and when authors [8] completed GC/O upon two celery genotypes also used in this study (12, 22), the average odour intensity of these compounds was high throughout maturity. Growing celery in the UK in 2018 produced genotypes with a higher phthalide composition, particularly high in 3-n-butylphthalide and sedanenolide, comprising an average percentage of 7.1% and 11.6%, respectively. The average percentage of these compounds was lower in celery growing in Spain in 2019, with 3-n-butylphthalide and sedanenolide contributing an average of 5.3% and 2.6%, respectively. However, (*Z*)-neocnidilide was expressed at a higher composition in Spanish celery, comprising an average of 0.92% of the aroma profile. Pino, Rosado and Fuentes [39] identified sedanenolide to comprise much of the volatile profile of celery leaf oil, comprising 32.1% of the composition. The significantly higher abundance of these phthalide compounds, reflected in Table 1, will allow assumptions to be drawn that these genotypes have a stronger typical celery aroma [3].

A similar pattern was observed within sesquiterpenes, whereby celery grown in the UK exhibited a significantly higher proportion of sesquiterpenes compared to Spanish grown celery. β-Caryophyllene and β-selinene comprised the highest proportion of the sesquiterpene profile for both geographical locations, and these two are the most reported sesquiterpenes in celery [7,36,37,40]. A similar sesquiterpene trend was observed in another study [10] between two harvest years (2018 and 2020) for the same eight genotypes, whereby the sesquiterpene content comprised a higher proportion of the volatile profile of celery grown in 2018, a significantly warmer season than 2020 [10]. Pino, Rosado and Fuentes [39] identified β-caryophyllene to comprise 13.5% of the volatile profile of Cuban celery leaf oil, whereas Lund, Wagner and Bryan [41] identified β-caryophyllene and β-selinene to comprise an average of 1.5% and 3.4%, respectively. Lund, Wagner and Bryan [41] also identified β-selinene to have a celery-like odour.

Whilst monoterpenes formed much of the composition of UK grown celery, aldehydes were observed to contribute a high proportion in Spanish-grown celery for all genotypes, except genotypes 10 and 12, comprising an average of 38.5% of the aroma composition. Hexanal and (*E*)-2-heptenal were the most abundant compounds in this group for both geographical locations and genotypes, with odour characteristics of fresh, green and fatty. Although not identified in UK grown celery, benzaldehyde and (*E*)-2-octenal composed a high proportion of the volatile composition, with odour characteristics of almond, cherry, and cucumber, averaging to comprise 2.0% and 2.7%, respectively. Aldehyde content within celery has not been discussed thoroughly, with only a few studies detecting the compound group. Gold and Wilson [9] identified a range of aldehydes including hexanal, octanal and heptanal, yet Shojaei, Ebrahimi and Salimi [40] only identified benzeneacetaldehyde and nonanal within three ecotypes of wild celery. A large proportion of aldehydes that were identified in the current study were detected, using GC/O, to be prominent throughout celery maturity [8]. Hexanal was one of the compounds contributing the most to the aldehyde content in celery for all genotypes across both locations, with odour characteristics including fresh, green and apple, as well as being identified throughout celery maturity [8].

Similarly, the ketone content of celery has rarely been discussed and only few studies have reported these compounds [8,9,40]. Accompanying the identification of aldehydes, Shojaei, Ebrahimi and Salimi [40] further detected *p*-methyl acetophenone and 2-undecanone within the three wild celery ecotypes. An explanation for the variation in ketone content between geographical location could involve investigating the formation of phthalides. The metabolic pathway involved in the synthesis of phthalides has yet to be confirmed and, currently, there are multiple suggestions looking into how phthalides are synthesised [7]. Phan, Kim, and Dong [42] identified a method of synthesising phthalides through ketone hydroacylation. Here, the hydroacylation of ketones led to the formation of five-membered lactones, inducing the synthesis of 1(3H)-isobenzofuranone, the simplest phthalide structure. From here, various phthalides can be formed according to the substitution at C3 [7,42]. The large variety of ketones identified (Table 1) may be an indication of the potential for the Spanish crop to synthesise phthalides. Many ketones were identified by the authors [8] to be important to celery aroma when using GC/O to measure the change in aroma during celery maturity. The compounds 3-Pentanone, 2-hexanone and 3-octen-2-one were detected at higher intensities in immature celery, displaying the crop's potential to synthesis phthalide compounds, whereas 1-octen-3-one was identified by GC/MS with a relative abundance of 6.7 and 4.7 AU, respectively, in post-mature celery.

Principal Component Analysis of Volatile Compounds in UK and Spanish Celery Samples

Principal component analysis allowed for the visual comparison of the volatile composition of the eight celery genotypes grown in UK and Spain (Figure 1) and to examine any correlations occurring between genotype, geographical location and chemical compounds. Using only the significant compounds for geographical location (G), genotype (*E*) and their interaction (GxE), a clear divide between the compounds associated with each year was observed. Principal component one (F1) and two (F2) explained 72.32% of the total variation present in the data, and it can be observed that the first axis separated samples from the geographical location (UK and Spain), whereas the second axis separated the various genotypes within a location. Differences between geographical location were apparent, as they separated along the F2 component.

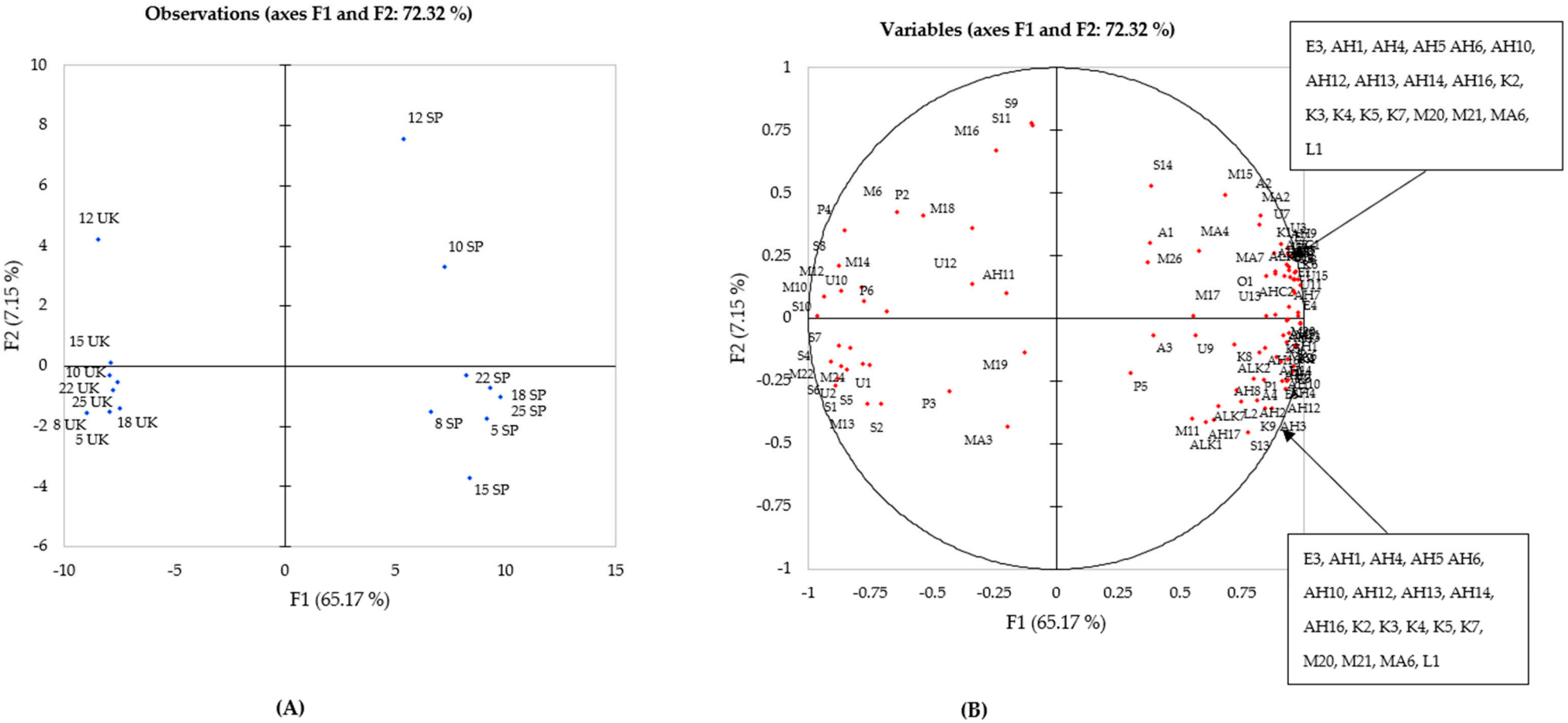


Figure 1. Principal component analysis of eight celery samples harvested in the UK in 2018 and Spain in 2019 showing correlations with volatile compounds. (**A**) Projection of the samples; (**B**) Distribution of variables.

Genotype expressed a significant influence over both the UK- and Spanish-grown celery (Table 1), yet a more noticeable separation was observed in the Spanish-grown celery between genotypes, in addition to a strong association with more aroma compounds than UK celery (Figure 1). Genotype expressed significant differences (Table 1), but genotypes 12, 22 and 25 for Spain were positioned in a similar place on the opposite quadrant in the observation plot. Genotype 12 in both locations took the appearance of an outlier, displayed as the most significantly different from other genotypes used within this experiment. This was caused by the high abundance of sesquiterpene compounds present in the UK harvest, especially from β-selinene, and the high phthalide content within the Spanish harvest, with 3-n-butylphthalide and sedanenolide comprising 8.5% and 9.2%, respectively, of the total volatile content. Significant compound associations with Spanish grown celery were expressed within Figure 1 including all aldehydes (except AH11) and ketones, accompanied by monoterpenes (M11, 15, 17, 20, 26), sesquiterpenes (S13, 14), phthalides (P1, 5) and alcohols (A1, 2, 3). This was further reflected in Table 1. Conversely, less noticeable separation between the eight celery genotypes was observed by celery grown in the UK, in addition to fewer compound associations. Monoterpenes (M6, 10, 12, 13, 14, 16, 18, 22, 24), sesquiterpenes (S1, 2, 4, 5, 6, 7, 8, 10, 12) and phthalides (P2, 3, 4, 6) were positively correlated with samples grown in the UK. The spread of monoterpenes, sesquiterpenes and phthalides across the plot, together with ubiquity within all celery genotypes regardless of location of growth, harvest year [10] and maturity [8], confirmed the importance of these compound groups to celery and celery aroma. This was originally concluded by the authors [10], where eight genotypes of celery grown in the UK in 2018 and 2020 both exhibited these compounds, and in a similar pattern. Aldehydes and ketones appeared to be more strongly influenced by geographical location rather than genotype, explaining why these compounds are not commonly reported within the celery volatile composition.

Genotype and geographical location both expressed a significant influence over the volatile content of celery (Table 1), however, geographical location expressed a stronger influence upon the composition (Figure 1). Differences within the growing climate and agronomy applied to the celery increased the risk of variation, as similarly expressed between harvest years [10], whereby differences in air temperatures were likely the cause for the large variation expressed between years 2018 and 2020, altering the sensory profile of the crop. The differences in composition observed between the eight celery genotypes grown in the UK and Spain (Figure 1) and the impact that these have upon the sensory characteristics were investigated through sensory profiling.

3.2. Sensory Evaluation of Fresh Celery Samples

The sensory profile of the eight celery samples was generated by a trained panel who came to the consensus of 22 and 23 terms for the quantitative assessment of samples grown in the UK in 2018 and samples grown in Spain in 2019, respectively. The additional attribute for the samples grown in Spain in 2019 was salty taste, and we hypothesised that this was because of the saline soils present in this part of the country, as observed in other studies such as tomato [43], pepper [44] and cauliflower [45]. Mean panel scores for these attributes are presented in Table 2. Out of the 22 attributes that were profiled from the UK harvest, 14 of these were found to be significantly different between the genotypes, and seven out of 23 attributes were significantly different for the Spanish trial in 2019. Few significant assessor x sample interactions were identified for both UK and Spanish harvests, suggesting that the panellists scored samples in a consistent manner [46]. Statistical comparison of sensory differences between locations could not be completed due to the one-year difference between harvests, however, general trends will be discussed.

Table 2. Mean panel scores for sensory attributes of the eight celery samples harvested in UK 2018 and Spain 2019.

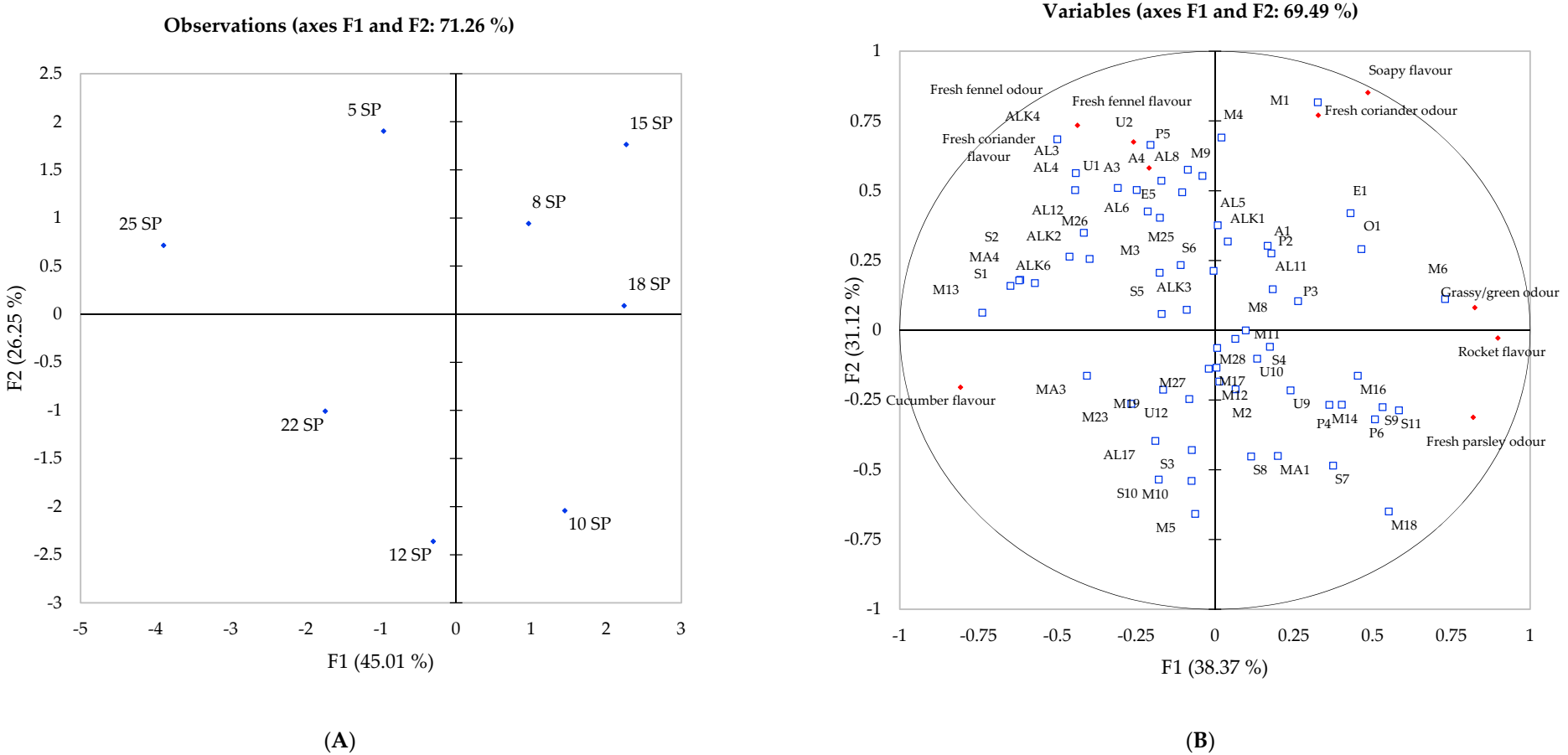
Attribute	Score [A]																	
	UK								P [B]	Spain								P [B]
	5	8	10	12	15	18	22	25		5	8	10	12	15	18	22	25	
Appearance																		
Colour	56.4 ^{b}	63.6 ^{ab}	62.6 ^{ab}	72.9 ^{a}	72.1 ^{a}	65.6 ^{ab}	70.5 ^{a}	26.8 ^{c}	***	45.6 ^{c}	51.2 ^{c}	50.0 ^{c}	69.9 ^{ab}	71.8 ^{a}	56.0 ^{bc}	71.6 ^{a}	26.7 ^{d}	***
Stalk thickness	49.8 ^{ab}	49.5 ^{ab}	55.8 ^{a}	20.9 ^{b}	58.7 ^{a}	62.5 ^{a}	61.3 ^{a}	55.0 ^{a}	***	42.4 ^{ab}	46.8 ^{ab}	38.2 ^{bc}	27.3 ^{c}	55.5 ^{a}	55.9 ^{a}	58.4 ^{a}	54.4 ^{a}	***
Ribbed	46.6 ^{bc}	61.0 ^{ab}	61.7 ^{a}	65.9 ^{a}	35.5 ^{cd}	25.4 ^{d}	34.2 ^{cd}	37.4 ^{cd}	***	66.7 ^{a}	64.0 ^{ab}	67.9 ^{a}	76.1 ^{a}	48.4 ^{c}	42.1 ^{c}	49.6 ^{bc}	49.5 ^{bc}	***
Odour																		
Fresh fennel	16.5	14.2	18.9	15.5	15.3	18.6	15.4	18.2	ns	19.5	18.4	16.8	15.4	24.8	19.9	15.8	13.7	ns
Grassy/green	32.6 ^{a}	31.0 ^{ab}	32.1 ^{ab}	36.3 ^{a}	30.7 ^{ab}	28.3 ^{ab}	35.3 ^{a}	21.1 ^{b}	***	11.6 ^{b}	19.4 ^{ab}	24.3 ^{a}	25.6 ^{a}	23.5 ^{a}	20.1 ^{ab}	23.2 ^{a}	19.2 ^{ab}	**
Fresh parsley	14.1	19.7	19.0	19.1	20.6	16.7	16.7	10.8	ns	11.5	15.5	16.8	16.1	18.5	16.6	14.1	11.4	ns
Fresh coriander	12.8	12.1	14.2	11.7	14.2	17.5	15.4	11.1	ns	17.9	18.9	21.5	15.1	22.8	22.7	17.7	14.3	ns
Taste/flavour																		
Bitter	23.1 ^{abc}	24.0 ^{abc}	24.7 ^{abc}	35.9 ^{a}	28.2 ^{abc}	31.3 ^{ab}	24.4 ^{abc}	15.5 ^{c}	ns	24.4 ^{ab}	30.9 ^{ab}	29.4 ^{ab}	30.9 ^{ab}	28.4 ^{ab}	36.4 ^{a}	26.1 ^{ab}	18.1 ^{b}	**
Salt	nd	nd	nd	nd	nd	nd	nd	nd	**	26.4	22.6	27.3	31.3	23.4	31.2	24.8	18.7	ns
Sweet	15.2 ^{bcd}	20.3 ^{ab}	21.6 ^{ab}	10.6 ^{d}	15.6 ^{bcd}	12.2 ^{cd}	20.0 ^{ab}	24.6 ^{a}	***	18.3	19.8	21.4	18.2	20.0	14.5	16.1	22.8	ns
Fresh fennel	11.9	10.3	12.6	11.0	7.7	13.6	11.6	11.3	ns	15.0	15.7	10.4	13.2	17.4	13.6	8.0	10.8	ns
Rocket	11.3 ^{bc}	13.4 ^{bc}	12.4 ^{bc}	23.8 ^{a}	16.6 ^{abc}	16.9 ^{abc}	10.4 ^{bc}	7.7 ^{c}	***	1.8	2.0	3.2	1.8	1.4	1.0	0.8	0.2	ns
Fresh coriander	17.5	16.3	16.0	9.6	15.0	18.1	18.9	14.1	ns	17.2	21.0	18.1	17.4	18.0	21.4	15.7	13.8	ns
Soapy	18.2 ^{ab}	12.4 ^{b}	16.4 ^{ab}	18.4 ^{ab}	15.4 ^{ab}	23.7 ^{a}	16.3 ^{ab}	13.0 ^{ab}	*	19.1	20.5	25.1	22.0	20.0	27.5	19.7	15.0	ns
Cucumber	25.7 ^{ab}	33.2 ^{ab}	30.4 ^{ab}	9.1 ^{c}	30.0 ^{ab}	22.4 ^{b}	27.9 ^{ab}	37.7 ^{a}	***	12.8	14.1	9.9	5.8	15.3	11.8	11.8	14.8	ns
Mouthfeel																		
Crunchy	65.4 ^{abc}	62.6 ^{bc}	64.9 ^{abc}	56.7 ^{c}	70.2 ^{ab}	66.4 ^{abc}	73.7 ^{a}	62.5 ^{bc}	***	64.0	67.4	67.8	61.9	70.5	66.2	70.3	65.5	ns
Stringy	40.8 ^{b}	46.6 ^{b}	40.1 ^{b}	64.1 ^{a}	33.2 ^{b}	40.6 ^{b}	35.1 ^{b}	35.2 ^{b}	***	60.2 ^{ab}	58.2 ^{ab}	59.9 ^{ab}	71.9 ^{a}	47.2 ^{bc}	57.3 ^{abc}	38.5 ^{c}	52.4 ^{abc}	***
Moist	50.6 ^{a}	47.2 ^{a}	50.0 ^{a}	29.7 ^{b}	53.1 ^{a}	44.3 ^{a}	51.4 ^{a}	54.8 ^{a}	***	49.9	55.8	45.1	35.5	58.6	47.8	52.1	56.2	ns
Firmness of first bite	63.7	59.9	63.3	59.2	68.9	65.7	67.6	58.6	ns	64.8	66.1	65.6	63.5	67.2	63.2	69.9	63.2	ns
Aftereffects																		
Numbness	13.1	8.6	13.8	11.5	10.0	14.0	9.8	9.0		17.0	19.3	20.9	16.4	21.1	23.1	16.0	11.4	ns
Bitter	17.4 ^{bc}	18.4 ^{bc}	18.3 ^{bc}	29.0 ^{a}	19.1 ^{bc}	25.7 ^{ab}	16.0 ^{bc}	12.0 ^{c}	***	16.7 ^{ab}	19.4 ^{ab}	24.3 ^{a}	21.8 ^{ab}	19.2 ^{ab}	25.0 ^{a}	17.2 ^{ab}	12.0 ^{b}	*
Soapy	16.9 ^{ab}	15.7 ^{ab}	16.7 ^{ab}	21.2 ^{ab}	19.9 ^{ab}	24.8 ^{a}	18.6 ^{ab}	12.9 ^{b}	*	18.3	21.5	22.7	20.8	21.7	25.5	18.8	11.7	ns
Grassy/green	27.7	27.0	30.3	27.6	28.4	26.4	31.4	19.0	ns	12.3	13.3	15.8	19.9	15.8	14.3	15.7	13.6	ns

[A] Means are from two replicate samples; differing small letters (a, b, c, d, e, f) represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$); nd, not detected. [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

Appearance attributes for both locations displayed significant differences caused by genotype, and similarities were observed between scoring for stalk thickness and colour attributes. A significant difference ($p < 0.001$) for ribbed appearance was apparent between locations for all genotypes. The genotype variation between ribbed appearance was more apparent for those harvested in the UK than those harvested in Spain, with scores ranging from 25.4 to 65.9. Mouthfeel attributes displayed a positive correlation with appearance attributes, and these attributes were the highest scoring attributes in all genotypes across both locations, apart from stringiness. Stringiness was scored higher in Spanish celery, with all genotypes of the Spanish celery recording an increase of at least 10, apart from genotype 22. Genotype 22 was scored significantly lower for stringiness when comparing other genotypes in both locations. Although not significantly different, grassy after-effect was scored higher within UK celery and exhibited a positive correlation with grassy odour, an attribute that was significantly different in both locations.Significant differences in the odour and flavour attributes evaluated in both genotypes and geographical location were observed but, more significantly, different attributes were identified in UK celery. The cucumber and rocket flavour with grass odour attributes were scored higher in the UK harvest, whereas Spanish-grown celery scored higher for fresh coriander odour, fennel and soapy flavour. The fresh coriander flavour attribute was scored alike for both locations, however genotype 12 displayed a higher score in coriander flavour when grown in Spain, going from a score of 9.6 to 17.4. Furthermore, genotype 12 was scored as most bitter with genotype 8 and 18 for both locations, but scored sweeter when grown in Spain. Genotype 18 was scored with the strongest soapy flavour, which expressed a positive correlation with fresh fennel. Where genotype 12 scored high for flavour/odour attributes (apart from cucumber), genotype 25 scored low for flavour/odour attributes, only scoring high in the cucumber flavour attribute in both locations.

Principal Component Analysis of Flavour Attributes and Volatile Compounds

PCA was used to visualise the sensory and chemical differences observed across the eight genotypes, with the volatile compounds identified (Table 1) and the sensory attributes related to odour and flavour used as variables (Figures 2 and 3). Celery grown in the UK expressed a large variation between the eight genotypes (Figure 2), whereby principal component one (F1) and two (F2) explained 69.49% of the total variation within the data. The first axis separated genotypes 5, 10, 18 and 22 from other genotypes, whereas the second axis separated genotypes 10, 12, 15 and 18. Genotype 25 was scored the lowest for all flavour attributes, only scoring high in cucumber flavour (Table 2), whereas genotype 12 opposed genotype 25 (Figure 2) and displayed strong association with a fresh parsley and grass odour along with a rocket flavour. Genotype 18 was positively correlated with fresh fennel and coriander flavour, with the soapy characteristics that accompany many members of the Apiaceae family [47]. A grouping of aroma compounds in the centre of the PCA was observed, whereas the sensory characteristics remained positioned on the outer rim of the biplot, with genotypes 5 and 22 grouped in the middle of the observation plot accompanied with no strong associations with any flavour/odour attribute (Figure 2). These genotypes exhibited a lower volatile content to genotype 12 (Table 1). Predominantly, monoterpenes and sesquiterpenes were negatively correlated with the first principal component (F1), and compounds belonging to compound classes such as alcohols and aldehydes were positively associated with F1. Phthalides were distributed around the plot, with (*Z*)-neocnidilide (P5) displaying positive association to fresh fennel, whereas sedanenolide and (*E*)-ligustilide (P4 and P6) express a positive correlation with fresh parsley.



(**A**) (**B**)

Figure 2. Principal component analysis of eight celery samples harvested in UK 2018 showing correlations with volatile compounds and sensory attributes. (**A**) Projection of the samples; (**B**) Distribution of variables.

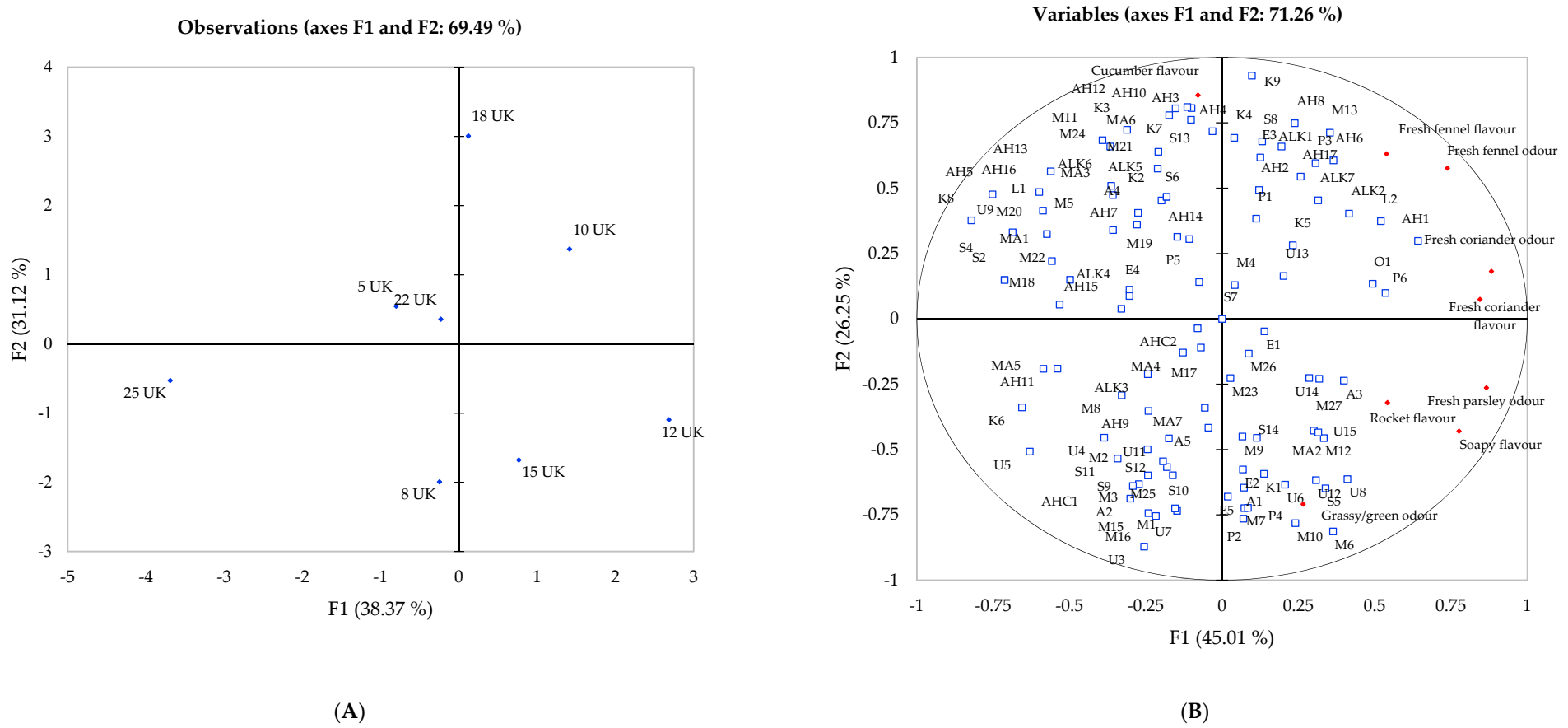


Figure 3. Principal component analysis of eight celery samples harvested in Spain 2019 showing correlations with volatile compounds and sensory attributes. (**A**) Projection of the samples; (**B**) Distribution of variables.

Principal component one (F1) and two (F2) explained 71.26% of total variation observed within the dataset for the samples grown in Spain, and the first axis separated genotypes 10, 12 and 22, whereas genotypes 5, 12, 22 and 25 are separated along the second axis. Genotype 25 in Spain exhibited a low association to all attributes apart from cucumber flavour, observed in UK 25, and genotype 12 in Spain expressed a significant association to grass odour, as observed in the UK. Furthermore, genotype 18 displayed a positive association with fresh coriander and fennel odour and flavour attributes when grown in Spain and the UK. The perception of genotypes 5, 8, 10, 15 and 22 was observed to change significantly between locations, caused by the chemical compositional changes.

The flavour attribute of cucumber displayed no significant correlations in UK compounds (Figure 2), yet significant correlations between compounds and this attribute were observed with multiple aldehydes (AH3, AH5, AH10, AH12 and AH13) that express odour characteristics such as fatty, cucumber and green (Figure 3). These compounds were not identified in the UK harvest. Compounds identified in UK celery (Figure 2) all displayed association with a flavour/odour attribute of sorts; however, this was not reflected within Spanish-grown celery. Plotto et al. [48] calculated the retronasal and orthonasal activity values for selected terpenes and aldehydes in an orange juice matrix, identifying limonene, β-pinene and γ-terpinene to have the highest thresholds in water and orange juice, whereas hexanal, octanal and nonanal, all aldehydes identified in celery (Table 1), expressed a much lower threshold. Due to the lower proportions of monoterpenes identified in Spanish-grown celery, the flavour characteristics contributed by these aldehydes (green, waxy, cucumber, honey [8]), allowed the panel to detect these more easily. This explains the differences observed in the sensory panel between the celery grown in the UK and in Spain. Furthermore, observed on the factor plot in the bottom left quadrant (Figure 3), a large group of compounds displayed no significant associations with any sensory attribute.

Celery harvested in Spain expressed a different aroma profile when compared to samples harvested in the UK, as observed in the significant difference of the aroma composition (Table 1), and although we cannot compare statistically UK and Spanish genotypes, differences in the scoring of attributes were observed. Genotypes 5, 8 and 15 displayed no association with herbal odour and flavour attributes in the UK (Figure 2) but were scored higher after growing in Spain, where strong associations to fresh fennel, coriander and parsley were displayed (Figure 3). Genotype 12 expressed close association with grass and fresh parsley odours, in addition to sedanenolide and 3-n-butylphthalide, compounds known for their celery odours, and displayed significant positive correlations with grass and parsley odour. On the other hand, genotype 25 expressed the lowest relative content of volatile compounds identified, apart from aldehyde compounds, and was scored with a significantly higher cucumber flavour than any other genotype in both locations. Here, we can assume this genotype does not exhibit a strong characteristic odour in comparison to genotype 12. As both these genotypes performed in a similar manner across locations, we would recommend these genotypes to breeders and fresh produce growers who plan to use the same cultivar across different locations, as they have expressed stability in volatile composition.

3.3. Environmental Differences between Geographical Location and Influence on the Aroma Profile

In this study, differences in the volatile composition and sensory profile were observed between eight genotypes and two geographical locations. Previously, Turner et al. [10] used the same genotypes grown in different years in the UK and identified that differences in temperatures (air and soil) played an important role in determining the overall flavour of celery. Environmental data including temperature, rainfall and relative humidity were collected at the nearest weather station to the farm of growth and provided by G's Fresh UK and Grupo G's España (Table 3) to compare the differences in the climate of geographical location. These environmental and geographical differences and how they influence the chemical composition of celery are only hypothesized due to the inadequate study of different growing conditions on celery. However, abiotic stresses from factors including

temperature, humidity, water and mineral availability have been commonly observed in literature to influence secondary metabolic profiles in plants [49–51].

Table 3. Environmental data recorded at the nearest weather station to the farm of growth and provided by G's Fresh (UK) and Grupo G's España.

	Ely, Cambridgeshire (UK)					Aguilas, Mercia (Spain)				
Weeks after Transplant	**Air Temp (°C)**	**Rainfall (mm)**	**Relative Humidity (%)**	**Wind Speed (m/s)**	**Dew Point (°C)**	**Air Temp (°C)**	**Rainfall (mm)**	**Relative Humidity (%)**	**Wind Speed (m/s)**	**Dew Point (°C)**
1	17.0	0.0	73.0	2.4	15.4	15.3	0.0	79.6	0.8	1.9
2	14.7	0.0	81.3	1.5	18.7	15.4	0.1	76.3	1.1	3.9
3	16.4	0.1	66.1	1.3	20.0	19.9	0.0	72.8	2.4	4.1
4	17.0	0.0	94.8	1.6	18.4	17.4	0.1	63.7	2.9	1.1
5	18.9	0.0	98.5	1.5	20.4	16.9	0.0	82.1	1.0	6.9
6	19.8	0.0	99.7	3.0	16.3	16.4	0.0	81.2	1.9	6.1
7	18.2	0.0	99.4	1.4	6.5	16.6	0.0	82.5	1.2	6.3
8	20.4	0.0	99.0	1.9	16.3	18.5	0.0	84.7	0.8	8.2
9	21.4	0.1	70.5	2.1	18.2	18.9	0.0	78.3	1.3	6.9
10	20.9	0.0	71.8	2.6	13.9	19.8	0.0	79.4	1.4	7.2
11	17.3	0.2	99.9	1.0	12.4	17.9	0.3	71.1	2.2	5.1
12	18.4	0.0	98.6	2.3	12.9	16.9	1.8	78.3	2.1	8.0
13	15.8	0.0	93.9	2.0	12.4	19.0	0.6	74.3	2.4	6.6
Average	18.2	0.0	88.1	1.9	15.5	17.6	0.4	77.3	1.7	6.0

Utilising two seasons for growing and using the same eight genotypes, Turner et al. [10] identified that warmer temperatures had a positive correlation with sesquiterpene and phthalide generation, whereas growing in lower temperatures led to celery with a higher monoterpene content. As similarly discussed by the authors [10], data from two harvests are insufficient when stating any relationships between environment and volatile composition, however, collating the data collected in this investigation, the dataset is completed with eight genotypes in a multi-site and multi-year experiment. Similarities in the chemical profile were observed in genotypes 12, 18, 22 and 25 in how they reacted to being grown in an alternative environment, suggesting that genotype predetermines the protective or coping mechanisms for the crop when exposed to abiotic and biotic stresses.

Celery grown in 2018 in the UK was subjected to temperatures much warmer than considered normal for the UK, and the environmental values do not express any significant differences between geographical location (Table 3) apart from the dew point; UK grown celery was grown in an environment where the average dew point value was 15.5 °C, substantially higher when compared to the 5.7 °C experienced by Spanish-grown celery. The observed dew point temperature indicates the temperature required for the air to cool to reach a relative humidity of 100%. The average daily temperature of UK grown celery is 18.2 °C and much closer to the dew point value, confirming the increased humidity experienced by UK grown celery. Exposure to high dew points promotes the growth of pathogens, inhibiting crop growth and, subsequently, compromising the crop to biotic stresses [52]. Specific stresses such as those caused by a pathogen will cause the crop to prepare a stress response and, additionally, increase the rate of plant-to-plant signalling as a form of communication, perhaps explaining the increased content of monoterpene compounds observed by the UK grown crop (Table 1). Sampaio, Edrada-Ebel and Da Costa [53] studied the influence of environmental factors on the secondary metabolic profile of *Tithonia diversifolia*, observing a variation within the metabolic profile in the leaves and stems, expressing a stronger association with rainfall and humidity levels than with temperature and solar radiation. The primary metabolite content of *Tithonia diversifolia* expressed a strong positive correlation with relative humidity, whereas secondary metabolite content expressed a strong negative correlation with humidity. A similar reaction was observed in the present study, whereby more secondary metabolites in the form of volatile compounds were identified in Spanish grown celery, where relative humidity was lower (Table 3).

Due to minimal differences in the climate data, investigating differences in agriculture, including water and soil composition, must be included in the discussion, as these factors will also influence the flavour outcome. As a consequence of the arid and semi-arid conditions of Aguilas, Spain and the increasing shortage of water for crop irrigation, desalinated seawater is often used in southern regions of Spain [54]. Conversely, the crop irrigation system in place within the UK is by fresh water from a nearby reservoir, supplied by the river Little Ouse, in this instance. Although rigorous pre-treatment processing and filtration steps would have been completed upon both water supplies, the mineral composition of water will be vastly diverse due to differences in the original source. This will lead to variances in the soil for uptake in minerals such as calcium, sodium, magnesium, zinc and iron.

Growing in different geographical locations involves growing on different soil types. This will lead to differences in the soil properties including water holding capacity and mineral composition. UK celery was grown on loamy and sandy soils with naturally high groundwater, allowing for high water availability and nutrient uptake, whereas the Calcisol soils of Spain are known for their accumulation of calcium carbonate from precipitation brought about by evaporation under arid and semi-arid conditions [55]. The presence of surplus calcium carbonate in the soil could ultimately cause a stress response by the crop. To promote healthy growth, the crop must uptake soil, waterborne micronutrients and inorganic elements which are necessary for functional growth and involved in an array of essential pathways, including the synthesis of secondary metabolites such as isoprenoid through the non-mevalonate pathway, i.e., the building block for monoterpenes and sesquiterpenes. Primarily, carbon-, nitrogen-, sulphur- and phosphorous-fixation is involved in the synthesis of substrates and precursors involved in primary and secondary metabolism [56]. The micronutrient and element content of the soil and its permeability will influence the uptake of water and minerals from the soil to be utilised within the crop. These micronutrients can be applied by the plant for a range of uses; for example, copper has been identified to improve the flavour of fruits and vegetables along with increasing sugar and lignin content, zinc promotes the transformation and consumption of carbohydrates in plants and iron is a prominent micronutrient involved in the synthesis of organic acids [57,58]. Applying fertilisers (organic or inorganic) will increase the soil micronutrient content leading to the desired elements being available for crop uptake. Calcium and boron deficiencies, known causes of black heart and hollow stem in celery, are both nutrient-deficient illnesses that can be avoided through the application of appropriate sprays and fertiliser [59]. However, van Wassenhove, Dirinck, Schamp and Vulsteke [12] identified the negative impact of using nitrogen-based fertilizer on celery and its volatile composition. Contrary to what has been discussed above, an increased application of a nitrogen fertilizer (organic and/or mineral nitrogen) led to a reduction in the aroma-determining compounds in two celery cultivars. In fact, applying no fertilizer resulted in a higher content of volatile compounds including phthalides, whereas an overall decrease was observed between 1000 and 2000 μg kg^{-1} of fresh material when a nitrogen fertilizer was applied. D'Antuono, Neri and Moretti [60], similarly, observed a decrease in volatile content as nitrogen fertilizer volume was increased, especially in compounds such as limonene, myrcene and β-selinene. However, total phthalide content along with β-caryophyllene and α-selinene were identified in high proportions when 300 kg ha^{-1} of nitrogen was used on celery. It is possible that Spanish grown celery was exposed to higher levels of nitrogen, thus leading to a lower proportion of monoterpenes, sesquiterpenes and phthalides within the aroma composition.

Factors that accompany field placement will be a less significant cause of variation, but when these factors are combined, they will play a more significant role in determining the secondary metabolite content in celery. Possibly the most obvious difference between geographical location would be the altitude of each field: UK celery was grown on an east-facing field that was −1 to 1 m above sea level, whereas the field in Aguilas was south-facing at 390 m above sea level. Higher altitudes will result in lower temperatures

and limitations on light exposure [61]. Cui et al. [61] investigated the physiological changes of *Leymus secalinus* and the effect of altitude, observing an increase in soluble sugars as elevation increased but a decrease in chlorophyll *a* and *b*, leading to a decrease in the crop's ability to absorb light. Both these reactions were noted as defence mechanisms and adaption strategies to the change in environment. It is possible that these environmental differences led the Spanish celery to synthesise ketones and aldehydes in response to these abiotic stresses. The solar radiation would be significantly higher in the UK-grown celery due to the lower altitude along with growing in the summer months. This will increase the duration of light exposed to the crop and, thus, increase the rate of photosynthesis. Although not discussed in celery, higher exposure to UV-B in tree foliage led to an increase in flavonoids as a protective mechanism [62], and it is possible that a similar mechanism occurred in UK celery but for monoterpene production.

Synthesising aromatic compounds is a typical response from the crop to abiotic and biotic stresses for protection and adaption to the growing environment, and it is clear the celery grown in the UK reacted differently to the celery grown in Spain. Turner et al. [10] previously suggested that increased sesquiterpene and phthalide content was due to temperature stress, yet similar temperatures and other climate conditions were experienced by the Spanish crop, leading to variation in the chemical composition. Differences in soil, water and fertilizer composition used upon the UK- and Spanish-grown celery caused a change in the availability of minerals and elements to be used for primary and secondary metabolite production and, along with the placement of the field which altered the duration of light, caused a change in the crop's defence mechanism and adaption strategy.

4. Conclusions

Geographical location displayed a strong influence over the aroma composition of eight celery genotypes, and the influence expressed by genotype remained significant. Changes in composition caused by these factors led to differences in the aroma profile and, hence, sensory differences between genotypes and celery grown in different geographical locations were identified. Completing volatile analysis and sensory evaluation of the eight genotypes of celery demonstrated that celery genotypes grown and harvested in the UK were perceived with a strong green aroma and cucumber flavour compared to the celery grown and harvested in Spain. A wider range of compound families were identified within Spanish celery samples, imparting a significantly different aroma profile, which was perceived to be more closely associated with fresh fennel and coriander flavour. Identifying more compounds, including aldehydes and ketones in Spanish-grown celery, allowed for the explanation of the association to cucumber flavour.

Combining findings presented in this study and in the previous study completed by the authors [10], the genetic make-up of the crop regulates the synthesis of primary and secondary metabolites in response to abiotic and biotic stresses. Nonetheless, the environmental stresses experienced by the UK and Spanish crops were different and, thus, a different defence mechanism was required. This was reflected by the number of compounds expressing significant differences between genotypes and the variation caused by genotype in the UK crop, as well as the variation in perception between genotypes from sensory evaluation. The influence of geographical location on the aroma composition was also evident through the variation observed due to the location, in addition to most compounds also expressing significant differences caused by geographical location. The chemical composition was different in both locations, mostly caused by the aldehyde and ketone contents that were expressed in a significantly higher proportion of the volatile composition when sampling celery grown in Spain. A similar response was observed between harvest years, whereby significant compositional differences when the warmer temperatures of 2018 celery were observed, ultimately leading to an increased sesquiterpene and phthalide content in the eight genotypes when grown in a considerably warmer climate in response to stress.

All eight genotypes used within these studies were observed to be influenced by both genotype and external factors, including the environment (air temperatures, soil temperatures, relative humidity), geographical location (altitude and placement of field) and agronomic techniques (application of fertilisers, water availability and irrigation systems). Two genotypes (12 and 25) demonstrated consistency in their performance across harvest year and location; 12 remained a high "extreme", profiled with strong fresh coriander and fennel attribute notes, which was reflected through its abundance in strong aroma compounds. On the other hand, genotype 25 was presented as a low "extreme" and was only profiled with a cucumber flavour, expressing significant correlations with related compounds, predominantly, aldehydes and ketones. This consistency makes these lines strong candidates to drive breeding programmes aimed at developing celery with distinct flavour profiles that will appeal to different consumer groups.

With apparent differences in the aroma and sensory profile, identifying which harvest year, environment, geographical location and agronomy produced the most appealing celery is impossible to accomplish without carrying out consumer preference trials combined with sensory profiling. Combining the data collected from this study and experiences alike with consumer preference tests would aid in the identification of attributes that consumers find important in celery products, including preferences for sweet, bitter and flavour intensity. The findings from this study could be offered to celery breeders and fresh produce growers to guide celery production with aroma profile targets in mind. Furthermore, by educating breeders about the environment, including location, genotype and agronomy, a deeper understanding will be provided on the role these factors play in determining and influencing the aroma profile and, therefore, the sensory perception of celery. Combining all these considerations will lead to a higher quality and better tasting product. Additionally, selecting cultivars according to the growing environment rather than using the same cultivar across circumstances will allow for a more consistent product.

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Institutional Review Board Statement: Ethical review and approval were not necessary for this study as the study involved tasting fresh celery samples harvested under standard commercial practices by a trained sensory panel, the members of which are employees and have consented to taste and rate food as part of their job. Ethics approval and separate consent is only required from the trained panel where they are tasting non-standard, non-commercial or novel food ingredients. The trained panel work was within the ethical and professional practices set out by the IFST: https://lwww.ifst.org/membership/networksand-communities/special-interest-groups/sensory-science-group/ifst-guidelines (accessed on 4 November 2021).

Informed Consent Statement: Our trained employed sensory panel provided consent to taste and rate food as part of their job; they only provide separate written consent to a specific study where they are tasting non-standard, non-commercial or novel food ingredients.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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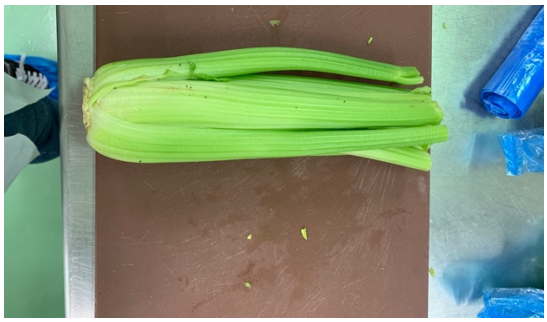
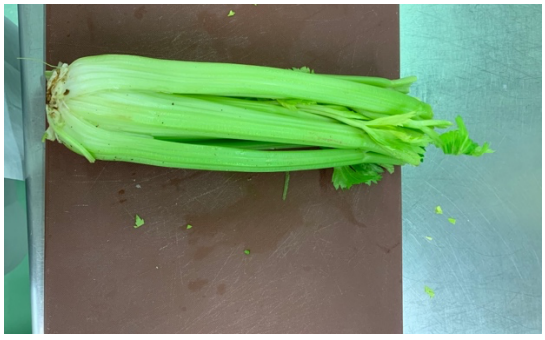
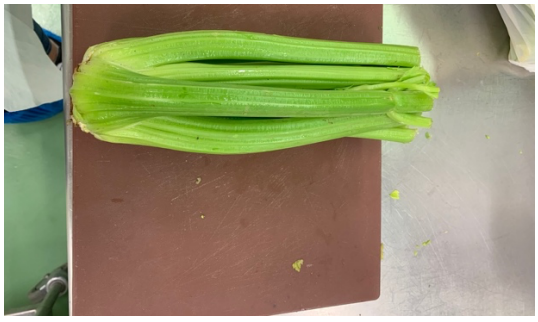
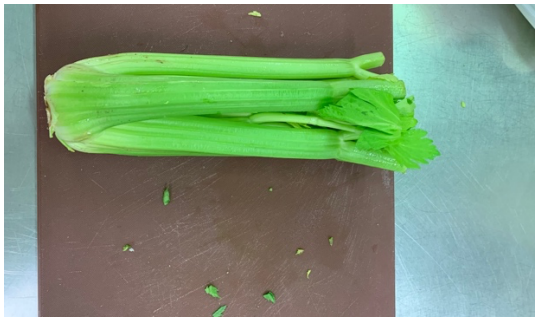
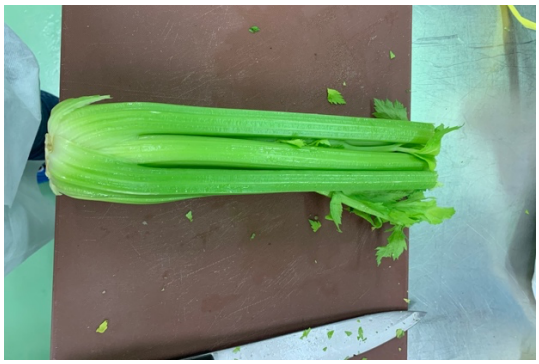
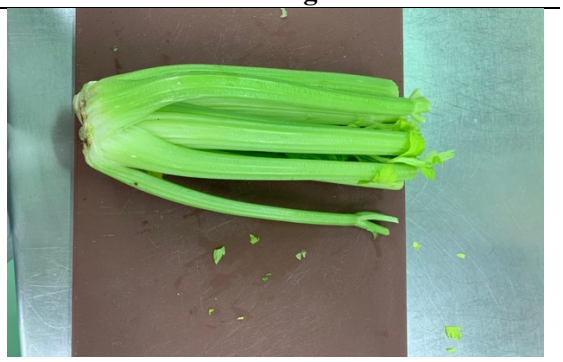
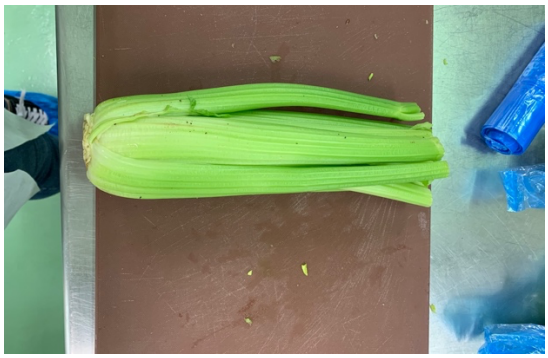
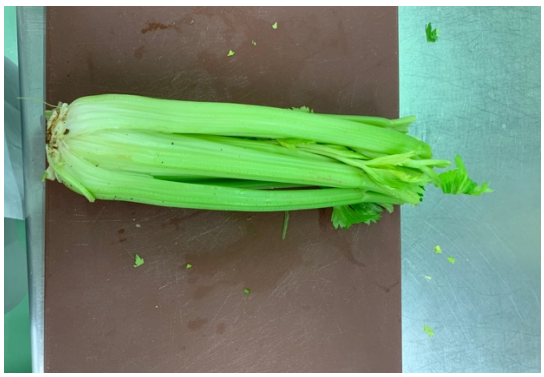
5815 Appendix IX - Origin and images of the eight celery samples used in this study and harvested in Ely,
5816 United Kingdom and Águilas, Spain.
5817

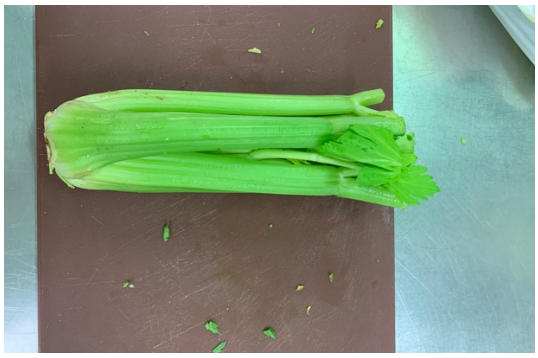
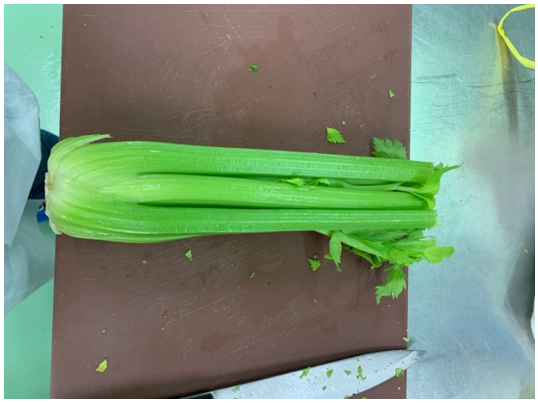
Line	Origin	Harvest UK	Harvest Spain
5	USA		
8	AUS		
10	UK		
12	UK		
15	USA		

18	EU		
22	USA		
25	EU		

5818

5819 Appendix X - Origin and images of the eight celery samples used in this study and harvested in
5820 Cartagena and Águilas, Spain.

Line	Origin	Harvest Cartagena	Harvest Águilas
5	USA		
8	AUS		
10	UK		
12	UK		

15	USA		
18	EU		
22	USA		
25	EU		

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Influence of harvest maturity on the aroma quality of two celery (*Apium graveolens*) genotypes

Lucy Turner [a], Dhriti Dawda [a], Carol Wagstaff [a], Frances Gawthrop [b], Stella Lignou [a,*]

[a] Department of Food and Nutritional Sciences, University of Reading, Harry Nursten Building, Pepper Lane, Whiteknights, Reading RG6 6DZ, UK
[b] A.L. Tozer Ltd, Pyports, Downside Bridge Road, Cobham KT11 3EH, UK

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ABSTRACT

Celery is a fibrous horticultural vegetable grown globally and widely consumed due to its health benefits, distinct flavours and culinary versatility. Currently, few datasets examine its aroma development across maturity which could help guide growers towards optimising harvest times whilst identifying potential consequences of harvesting outside commercial maturity. Freeze-dried celery of two genotypes, selected for biochemical and sensory differences, were harvested at three time-points and investigated using solid-phase microextraction gas chromatography/mass spectrometry (SPME GC/MS) and gas chromatography/olfactometry (GC/O). Both maturity and genotype showed significant ($P < 0.05$) interactions between compounds, and harvest stage exhibited greater impact upon aroma quality than plant genotype. Thus, indicating that agronomic practice is key in determining crop quality. Monoterpenes, sesquiterpenes and phthalides begun to decrease once commercial maturity was reached, whereas alcohols were more prominent in post-mature celery. GC/O results confirmed the importance of phthalides to mature celery aroma and aroma differences caused by genotype.

1. Introduction

Apium graveolens, is a popular biennial crop that is grown and consumed globally; in salads as a raw ingredient or in cooking, whereby it forms the base of many soups, stocks and sauces (Rozek, 2007). Celery has a distinct flavour profile that has been investigated extensively, with studies looking at the aroma profile of various cultivars in a variety of forms, such as fresh, dried or as an essential oil. Regardless of the material under investigation, a wide range of compounds that contribute to its strong flavour, including alcohols, aldehydes, monoterpenes, sesquiterpenes and phthalides have been identified (Gold & Wilson, 1963; van Wassenhove, Dirinck, Vulsteke & Schamp, 1990). The latter are seen as characteristic compounds. Phthalides are mainly found in members of the Apiaceae family, predominantly *Ligusticum* and *Angelica* (Karmakar, Pahari, & Mal, 2014). Phthalides including 3-*n*-butylphthalide, sedanenolide and *cis* and *trans*- ligustilide have been identified in celery, possessing odour descriptors such as "celery", "herbal" and "green" (Macleod & Ames, 1989; Kurobayashi, Kouno, Fujita, Morimitsu & Kubota, 2006)(Macleod & Ames, 1989; Kurobayashi, Kouno, Fujita, Morimitsu & Kubota, 2006).

Sellami, Bettaieb, Bourgou, Dahmani, Limam & Marzouk (2012) identified more than 25 volatile compounds in the roots, petioles and leaves of celery in the form of essential oil. Although more compounds were identified in the roots, the leaves exhibited a high concentration of aroma compounds, including phthalides. Similarly, Kurobayashi et al. (2006) utilised a combination of analytical techniques including gas chromatography / olfactometry (GC/O) to analyse the odorants that characterise the aroma in raw and boiled celery and identified a much higher proportion of phthalides in the leaves rather than the petioles. Using GC/O, Kurobayashi et al. (2006) stated that sedanenolide, 3-*n*-butylphthalide and *cis*- and *trans*-sedanolide were the most distinguishing components of the celery aroma and through aroma extract dilution analysis (AEDA) quantified these compounds (3,200, 140 and 78 μg/kg respectively) to be the most abundant odour active compounds in raw celery petioles. Through sensory analysis and GC/O, these compounds were found to contribute odour characteristics such as "fragrant", "green" and "spicy" to celery.

Being such a widely consumed horticultural crop, research into the development across maturity of these key odour active compounds with celery is unexpectedly low. Yommi, Di Gerónimo, Carrozzi, Quillehauquy, Goñi & Roura (2013) monitored the quality changes (structural and textural) of self-blanching celery every seven days from day 80 (after

* Corresponding author.
E-mail addresses: l.turner@pgr.reading.ac.uk (L. Turner), c.wagstaff@reading.ac.uk (C. Wagstaff), s.lignou@reading.ac.uk (S. Lignou).

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transplanting) until day 129. It was concluded that the optimum yield and quality balance of the cultivar was attained at 122 days after transplanting, noting that a later harvest was strongly associated with lower quality due to textural changes. Ultimately, there has been inadequate focus on the internal quality aspects of celery during maturity and a possibility for this could simply be the flavour attribute labelled as 'characteristic flavour' as used by Yommi et al. (2013). This is not an appropriate descriptor as the flavour profile is more complex than this. Furthermore, a more analytical method such as solid-phase microextraction (SPME) or solvent-assisted flavour extraction (SAFE) could be required to monitor changes in the volatile content across maturity.

While quality standards are usually based on visual evaluation (petiole shape, appearance, health) (Raffo, Sinesio, Moneta, Nardo, Peparaio & Paoletti, 2006), it can be argued that aroma and, therefore, flavour are attributes that should be considered when determining quality, as these also play an important role in consumer product acceptance. The purpose of this study was to investigate the development of aroma over maturity by utilising two different genotypes of *A. graveolens*, harvested at three different time points during plant development. The relationship between genotype and odour as well as maturity and odour were investigated using SPME and gas chromatography/mass spectrometry (GC/MS) and GC/O. From this, time points during maturation when key families of compounds were at their most abundant, such as monoterpenes that contribute fresh and citrus notes or phthalides that give the strong, characteristic herbal and celery odour could be recognised. Eventually, this could help guide the fresh produce industry to introduce more flavour variation for celery and other vegetable products.

2. Materials & methods

2.1. Celery material and minimum information about a plant aroma experiment (MIAPAE) standard[1]

2.1.1. Sample information

The two varieties used in this experiment were chosen due to their vast differences in physical and chemical attributes. Although commercial confidentiality precludes revealing the exact genetic identity of each line in this paper, the sensory properties of these genotypes can be revealed as these (along with others) were evaluated by the trained panel at the Sensory Science Centre (n = 12) (University of Reading, UK) using Quantitative Descriptive Analysis (QDA™). Prior to GC/MS and GC/O analysis, celery material was freeze-dried to ensure consistent aroma quality throughout instrumental analysis.

The first genotype, coded as line 12, has United Kingdom origins. Green and pink in colour with long, narrow petioles and ribs that appear compact and very prominent (Supplementary data, Figure S1). This genotype is characterised by a fibrous physiology, revealing strings of vascular tissue when a petiole is snapped, and bitter tasting.

The second genotype, coded as line 22, has North American origins with light green, compact petioles (Supplementary data, Figure S2). This genotype had a more typical celery appearance and is less bitter than the line above. It is not stringy, and the petiole breaks cleanly in half when snapped.

2.1.2. Timing, Location, and environment

Celery seed (*Apium graveolens*) of two parental lines supplied by Tozer Seeds Ltd (Pyports, United Kingdom) were grown in commercial conditions and harvested in Cambridgeshire, United Kingdom by G's Fresh Ltd (Barway, United Kingdom) (52°21′12.9″N 0°17′15.6″E) during spring/summer 2018. Celery was grown in a field with commercial celery products and treated to the same agronomic techniques and conditions as commercial celery.

Plants were transplanted after 26 days of growing in the nursery. The first harvest occurred on day 63 after transplanting, in late July 2018 (premature, M1), the second harvest occurred on day 76 after transplanting, in mid-August 2018 (mature, M2) and the final harvest occurred on day 89 after transplanting, in late August 2018 (post-mature, M3). Average climate conditions from day one of transplanting to day 89 after transplanting were as follows: air temperature was 18 °C, average soil temperature was 22 °C and average rainfall was 0.04 mm. 20 to 25 mm of overheard irrigation was used and standard commercial fertiliser, pest and disease control regimes were applied.

2.1.3. Raw material collection, processing and storage

Within the field, the celery was grown in three randomised blocks (10 plants m^{-2}) and were harvested using a celery knife. M1 celery were cut to 10 cm from the base, M2 and M3 were cut to 13 cm from the base, ensuring that no knuckles or leaves were included in the petiole cuttings. Three biological replicates were harvested from each block. Once cut, the petioles were sealed in labelled bags for immediate transportation to the University of Reading (United Kingdom). Celery for aroma analysis was frozen at −80 °C and freeze-dried for five days. Celery was then milled to a fine powder using a milling machine (Thomas Scientific, Swedesboro, NJ) and stored in an airtight container out of sunlight exposure at room temperature for a maximum of 2 weeks before instrumental analysis.

2.2. Chemical reagents

For GC/MS analysis, calcium chloride solution was prepared with HPLC-grade water and added to the sample with 100 ppm propyl propanoate in methanol, as the internal standard. For GC/O analysis, HPLC-grade water was used to rehydrate the samples and dry ice obtained from the University of Reading. The alkane standards C_6-C_{25} in diethyl ether was used for both GC/MS and GC/O analysis. All reagents were purchased from Merck (Poole, United Kingdom).

2.3. Solid-phase microextraction followed by GC/MS to identify changes in the aroma profile of different celery maturities and genotypes

Celery (0.5 g) was combined with 0.5 mL of saturated calcium chloride solution and filled to 5 mL using HPLC-grade water with 50 μL of 100 ppm propyl propanoate (internal standard) in a 15 mL SPME vial fitted with a screw cap. Analysis was carried out by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA) as described by Turner, Lignou, Gawthrop & Wagstaff (2021).

Volatiles were identified by comparing each mass spectrum with spectra from authentic compounds analysed in our laboratory (The Flavour Centre, University of Reading), or from the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). To confirm the identification, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a homologous series of C_6–C_{25} *n*-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions. The approximate quantification (AU) of volatiles collected from the headspace were calculated from GC peak areas, by comparison with the peak area of the propyl propanoate standard.

2.4. Odour analysis using GC/O to identify changes in the perception of aroma compounds as celery matures

Celery (0.5 g) and 4.5 mL of HPLC grade water was placed in a SPME vial of 15 mL fitted with a screw cap. After equilibration at 37 °C for 10 min, the SPME device (divinylbenzene/Carboxen™ on polydimethylsiloxane) was exposed to the headspace above the sample for 30 min. After extraction, the SPME device was inserted into the injection

[1] MIAPAE standards refer to Minimum Information About a Plant Aroma Experiment as described in Turner et al. (2021) Food Chemistry 345: 128673.

port of an Agilent 7890B Series ODO 2 (SGE) GC/O (Agilent, Santa Clara, CA) system equipped with a DB5 column (30 m × 0.25 mm × 0.25 μm). The outlet was split between a flame ionisation detector and a humified sniffing port (1:1). The fibre contents were desorbed for 2 min onto five small loops of the column in a coil, which were cooled in solid carbon dioxide, contained within a 250 mL beaker. The injector and detector temperatures were maintained at 280 °C and 250 °C respectively. The oven was held at 40 °C during desorption. After desorption, the solid carbon dioxide was removed from the oven. The temperature program used was: 40 °C for 2 min isothermal, an increase of 4 °C/min to 200 °C, and an increase at 8 °C/min to 300 °C. Helium was the carrier gas with a flow rate of 2.0 mL/min. A standard of C_6–C_{25} *n*-alkanes was used to collect linear retention index (LRI) values.

Three assessors were used for the detection and verbal description of the aroma compounds. All assessors were subjected to multiple training sessions with different materials on the GC/O prior to scoring using celery material, accounting to seven hours in training. Two assessors were already considered to be well trained on the GC/O. Further training, including odour identification using 12 flavour compounds, threshold and discrimination tests using Sniffin' Sticks (Burghardt®, Wedel, Germany) were also completed prior to assessment. Assessors smelt each sample in duplicate and documented the odour description, time and odour intensity (OI) using a seven-point scale (2–8) where 3 = weak, 5 = medium and 7 = strong. Each session lasted 40 min and assessors were advised to refrain from drinking coffee and eating at least 30 min before the scoring session.

2.5. Statistical analysis and data pre-treatment

Quantitative data from the SPME GC/MS analysis were analysed by both one- and two-way analysis of variance (ANOVA) and principal component analysis (PCA) following Spearman's correlation, using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($P < 0.05$) between harvest maturities and the celery parental lines. Only those compounds exhibiting significant differences between maturity, genotype and their interaction (maturity × genotype) were included in the principal component analysis plots.

3. Results and discussion

3.1. Biochemical profile is more influenced by maturity than genotype

In total, 94 compounds were determined in the headspace across two celery parental lines (Table 1) and 91 of these were identified. Ninety-three compounds were shown to be significantly influenced by plant maturity whereas 71 compounds by plant genotype. Identified compounds include 20 monoterpenes, 13 monoterpenoid alcohols, 11 sesquiterpenes, nine alcohols and nine aldehydes, six phthalides and a range of other compounds counting esters and ketones. Monoterpenes, followed by phthalides and sesquiterpenes, comprise the majority of the total volatiles collected from the headspace of the two genotypes and three maturities (Table 1) and are at their highest total volatile content at M1 for line 12 and M2 for line 22. Alcohols displayed an increase as the crop developed and became most abundant at M3; similar trend also observed for the aldehyde content in line 22. Sesquiterpenes and phthalides were at their highest total volatile content at M2.

GC/MS analysis identified groups of compounds that fluctuate throughout maturity and between genotype (Table 1). All compounds apart from *p*-cymen-8-ol, were influenced by maturity and fewer significantly influenced by genotype. Similar patterns can be observed between genotypes as the crop develops, but certain compounds prevent these patterns from occurring consistently between genotypes. For example, hexanal and propyl 3-methylbutanoate dramatically increased in line 22 at M2, causing the total aldehyde and ester content to increase accordingly.

Monoterpene content in line 12 was the highest at M1, with limonene, the most abundant compound, identified across both lines and maturities. Limonene's content decreased as celery developed. Most monoterpenes followed this pattern including γ-terpinene, m-cymene and β-pinene and is most noticeable in line 12. These compounds remained the most abundant monoterpenes in line 22, however, there is less of a noticeable change between M1 and M2. These compounds are known to have odour descriptors that include citrus, pine and sweet. Throughout literature, monoterpenes have been shown to be the most abundant compounds reported in various celery genotypes as shown previously by Turner, Lignou, Gawthrop & Wagstaff (2021). Orav, Kailas & Jegorova (2003) analysed the composition of Estonian grown celery essential oil and similarly, identified monoterpenes to comprise the majority of the flavour profile (85.3%). Likewise, MacLeod & Ames (1989) identified 18 monoterpenes, representing around 46% of the aroma profile of fresh supermarket bought celery and identified limonene as the major component in the celery isolate, similar to this study.

Additional monoterpenes such as p-mentha-1,5,8-triene and L-carvone in M2 and *(E)*-dihydrocarvone and *p*-cymene were identified in both genotypes as maturity developed whereas dehydrosabinene only appeared in line 22 at M3. These compounds could signal the deterioration of the crop through the development of the aroma from fresh and green, to woody and pine. Similarly, further monoterpenoid alcohols such as p-mentha-2,8-dien-1-ol, dihydrolinalool, terpinen-4-ol and (Z)-carveol were identified as maturity developed. Linalool, pinocarveol, thymol and carvacrol exhibited their highest abundance at M3. These compounds are responsible for floral, herbal, pine odours. For both genotypes, fenchol was the most abundant monoterpenoid alcohol with odour descriptors such as minty, medicinal and camphoreous. Compared to M1, fenchol's content at M3 was significantly lower. Monoterpenoid alcohols presented to be least influenced by genotype compared to other compound groups.

Sesquiterpenes, while fewer were identified and with lower relative abundances, contribute woody, herbal and floral notes to celery aroma. Maturity showed to have a significant influence for all sesquiterpenes. Lund, Wagner & Bryan (1974) and MacLeod & Ames (1989) both identified β-selinene to be an important compound to the celery aroma, although not a characteristic compound. β-Selinene and β-caryophyllene were identified as non-phthalide compounds with the highest concentrations in celery essential oil, however, β-selinene was characterised with a celery-like odour. Using odour evaluation, β-selinene was shown to have a threshold of 1 ppm which is low compared to 3-*n*-butylphthalide with an odour threshold of 10 ppm (Lund, Wagner & Bryan, 1974). Furthermore, Ehiabhi et al. (2006) reported both β-selinene and β-caryophyllene to be major constituents of Nigerian grown *A. graveolens* and were reported to make up as much as 16.3 and 10.5% respectively, of the aroma profile.

Findings in the present study are in agreement with Ehiabhi et al. (2006), β-selinene and β-caryophyllene expressed their highest relative abundance at M2 and decreased once commercial maturity reached (Table 1). A similar pattern was observed for other sesquiterpenes including α-selinene and α-copaene and monoterpenes in line 22. α-humulene was most abundant at M1 with curcumene and kessane only detected at M1. Kessane was also identified by Philippe, Suvarnalatha, Sankar & Suresh (2002) in the essential oil of Indian celery seed. During M3, the abundance of sesquiterpenes remained relatively low compared to monoterpenes and phthalides, however, (Z)-β-nerolidol was only identified at M3 for both genotypes. Kessane, curcumene and (Z)-β-nerolidol were all determined by Nurzyńska-Wierdak, Gruszecki and Kosior (2018) in varying amounts of celery essential oil of two varieties grown in Poland. These had been preserved through various drying techniques and harvested in July and October. Only the July harvest showed the presence of these compounds.

Phthalides exhibited a similar pattern to sesquiterpenes, showing

Table 1
Approximate quantities of volatile compounds identified in the headspace of celery using SPME GCMS harvested at three different maturity stages.

Code	Compound	LRI expt[a]	ID[b]	Mean relative abundance (AU) [f]						P-value [g]		
				Line 12			Line 22					
				M1[c]	M2[d]	M3[e]	M1	M2	M3	M[h]	L[i]	MxL[j]
	Alcohols											
A1	3-methyl-3-buten-1-ol	730	A	n.d. ^{C}	4.6±1.3 ^{A}	8.6±0.91 ^{A}	n.d. ^{C}	3.7±0.40 ^{B}	4.3±0.76 ^{B}	***	***	***
A2	1-pentanol	763	A	0.19±0.03 ^{E}	3.7±0.53 ^{BC}	2.5±0.24 ^{CD}	0.5±0.12 ^{E}	5.7±0.85 ^{AB}	7.9±1.7 ^{A}	***	***	***
A3	1-hepten-3-ol	893	A	n.d. ^{C}	n.d. ^{C}	1.7±0.10 ^{B}	n.d. ^{C}	n.d. ^{C}	5.2±0.45 ^{A}	***	***	***
A4	*(E)*-2-hexen-1-ol	867	A	0.37±0.02 ^{C}	n.d. ^{C}	4.5±0.50 ^{B}	0.68±0.12 ^{C}	n.d. ^{C}	8.1±0.88 ^{A}	***	***	***
A5	(*E*)-2-octen-1-ol	1069	A	n.d.	n.d.	1.8±1.8	n.d.	n.d.	1.7±1.2	***	ns	ns
A6	1-octanol	1073	A	1.5±0.30	n.d.	n.d.	1.8±0.27	n.d.	n.d.	***	*	ns
A7	1-nonanol	1176	A	6.0±1.7 ^{A}	4.1±0.59 ^{AB}	5.1±0.57 ^{AB}	2.1±0.57 ^{AB}	1.4±0.17 ^{B}	3.7±1.0 ^{AB}	***	***	**
A8	1-decanol	1272	A	n.d. ^{C}	2.9±0.64 ^{A}	n.d. ^{C}	n.d. ^{C}	1.6±0.39 ^{B}	n.d. ^{C}	***	*	*
A9	1-dodecanol	1469	A	1.1±0.16 ^{A}	n.d. ^{C}	0.63±0.16 ^{B}	0.65±0.10 ^{B}	n.d. ^{C}	0.83±0.18 ^{AB}	***	ns	**
	Total			**9.2**	**15.3**	**24.8**	**5.7**	**12.4**	**31.7**			
	Aldehydes											
AH1	*(E)*-2-pentenal	754	A	4.7±0.57 ^{C}	4.1±0.99 ^{C}	7.6±1.4 ^{BC}	6.5±2.4 ^{BC}	13.6±3.2 ^{A}	11.3±1.9 ^{AB}	*	***	*
AH2	hexanal	802	A	3.1±0.32 ^{B}	14.3±3.3 ^{B}	7.1±1.1 ^{B}	5.7 ±0.60 ^{B}	134±32.3 ^{A}	153±2.2 ^{A}	***	***	***
AH3	*(Z)*-2-hexenal	855	A	1.3±0.05 ^{B}	1.7±0.10 ^{BC}	n.d. ^{D}	0.39±0.07 ^{CD}	2.5±0.45 ^{A}	n.d. ^{D}	***	**	***
AH4	*(Z)*-4-heptenal	902	A	n.d.	4.1±0.61	n.d.	n.d.	3.7±0.91	n.d.	***	ns	ns
AH5	*n*-octanal	1007	A	8.9±0.47 ^{A}	5.1±1.1 ^{B}	4.9±0.96 ^{B}	4.0±0.72 ^{B}	5.6±1.2 ^{B}	4.3±0.54 ^{B}	*	**	***
AH6	benzeneacetaldehyde	1049	A	6.9±0.92 ^{BC}	4.4±0.57 ^{C}	4.5±0.25 ^{C}	15.8±2.4 ^{A}	8.4±1.9 ^{B}	3.8±0.33 ^{C}	***	***	***
AH7	2-hydroxybenzaldehyde	1056	A	n.d. ^{B}	n.d. ^{B}	4.8±0.05 ^{B}	n.d. ^{B}	n.d. ^{B}	34.6±6.3 ^{A}	***	***	***
AH8	*(E,Z)*-2,6-nonadienal	1156	A	2.1±0.38 ^{A}	n.d. ^{C}	n.d. ^{C}	1.0±0.23 ^{B}	n.d. ^{C}	n.d. ^{C}	***	***	***
AH9	*(E,E)*-2,4-nonadienal	1221	A	3.0±0.41 ^{A}	1.1±0.09 ^{C}	n.d. ^{D}	1.2±0.27 ^{BC}	0.44±0.28 ^{B}	n.d. ^{D}	***	**	*
	Total			**30**	**34.8**	**28.9**	**34.6**	**168.2**	**207**			
	Ketones											
K1	3-hexanone	779	A	n.d. ^{C}	n.d. ^{C}	1.3±0.12 ^{B}	n.d. ^{C}	n.d. ^{C}	2.1±0.45 ^{A}	***	***	**
K2	1-octen-3-one	978	A	n.d. ^{C}	n.d. ^{C}	6.7±1.3 ^{B}	n.d. ^{C}	n.d. ^{C}	4.7±1.0 ^{A}	***	ns	*
K3	2-nonanone	1090	A	2.4±0.14	n.d.	n.d.	1.6±0.51	n.d.	n.d.	***	ns	ns
	Total			**2.4**	**n.d.**	**28.6**	**1.6**	**n.d.**	**6.8**			
	Esters											
E1	methyl butanoate	720	A	n.d. ^{C}	0.53±0.05 ^{B}	n.d. ^{C}	n.d. ^{C}	2.3±0.09 ^{A}	n.d. ^{C}	***	***	***
E2	propyl 3-methylbutanoate	947	A	1.5±0.26 ^{C}	9.8±0.69 ^{C}	8.8±1.2 ^{C}	1.5±0.45 ^{C}	52.5±10.8 ^{A}	23.1±0.31 ^{B}	***	***	***
E3	bornyl acetate	1297	A	0.71±0.15 ^{B}	n.d. ^{B}	n.d. ^{B}	0.41±0.03 ^{B}	n.d. ^{B}	2.4±0.67 ^{A}	***	***	***
E4	(*E*)-pinocarvyl acetate	1304	A	8.3±1.1 ^{A}	n.d. ^{C}	7.9±0.95 ^{A}	4.8±1.2 ^{B}	n.d. ^{C}	7.3±1.7 ^{AB}	***	*	*
E5	carveol acetate	1339	A	8.7±0.54 ^{A}	n.d. ^{C}	10.5±0.47 ^{B}	4.2±1.1 ^{B}	n.d. ^{C}	5.2±1.5 ^{B}	***	***	***
E6	hexyl hexanoate	1385	A	0.36±0.07 ^{CD}	1.5±0.12 ^{B}	n.d. ^{D}	0.92±0.36 ^{BC}	2.6±0.69 ^{A}	n.d. ^{D}	***	**	*
E7	hexyl octanoate	1584	A	0.67±0.15	n.d.	n.d.	0.57±0.12	n.d.	n.d.	***	ns	ns
	Total			**20.2**	**11.8**	**27.2**	**12.4**	**57.4**	**38**			
	Monoterpenes											
M1	α-thujene	932	A	12.5±1.5 ^{A}	4.6±0.34 ^{B}	1.3±0.10 ^{D}	3.4±0.32 ^{BC}	4.3±0.54 ^{B}	1.6±0.36 ^{CD}	***	***	***
M2	α-pinene	939	A	15.8±3.7 ^{A}	8.8±0.86 ^{BC}	11.4±1.3 ^{AB}	5.9±0.60 ^{C}	6.7±1.4 ^{BC}	5.0±0.40 ^{C}	*	***	**
M3	camphene	958	A	3.7±0.64 ^{C}	4.9±1.3 ^{BC}	6.8±0.97 ^{AB}	2.2±0.40 ^{C}	8.0±1.7 ^{A}	7.8±0.76 ^{A}	***	ns	**
M4	dehydrosabinene	960	A	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	0.5±0.14 ^{A}	***	***	***
M5	sabinene	976	A	13.3±2.5 ^{A}	5.5±1.0 ^{B}	4.6±0.17 ^{B}	3.7±0.45 ^{B}	6.7±1.2 ^{B}	3.5± 0.73 ^{B}	***	***	***
M6	β-pinene	980	A	190±37.9 ^{A}	86.9±10.8 ^{B}	14.9±2.4 ^{C}	39.3±5.6 ^{C}	16.9±2.7 ^{C}	17.4±3.2 ^{C}	***	***	***
M7	myrcene	991	A	122±25.7 ^{A}	49.6±11.8 ^{B}	15.1±2.4 ^{C}	20.3± 5.7 ^{BC}	12.3± 2.8 ^{C}	6.9± 2.3 ^{C}	***	**	***
M8	α-terpinene	1018	A	7.2±1.9 ^{A}	4.8±1.0 ^{AB}	0.84±0.02 ^{C}	3.3±0.77 ^{BC}	3.9±0.43 ^{B}	2.5±0.29 ^{BC}	***	*	**
M9	m-cymene	1027	A	185±32.7 ^{A}	71.5±10.6 ^{B}	40.8±9.2 ^{B}	59.1±26.3 ^{B}	59.2±8.0 ^{B}	25.8±0.68 ^{B}	***	***	***
M10	limonene	1034	A	1068±207 ^{A}	598±41.8 ^{B}	264±61.8 ^{C}	581±93.7 ^{B}	605±88.8 ^{B}	264±7.4 ^{C}	***	**	**
M11	γ-terpinene	1063	A	256±34.4 ^{A}	112±20.3 ^{B}	21.7±2.5 ^{C}	63.7±34.6 ^{BC}	54.0±12.9 ^{BC}	42.3±12.8 ^{C}	***	***	***
M12	terpinolene	1093	A	9.6±0.15 ^{B}	8.0±0.89 ^{BC}	15.1±2.0 ^{A}	4.4±0.74 ^{D}	7.3±1.0 ^{BCD}	6.4±1.0 ^{CD}	***	***	***
M13	p-cymene	1099	A	n.d. ^{C}	n.d. ^{C}	3.7±0.35 ^{A}	n.d. ^{C}	n.d. ^{C}	2.9±0.27 ^{B}	***	**	**
M14	β-thujone	1119	A	1.6±0.50	4.2±0.82	0.96±0.20	0.77±0.18	3.0±0.45	0.86±0.13	***	**	ns
M15	*p*-mentha-1,5,8-triene	1113	A	n.d. ^{C}	1.3±0.26 ^{B}	1.9±0.35 ^{A}	n.d. ^{C}	1.4±0.16 ^{B}	1.4±0.05 ^{B}	***	ns	*
M16	citronellal	1159	A	25.4±4.2 ^{A}	9.3±2.4 ^{B}	2.8±0.12 ^{C}	4.2±0.83 ^{BC}	6.5±1.4 ^{BC}	1.2±0.06 ^{C}	***	***	***
M17	(*E*)-dihydrocarvone	1195	A	n.d.	n.d.	2.9±0.64	n.d.	n.d.	2.8±0.18	***	ns	ns
M18	β-cyclocitral	1232	A	1.2±0.27	1.9±0.42	1.8±0.10	0.88±0.28	1.9±0.21	1.1±0.15	***	*	ns
M19	carvone	1246	A	9.2±1.7 ^{B}	18.1±3.3 ^{A}	2.1±0.41 ^{C}	7.0±1.5 ^{BC}	10.2±1.7 ^{B}	4.1±1.2 ^{C}	***	*	*
M20	L-carvone	1257	A	n.d. ^{C}	3.6±0.74 ^{B}	4.9±0.93 ^{B}	n.d. ^{C}	4.4±0.80 ^{B}	7.1±0.84 ^{A}	***	**	**
	Total			**1921**	**993**	**418**	**799**	**812**	**405**			
	Monoterpenoid alcohols											
MA1	linalool	1103	A	1.3±0.23 ^{CD}	1.6±0.34 ^{CD}	1.7±0.36 ^{C}	0.84±0.13 ^{D}	3.7±0.35 ^{A}	2.8±0.19 ^{B}	***	***	***
MA2	*p*-mentha-2,8-dien-1-ol	1122	A	n.d.	1.2±0.15	0.8±0.15	n.d.	1.1±0.20	1.1±0.29	***	ns	ns
MA3	fenchol	1127	A	16.9±1.5 ^{A}	5.6±1.0 ^{B}	1.8±0.27 ^{B}	22.5±5.5 ^{A}	1.9±0.27 ^{B}	3.9±0.86 ^{B}	***	ns	*
MA4	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	1129	A	6.8±1.6^{AB}	9.7±1.9 ^{AB}	1.8±0.35 ^{B}	7.5±1.6 ^{A}	9.3±1.1 ^{B}	1.7±0.13 ^{B}	***	ns	ns
MA5	dihydrolinalool	1136	A	n.d. ^{B}	n.d. ^{B}	6.3±1.0 ^{AB}	n.d. ^{B}	n.d. ^{B}	5.0±1.7 ^{A}	***	ns	ns
MA6	pinocarveol	1152	A	3.1±0.68 ^{B}	4.0±0.84 ^{AB}	4.2±0.22 ^{AB}	1.2±0.35 ^{C}	1.1±0.05 ^{C}	5.4±0.43 ^{A}	***	***	***
MA7	terpinen-4-ol	1184	B^{A}	n.d. ^{C}	1.7±0.30 ^{B}	2.9±0.68 ^{A}	n.d. ^{C}	n.d. ^{C}	2.7±0.61 ^{AB}	***	***	**

(*continued on next page*)

Table 1 (*continued*)

Code	Compound	LRI expt[a]	ID[b]	Mean relative abundance (AU) [f]						P-value [g]		
				Line 12			Line 22					
				M1[c]	M2[d]	M3[e]	M1	M2	M3	M[h]	L[i]	MxL[j]
MA8	p-cymen-8-ol	1202	A	4.1±0.79	3.8±0.03	4.2±0.91	2.0±0.63	2.8±0.29	2.7±0.78	ns	***	ns
MA9	γ-terpineol	1210	A	2.6±0.71 ^{A}	n.d. ^{C}	1.8±0.40 ^{AB}	1.2±0.44 ^{A}	2.0±0.19 ^{AB}	2.5±0.42 ^{A}	***	ns	***
MA10	*(Z)*-carveol	1220	A	n.d.	7.5±1.5	5.8±0.92	n.d.	4.9±1.0	4.2±1.1	***	**	ns
MA11	thymol	1290	A	0.87±0.15 ^{BC}	2.8±0.30 ^{A}	3.2±0.74 ^{A}	0.31±0.07 ^{C}	n.d.^{C}	1.4±0.37 ^{B}	***	***	**
MA12	carvacrol	1311	A	2.8±0.60 ^{B}	11.2±1.7 ^{A}	13.1±0.78 ^{A}	0.80±0.09 ^{B}	2.8±0.30 ^{B}	2.2±0.38 ^{B}	***	***	***
MA13	*(E)*-8-hydroxylinalool	1342	A	0.90±0.26 ^{A}	n.d. ^{C}	n.d. ^{C}	0.38±0.05 ^{B}	n.d. ^{C}	n.d. ^{C}	***	**	**
	Total			**39.4**	**49.1**	**47.6**	**36.7**	**29.6**	**35.6**			
	Sesquiterpenes											
S1	(+)-cyclosativene	1378	A	n.d. ^{C}	1.1±0.12 ^{B}	n.d. ^{C}	n.d. ^{C}	3.8±0.75 ^{A}	n.d. ^{C}	***	***	***
S2	α-copaene	1389	A	0.36±0.10 ^{B}	1.6±0.43 ^{B}	n.d. ^{B}	2.1±0.30 ^{B}	10.5±1.9 ^{A}	n.d. ^{B}	***	***	***
S3	β-caryophyllene	1440	A	35.9±12.1 ^{A}	46.5±11.4 ^{AB}	12.8±3.3 ^{B}	15.9±3.8 ^{B}	25.6±1.1 ^{B}	6.6±2.1 ^{B}	***	***	ns
S4	α-humulene	1475	A	9.8±2.3 ^{A}	8.5±1.1 ^{BC}	5.2±1.6 ^{B}	2.2±0.29 ^{BCD}	2.0±0.41 ^{D}	1.3±0.17^{CD}	**	***	ns
S5	(+)-aromadendrene	1447	A	1.1±0.18 ^{ABC}	1.5±0.16 ^{A}	0.60±0.10 ^{C}	0.66±0.11 ^{C}	1.3±0.33 ^{AB}	0.97±0.18 ^{BC}	***	ns	**
S6	curcumene	1486	A	2.0±0.21 ^{A}	n.d. ^{C}	n.d. ^{C}	1.0±0.11 ^{B}	n.d. ^{C}	n.d. ^{C}	***	***	***
S7	β-selinene	1505	B^{C}	57.0±13.3	79.2± 14.6	26.4±4.5	21.6±4.2	50.5±11.5	15.0±2.0	***	***	ns
S8	valencene	1516	A	n.d. ^{B}	54.5±9.7 ^{A}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
S9	α-selinene	1518	A	8.3±1.6	14.2±2.4	4.0±0.72	3.5±0.12	9.3±2.1	3.3±0.84	***	***	ns
S10	*(Z)*-β-nerolidol	1535	A	n.d.	n.d.	3.2±0.34	n.d.	n.d.	3.4±0.56	***	ns	ns
S11	kessane	1554	A	60.3±7.8 ^{A}	n.d. ^{B}	n.d. ^{B}	0.64±0.23 ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
	Total			**175**	**207**	**52.2**	**47.5**	**103**	**30.6**			
	Phthalides											
P1	3-propylidene phthalide	1600	A	1.4±0.23	2.1±0.29	1.3±0.36	0.4±0.03	1.4±0.32	0.17±0.03	***	***	ns
P2	3-*n*-butylphthalide	1658	A	37.2±4.5 ^{C}	124±20.2 ^{A}	103±5.5 ^{AB}	26.8±6.7 ^{C}	148±27.3 ^{A}	68.0±22.9 ^{BC}	***	ns	*
P3	*(Z)*-butylidenephthalide	1685	B^{C}	n.d. ^{C}	2.9±0.60 ^{B}	1.5±0.28 ^{C}	n.d. ^{C}	4.3±0.84 ^{A}	0.84±0.07 ^{CD}	***	ns	**
P4	sedanenolide	1730	A	102±16.1 ^{C}	279±21.3 ^{A}	221±42.2 ^{AB}	56.8±12.3 ^{CD}	202±27.1 ^{B}	18.1±4.0 ^{D}	***	***	***
P5	neocnidilide	1753	B^{c}	1.1±0.13 ^{C}	2.9±0.53 ^{BC}	3.2±0.63 ^{BC}	3.0±0.62 ^{BC}	10.0±1.8 ^{A}	3.8±0.52 ^{B}	***	***	***
P6	*(E)*-ligustilide	1758	B^{B}	1.4±0.25 ^{B}	3.8±0.61 ^{A}	3.0±0.55 ^{A}	0.89±0.20 ^{B}	2.9±0.56 ^{A}	0.42±0.07 ^{B}	***	***	**
	Total			**143**	**415**	**333**	**87.9**	**369**	**91.3**			
	Alkanes											
ALK1	nonane	900	A	5.9±1.2 ^{AB}	9.7±2.0 ^{A}	6.8±1.1 ^{AB}	5.5±1.9 ^{AB}	n.d. ^{C}	9.3±1.2 ^{AB}	**	**	***
ALK2	decane	1000	A	n.d. ^{D}	6.4±1.2 ^{BC}	5.1±0.74 ^{CD}	n.d. ^{D}	22.5±4.2 ^{A}	11.1±1.6 ^{B}	***	***	***
ALK3	undecane	1100	A	2.4±1.5	2.3±0.17	n.d.	1.7±0.21	3.2±0.76	n.d.	***	ns	ns
ALK4	dodecane	1200	A	0.56±0.08 ^{D}	6.2±1.6 ^{A}	5.5±0.79 ^{A}	1.7±0.21 ^{CD}	4.6±1.0 ^{AB}	3.0±0.60 ^{BC}	***	*	*
ALK5	tridecane	1300	A	n.d. ^{B}	n.d. ^{B}	3.1±0.57 ^{A}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
ALK6	tetradecane	1400	A	0.51±0.13 ^{C}	0.99±0.21 ^{B}	n.d. ^{D}	0.39±0.04 ^{C}	2.0±0.14 ^{A}	n.d. ^{D}	***	***	***
	Total			**9.4**	**25.6**	**20.5**	**9.3**	**32.3**	**23.4**			
	Ether											
ET1	dill ether	1184	A	n.d. ^{C}	n.d. ^{C}	3.5±1.4 ^{A}	n.d. ^{C}	n.d. ^{C}	1.6±0.36 ^{B}	***	ns	*
	Oxide											
O1	*(Z)*-limonene oxide	1145	A	12.8±3.4	n.d.	n.d.	10.8±0.53	n.d.	n.d. ^{B}	***	ns	ns
	Phenol											
PH1	eugenol	1363	A	n.d.	1.8±0.22	2.7±0.23	n.d.	2.3±0.29	2.7±0.42	***	ns	ns
	Unknowns											
U1	unknown	935		3.9±0.58 ^{A}	n.d. ^{D}	1.1±0.21 ^{C}	2.1±0.18 ^{B}	n.d. ^{D}	1.6±0.l6 ^{C}	***	***	***
U2	unknown	1009		n.d. ^{C}	n.d. ^{C}	13.6±1.2 ^{A}	n.d. ^{C}	n.d. ^{C}	10.9±1.1 ^{B}	***	*	**
U3	unknown	1133		n.d. ^{B}	n.d. ^{B}	0.72±0.14 ^{B}	n.d. ^{B}	n.d. ^{B}	2.0±0.71 ^{A}	***	*	**
U4	unknown	1239		n.d. ^{B}	n.d. ^{B}	2.1±0.18 ^{B}	n.d. ^{B}	n.d. ^{B}	22.2±4.38 ^{A}	***	***	***
U5	unknown	1277		n.d. ^{B}	1.4±0.34 ^{B}	4.6±2.0 ^{A}	n.d. ^{B}	1.7±0.25 ^{B}	2.1±0.56 ^{B}	***	ns	*
U6	unknown	1466		n.d. ^{C}	2.6±0.57 ^{A}	n.d. ^{C}	n.d. ^{C}	1.5±0.05 ^{B}	n.d. ^{C}	***	**	***
U7	unknown	1698		n.d. ^{B}	51.8±7.7 ^{A}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	n.d. ^{B}	***	***	***
	Total			**64.2**	**55.8**	**22.1**	**2.7**	**3.2**	**38.8**			

[a] Linear retention index on a DB-5 column

[b] A – Experimental LRI, identification of compound whereby the mass spectrum and LRI agree with those of authentic compound (A) Identification, mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectra database or (B) LRI agree with those in the literature [(A)] Mévy et al. (2006) [(B)] Asuming et al., 2005, [(C)] Andriamaharavo (2014), [(D)] Jalali-Heravi et al., 2006

[c] Premature time-point

[d] Commercial maturity time-point

[e] Post-maturity time-point

[f] Estimated quantities (mg) collected in the headspace of celery samples containing 0.5 mL of saturated calcium chloride and filled up to 5 mL with HPLC-grade water, calculated by comparison with of 100 μg/mL propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; n.d. - not detected; ns - not significant probability obtained by ANOVA, * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level

[h] Maturity

[i] Line

[j] Maturity and line interaction. Tukey's HSD - means not labelled with letters are not significantly different ($p < 0.05$) according maturity/line interaction.

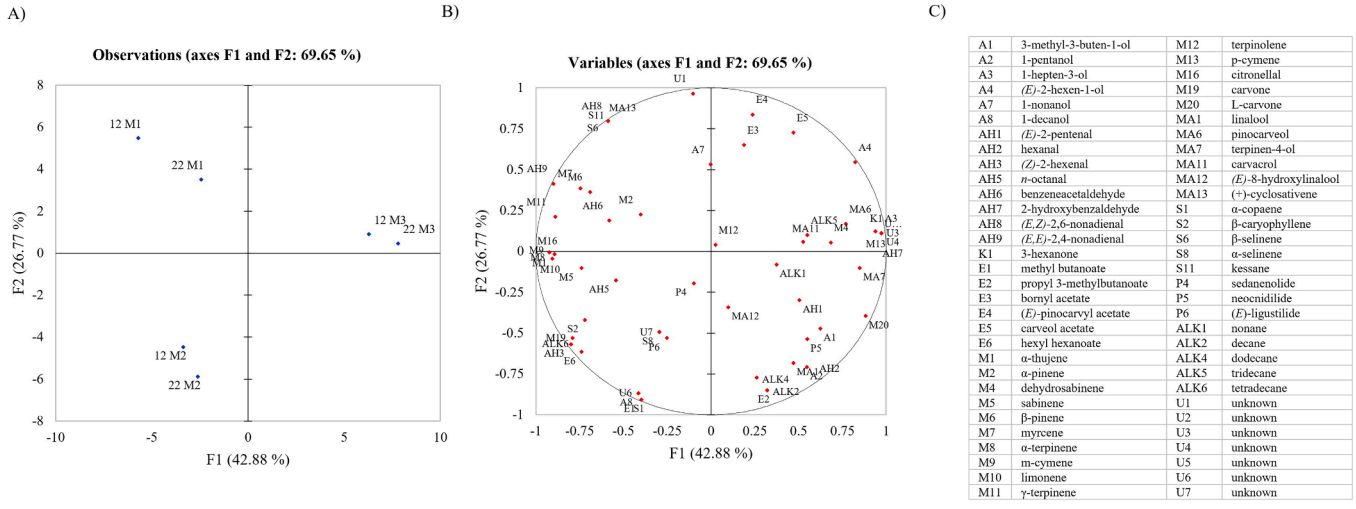
their highest level of abundance at M2. Abundance variation within the phthalides identified were observed between maturities, with line 12 showing a much higher phthalide content than line 22. As shown by both Kurobayashi et al. (2006) and Sellami et al. (2012), phthalide compounds are important contributors to the typical *A. graveolens* aroma and therefore, having a lower abundance of these compounds at a later maturity may mean that the odour these genotypes exhibit is a much less typical celery odour. Focussing further on the phthalide compounds, a significant difference between the maturities for the majority of these compounds can be observed, with sedanenolide showing the most significant increase from M1 to M2 and then decreasing at M3. Apart from neocnidilide in line 22, all phthalides were at the highest abundance at this time point. 3-*n*-Butylphthalide and (Z)-butylidene phthalide showed no significant difference between genotype, only maturity, and (Z)-butylidene phthalide was not identified at M1.

The relative abundance of alcohols increased as the crop developed for both genotypes. At M3 more alcohols were identified and in most cases at a higher abundance. Compounds 1-nonanol and 1-dodecanol for line 12 were shown to be of lower abundance at M3 when compared to M1 and 1-octanol and 1-decanol were not identified in either genotype at M3. For monoterpenes, sesquiterpenes and phthalides, line 12 has been shown to have the highest abundance of these compounds when compared to line 22. However, for alcohols, aldehydes and esters, line 22 has a significantly higher abundance of these and exhibited a different pattern to line 12. At M1, line 22 expressed a similar aldehyde and ester content to line 12 at M2 and at M3, a much higher abundance of these compounds is observed. The biggest cause of this difference in esters was attributed to the large increase of propyl 3-methylbutanoate, known for its fruity, apple odour. Seven aldehydes were identified at both M1 and M2 compared to the five identified at M3. Compounds contributing to green, fresh odours such as *(Z)*-2-hexenal, (*Z*)-4-heptenal, *(E,Z)*-2,6- and *(E,E)*-2,4-nonadienal were not found in M3. Conversely, 2-hydroxybenzaldehyde was only identified at M3 and at much higher abundance in line 22, again this could possibly be indication for aroma deterioration. Line 22 exhibited a higher abundance in compounds such as hexanal at all maturities, particularly at M3 where hexanal increased in relative abundance, whereas in line 12 this began to decrease after M2.

As these lines were transplanted in the same field at the same time and were grown under the same environmental conditions, minimal significant differences caused by environmental factors were expected. Therefore, any differences observed should be attributed to differences in the genotype and maturity. From the results so far, it seems that maturity has a higher impact on aroma profile differences than genotype however, the difference between genotypes in terms of patterns for different compounds across maturities is apparent. This was expected due to the differences identified by Yommi et al. (2013) and Fellman, Miller and Mattinson (2000). They observed the influence of genetics and harvest maturity on volatile compounds in different apple varieties, stating that the nature and amount of aroma compounds present in apples were cultivar dependent.

Principal component analysis was used to visualise graphically the differences in the volatile compounds in the three maturity stages and the two genotypes and to examine any correlations occurring between maturity, genotype and chemical compounds (Fig. 1). Using only the significant compounds for maturity, genotype and their interaction, a clear separation between the maturities and the chemical compounds associated can be observed. Principal component one (F1) and two (F2) explained 69.95% of the total variation present within the data and it can be observed that the first axis discriminates M3 from M1 and M2, whereas M2 is discriminated from M1 and M3 by the second axis. Predominantly, monoterpene content expresses a strong association with F1 (42.88%) whereas other compound groups including aldehydes, esters and phthalides are measured through F2 and explaining a lower proportion of the variation present within the data (26.77%).

Genotype shows a stronger influence upon M1 where a larger separation can be seen between the two genotypes and a stronger association with the volatile compounds associated with line 12 M1. M1 displays a strong positive association with the majority of monoterpenes, such as α-pinene (M2), sabinene (M5), β-pinene (M6), myrcene (M7) and (M11) γ-terpinene, and aldehydes such as 1-octanol (AH5) benzeneacetaldehyde (AH6), (*E,Z*)-2,6-nonadienal (AH8) and *(E,E)*-2,4-nonadienal (AH9). These are compounds are known to exhibit fresh, waxy, green notes, similar to cucumber odour. The highest number of esters were identified at M1 (Table 1) and these compounds contribute fruity and fresh notes however, these are at low relative abundance compared to the other maturities as seen in Table 1, explaining the low association of these compounds in all PCA plots. Nurzyńska-Wierdak et al. (2018) observed both increases and decreases in the ester content of celery essential oil when comparing freeze-dried with convection drying,



A1	3-methyl-3-buten-1-ol	M12	terpinolene
A2	1-pentanol	M13	p-cymene
A3	1-hepten-3-ol	M16	citronellal
A4	*(E)*-2-hexen-1-ol	M19	carvone
A7	1-nonanol	M20	L-carvone
A8	1-decanol	MA1	linalool
AH1	*(E)*-2-pentenal	MA6	pinocarveol
AH2	hexanal	MA7	terpinen-4-ol
AH3	*(Z)*-2-hexenal	MA11	carvacrol
AH5	*n*-octanal	MA12	*(E)*-8-hydroxylinalool
AH6	benzeneacetaldehyde	MA13	(+)-cyclosativene
AH7	2-hydroxybenzaldehyde	S1	α-copaene
AH8	*(E,Z)*-2,6-nonadienal	S2	β-caryophyllene
AH9	*(E,E)*-2,4-nonadienal	S6	β-selinene
K1	3-hexanone	S8	α-selinene
E1	methyl butanoate	S11	kessane
E2	propyl 3-methylbutanoate	P4	sedanenolide
E3	bornyl acetate	P5	neocnidilide
E4	*(E)*-pinocarvyl acetate	P6	*(E)*-ligustilide
E5	carveol acetate	ALK1	nonane
E6	hexyl hexanoate	ALK2	decane
M1	α-thujene	ALK4	dodecane
M2	α-pinene	ALK5	tridecane
M4	dehydrosabinene	ALK6	tetradecane
M5	sabinene	U1	unknown
M6	β-pinene	U2	unknown
M7	myrcene	U3	unknown
M8	α-terpinene	U4	unknown
M9	m-cymene	U5	unknown
M10	limonene	U6	unknown
M11	γ-terpinene	U7	unknown

Fig. 1. Principal component analysis of two different celery genotypes at three different maturities showing correlations with volatile compounds that are significant according to factors of maturity, genotype and the interaction of maturity × genotype: (A) Projection of samples (B) Distribution of volatile compounds (C) Key of compounds used to construct the PCA.

however these were not significant differences. Phthalides show no association with M1 in Fig. 1 and only sesquiterpenes β-selinene (S6) and kessane (S11) show association with M1.

Developing into M2, the aroma profile shifted, with strong associations with phthalides such as sedanenolide (*P4*) and *(E)*-ligustilide (*P6*), and sesquiterpenes such as α-copaene (S1), β-caryophyllene (S2) and α-selinene (S8). The presence of these compounds allows stronger odours that are woodier, herbal and celery-like to seem more apparent, descriptors that are more common when describing *A. graveolens* aroma. At this stage, the highest number of sesquiterpenes and phthalides were observed for both genotypes (Table 1).

Once M3 is reached, the spread of compounds within the quadrant (Fig. 1) is much less compared to other maturities, with the compounds more localised. Furthermore, where more obvious groupings of compounds by M1 and M2 can be seen clearly, this is less apparent for M3. Compounds including 2-hydroxybenzaldehyde (AH7), dehydrosabinene (M4), *p*-cymene (M13) and terpinolene (M12) are strongly associated with M3 as well as the monoterpenoid alcohols; pinocarveol (MA6), terpinen-4-ol (MA7), carvacrol (MA11) and (E)-8-hydroxylinalool (MA12). M3 displaying stronger associations with these compounds and weaker associations with monoterpenes, alcohols and phthalides (fresh, green and fruit odours) suggests that the odour of these genotypes are no longer of the same quality as M2 and therefore, deterioration of the crop is beginning. The presence of certain compounds (A3, K1, M4, M13) could act as an indicator of quality decline in celery. Within the same quadrant as M3, esters bornyl acetate (E3), (E)-pinocarvyl acetate (E4), carveol acetate (E5) express a closer association than previous maturities.

Furthermore, line 22 shows significantly higher abundances in certain compounds at M3 including AH2, M4 and AH7 whereas line 12, show higher abundances in other compounds at M3 including K2, M13 and MA5 (Table 1). Possibly due to genetic differences or because line 22 may have progressed through developmental stages differently compared to than line 12, it is possible that floral transition had occurred, and the plants were preparing to bolt. At the beginning of maturity, line 12 appears to be most aromatic (Fig. 1, Table 1) however, as maturity occurs line 22 M2 and M3 progresses into a more aromatic line, showing these two time points to be most significantly different when combined with genotype. Line 12 M1 and line 22 M2 celery share the most similarities in terms of aroma profile and independent of genotype, M1 and M2 appear to be the most similar.

Compounds including hexanal and *(E)*-2-hexen-1-ol are known as green leaf volatiles (GLVs); these are released in the early stages of maturity and increase as the plants develop, similar to monoterpenes. Over time, the bolting process begins and the crop invests more resources into reproduction and protecting the developing floral meristem from predatory attack, as shown by Rapparini, Baraldi & Facini, (2001). This is where the concentration of terpenes was highest (Table 1, M1) following flowering and in subsequent reproductive stages. As the plant develops, plant-plant and plant-insect interactions become more important, involving the synthesis of GLVs and other volatile compounds (Spinelli, Cellini, Marchetti, Mudigere & Piovene, 2011). This relationship could explain the increase of monoterpenes from M1 to M2 before the crop focuses on the synthesis of alcohols and aldehydes as maturity develops.

Overall, comparing the odours between the two genotypes and three maturities, it can be seen that line 12 has the highest abundance of volatile compounds and can be assumed to be a more aromatic variety. Harvesting at any time point will result in a crop with a significantly different aroma profile. Harvesting at an earlier, similar to M1 would result in low in phthalide and high monoterpene content, resulting in a more citrus-like profile. Over commercial maturity, phthalide content remains high, maintaining strong celery notes. In order to identify whether there has been aroma quality decline and whether compounds identified in M3 contribute to off-odours, sensory profiling using a trained panel can be completed. The differences support the hypothesis that the time point of harvest does have a significant influence over the aroma of celery as well as the genotype and that genotype will influence the synthesis of odours during deterioration. This relationship is discussed further when considering the GC/O data in section 3.2.

3.2. Human olfactory analysis using GC-O shows that genotype influences development of off-flavours

In total, 103 different odours were detected in the headspace of the two celery genotypes across three different maturities using GC/O. Out of these, 65 compounds were identified using a combination of GC/MS analysis, LRI comparison to authentic standards and using the aromas they were described with (Table 2). Similarly to the chemistry described by GC/MS (Table 1), differences between genotype as the crop developed is evident in Table 2, with the absence/presence of compounds within genotypes contributing different odours to the overall aroma profile and thus indicating that genotype plays a role in the synthesis of odours that may indicate quality decline.

Within the samples, 18 monoterpenes, 12 alcohols, 11 aldehydes, ten ketones, nine monoterpenoid alcohols and phthalides and other compounds including esters (acetates and non-acetates) and sesquiterpenes were identified respectively. Out of the 103 odours that were identified, only nine of these compounds appeared in both genotypes and across the three maturities (Table 2). Across these compounds, it can be observed that line 12 had the highest recorded intensity for all of these compounds apart from hexanal and *(E,E)*-2,6-nonadienal. In the majority of the cases, the compounds were at their highest intensity at M1 and started to decrease thereafter, with a subset then showing an increase between M2 and M3.

In M1, 43 and 51 compounds were identified in the two genotypes respectively, with the majority of these compounds being monoterpenes (sabinene, β-pinene, limonene and γ -terpinene) and alcohols (1-hepten-3-ol, 1-octen-3-ol and 1-nonanol), all averaging intensity scores of around five and six (Table 2). No sesquiterpenes were not detected in M1 line 12, however, α-copaene and β-selinene were both detected within M1 line 22 at an intensity of five. β-selinene was identified as having a high abundance in GC/MS (Table 1) for both line 12 and 22 across all maturities. The absence of these compounds is with agreement with the PCA plots, whereby monoterpenes show a high association with M1 with low sesquiterpene association. Aldehydes (benzeneacetaldehyde, (E,E)-2,6- and (E,Z)-2,6-nonadienal), ketones (3-pentanone, 2-hexanone and 3-octen-2-one) were detected to have a high average odour intensity in line 12, contributing cucumber, herbal and green odour notes however, only 2-pentanone was detected in line 22.

Among some of the compounds that were identified with a high average odour intensity, compounds with 'mushroom' and 'earthy' odours were very much apparent. These included 2- and 3-heptanol, 1-octen-3-ol, sabinene and β-pinene. It could be suggested that these mushroom smelling compounds are key contributors to a M1 celery odour. Out of these compounds, sabinene and β-pinene were identified by the GC/MS and exhibited high abundance at M1. In terms of phthalides, *(E)*-3-butylidenephthalide had an odour intensity of seven at M1 line 12 yet *(E)*-3-butylidenepht was not identified in line 22. Sedanenolide and sedanolide were identified throughout maturity and at a high average odour intensity for both genotypes, reflected in Table 1 also.

A study completed by Macleod and Ames (1989) identified *(E)*-3-butylidenephthalide, sedanolide and sedanenolide in supermarket purchased celery using GC/MS and GC/O. *(E)*-3-Butylidenephthalide was identified to have an odour of 'cooked celery', *(E)*-sedanolide and sedanenolide were both identified to have an odour of 'celery' as well as being 'pungent'. Although not identified in line 12, *(E)*-ligustilide appeared to be an important compound for line 22, showing a high average odour intensity at M1 with a gradual decrease to not being detected in M3. Neocnidilide exhibited a consistently high odour intensity across the different maturities in line 12, reaching and average

Table 2
Odour description and intensity of the volatile compounds detected by GC-O in the headspace of two celery genotypes harvested at three different maturity stages.

Odour Description	LRIexp[a]	Compound	ID[b]	Code[d]	Average Odour Intensity [c] Line 12 M1[e]	Line 12 M2[f]	Line 12 M3[g]	Line 22 M1	Line 22 M2	Line 22 M3
Alcohols										
Burnt, baked, dairy	660	1-butanol	B		–	–	4	3	4	–
Green/chemical	670	1-penten-3-ol	B		4	–	–	–	–	–
Green, plastic, fruity	706	3-pentanol	B		–	3	4	–	–	–
Soapy, green, sharp	733	3-methyl-3-buten-1-ol	A	A1	5	–	5	3	–	–
Fresh, green, fruity	859	(*Z*)-3-hexen-1-ol	B		5	–	4	–	–	–
Musty, moss	867	(*E*)-2-hexen-1-ol	A	A4	–	5	3	–	4	–
Earthy, mushroom, grass	889	1-hepten-3-ol	A	A3	8	–	4	–	5	–
Mushroom	907	2-heptanol	B, C		6	5	–	–	–	3
Mushroom, soil	978	1-octen-3-ol	B, C		7	5	6	4	7	5
Fresh, citrus, waxy	1001	3-octanol	B		7	–	5	5	6	–
Metallic, sweaty	1174	1-nonanol	A	A7	7	–	6	–	4	4
Tomato, herbal, fatty	1274	1-decanol	A	A8	–	–	5	–	5	–
Aldehydes										
Floral, green, waxy	760	(*E*)-2-pentenal	A	AH1	–	4	5	3	3	–
Fresh, green, apple	801	hexanal	A	AH2	5	5	3	6	6	4
Garbage, damp	855	(*E*)-2-hexenal	A	AH3	–	5	–	5	–	–
Biscuit, bread	901	(*Z*)-4-heptenal	A	AH4	5	–	5	4	–	–
Floral, rose, citrus	1005	*n*-octanal	A	AH5	–	7	–	6	–	3
Rose, honey, floral	1045	benzeneacetaldehyde	A	AH6	7	5	4	5	5	4
Baked, honey, make-up powder	1057	2-hydroxybenzaldehyde	A	AH7	6	–	5	4	4	5
Floral, smoky, cherry	1071	*p*-tolualdehyde	B		–	–	5	3	–	–
Woody, moss, cucumber	1155	(*E,E*)-2,6-nonadienal	B, C		6	5	6	7	5	5
Green, cucumber, parsley	1159	(*E,Z*)-2,6-nonadienal	A	AH8	6	5	–	7	7	5
Floral, woody	1224	(*E,E*)-2,4-nonadienal	A	AH9	–	5	–	–	–	–
Ketones										
Vanilla, creamy, butter	677	1-penten-3-one	B		–	3	–	–	–	–
Bread, floral, grass	687	2-pentanone	B		–	–	4	5	6	3
Green	693	3-pentanone	B		7	–	4	5	–	–
Waxy, green, plastic	776	3-hexanone	A	K1	6	–	–	5	–	–
Green, cut grass, apple	793	2-hexanone	B		7	3	4	4	–	–
Metallic, musty	978	1-octen-3-one	A	K2	–	–	–	4	4	–
Rose, honey, floral	1041	3-octen-2-one	B		7	–	5	–	–	–
Herbal, soil, spicy	1083	2-nonanone	A	K3	–	3	5	–	5	–
Make-up powder, floral, creamy	1146	3-nonen-2-one	B		–	–	6	6	5	–
Make-up powder, baked	1401	*p*-mentha-8-thiol-3-one	B		–	5	4	–	–	–
Esters										
Make-up powder, floral	947	propyl 3-methylbutanoate	A	E2	3	–	6	–	–	–
Woody, pencil shavings, liquorice	1247	linalyl acetate	B		6	–	6	–	5	–
Herbal, woody	1305	bornyl acetate	A	E3	–	–	4	–	–	4
Plastic, green, herbal	1332	carveol acetate	A	E5	–	–	4	7	–	–
Metallic, damp, musty	1381	hexyl hexanoate	A	E6	–	–	4	–	6	4
Monoterpenes										
Pine, minty, floral	931	α-thujene	A	M1	5	–	4	4	4	–
Herbal, citrus, waxy	959	camphene	A	M3	6	4	5	5	5	3
Earthy, mushroom, green	981	sabinene	A	M5	8	–	6	7	7	–
Herbal, earthy, woody	987	β-pinene	A	M6	8	7	4	7	5	5
Lemon, green, waxy	997	β-myrcene	A	M7	–	3	4	6	–	–
Musty, camphoreous	1025	α-terpinene	A	M8	6	–	4	–	–	–
Floral, fresh, mint	1031	limonene	A	M10	6	–	4	4	4	–
Waxy, woody, makeup powder	1062	γ -terpinene	A	M11	6	–	–	–	–	–
Make-up powder, floral, citrus	1094	terpinolene	A	M12	5	3	4	–	5	4
Floral, herbal, violet	1098	*p*-cymene	A	M13	6	–	3	–	–	–
Caramel, honey, floral	1109	*p*-mentha-1,5,8-triene	A	M15	5	–	6	–	–	4
Tomato, spicy	1112	β-thujone	A	M14	–	–	–	5	5	–
Floral, musty, green	1166	citronellal	A	M16	–	7	4	5	6	–
Make-up powder, herbal, floral	1195	(*E*)-dihydrocarvone	A	M17	6	–	4	4	6	5
Floral	1231	β -cyclocitral	A	M18	–	–	6	–	–	–
Spearmint	1245	carvone	A	M19	–	–	6	5	–	3
Herbal, pine, minty	1253	L-carvone	A	M20	–	7	6	6	4	6
Oily, woody	1259	D-carvone	B, C		5	–	5	–	–	–
Monoterpenoid alcohols										
Woody, red fruit	1103	linalool	A	MA1	3	–	–	4	–	–
Herbal, cooked	1116	(+)-(*E*)-*p*-mentha-2,8-dien-1-ol	A	MA2	–	–	4	4	–	–
Cucumber, floral, woody	1150	pinocarveol	A	MA6	–	–	6	7	–	4
Mushroom, earthy, metallic	1180	terpinen-4-ol	A	MA7	–	7	3	3	–	–
Herbal	1207	γ -terpineol	A	MA9	–	–	–	4	–	–
Bread, creamy	1214	(*Z*)-carveol	A	MA10	–	–	5	5	4	–
Pine, spicy	1292	thymol	A	MA11	–	3	4	–	–	–
Herbal, starchy	1314	carvacrol	A	MA12	–	–	5	–	–	–
Herbal	1346	(*E*)-8-hydroxylinalool	A	MA13	–	3	–	–	–	–
Sesquiterpenes										

(*continued on next page*)

Table 2 (*continued*)

Odour Description	LRIexp[a]	Compound	ID[b]	Code[d]	Average Odour Intensity [c] Line 12 M1[e]	Line 12 M2[f]	Line 12 M3[g]	Line 22 M1	Line 22 M2	Line 22 M3
Cucumber skin, fatty	1366	(+)-cyclosativene	A	S1	–	–	3	–	3	–
Damp, bread, woody	1390	α-copaene	A	S2	–	–	4	5	6	4
Sweet, earthy	1443	β-caryophyllene	A	S3	–	–	4	–	–	3
Floral, vegetative, woody	1478	α-humulene	A	S4	–	–	4	–	4	–
Floral, rose, woody	1495	β-selinene	A	S7	–	5	4	5	5	–
Creamy	1513	α-selinene	A	S9	–	3	–	–	–	–
Vegetative	1555	kessane	A	S11	–	–	3	–	–	–
Phthalides										
Celery, vegetables	1603	3-propylidene phthalide	A	PH1	–	3	–	–	–	–
Dried celery, parsley	1660	3-*n*-butylphthalide	A	PH2	–	5	5	–	–	–
Dried celery	1676	(*Z*)-butylidenephthalide	A	PH3	–	–	–	4	–	–
Dried celery	1698	*cis*-ligustilide	B, C		5	–	6	4	5	5
Fresh celery	1709	*(E)*-butylidenephthalide	B, C		7	5	6	–	–	3
Cooked celery	1715	sedanolide	B, C		6	6	6	4	5	5
Celery	1731	sedanenolide	A	PH4	6	7	6	5	5	5
Dried celery	1742	neocnidilide	A	PH5	6	7	5	–	–	–
Celery	1752	(*E*)-ligustilide	A	PH6	–	–	4	7	3	–
Furans										
Caramel, rose, strawberry	1081	furaneol	B, C		7	5	5	6	5	5
Unknowns										
Floral, fruity	608	unknown			–	–	3	–	–	–
Floral	625	unknown			–	–	3	–	–	–
Buttery, dairy	632	unknown			–	–	4	4	4	3
Plastic, green, musty	768	unknown			–	–	4	–	5	3
Fresh lime, citrus	808	unknown			4	–	–	–	–	–
Floral, fruity, green	817	unknown			–	–	4	–	6	3
Pungent, cheese	842	unknown			–	–	5	–	4	–
Lemon, soil	913	unknown			–	–	–	–	5	–
Bread	918	unknown			–	–	–	–	–	3
Mushroom, soil	971	unknown			–	–	6	–	–	–
Smokey	1130	unknown	A	UN3	–	–	–	5	–	–
Woody, floral	1284	unknown	A	UN5	–	–	–	5	6	–
Smoked tomato, musty	1324	unknown			–	5	–	–	–	–
Vegetative, woody	1631	unknown			–	5	4	–	–	–
Dried celery	1649	unknown			–	–	5	–	–	–
Fresh celery	1722	unknown			–	6	6	–	5	–
Rotten celery	1765	unknown			–	4	4	–	–	–
Celery	1780	unknown			6	–	4	6	3	–
Celery	1800	unknown			–	–	–	5	3	–
Cooked celery	1816	unknown			5	3	–	–	–	–
Celery	1855	unknown			5	–	–	–	–	–
Total compounds					43	39	77	51	48	31

[a] Linear retention index (LRI) on DB5 column, calculated from a linear equation between each pair of straight chain *n*-alkanes C_6-C_{25}. [b] Means of identifying compound (A- Mass Spectrometry B- LRI C- Aroma note recognitions). [c] Average odour intensity recorded by three assessors recording each maturity in duplicate except line 22 where only one was completed. (scoring scale: weak = 3, medium = 5, strong = 7), - = not detected. [d] Code corresponds to compounds identified in Table 1. [e] Prematurity time-point. [f] Commercial maturity time-point. [g] Post-maturity time-point. An average odour intensity was taken by collecting the average scores from the duplicates of each assessor and dividing by the number of GC/O runs completed for the genotype and maturity. The value of average odour intensity was rounded up/down to the nearest whole number.

odour intensity of seven at M2 before decreasing to five in M3.

At M2, 39 and 48 compounds were identified in line 12 and 22 respectively. A wide variety of compounds were observed at this time point, including a mixture of monoterpenes, alcohols, aldehydes and phthalides. Key odour descriptors for commercial mature celery include fresh, green, herbal and earthy. These odours are achieved by compounds such as hexanal, β-pinene and phthalides such as neocnidilide and sedanenolide, all scoring at an intensity five and above (Table 2). According to Table 2, the aroma profile of line 22 appeared to be more complex, with more compounds being identified at M2 than line 12 including more alcohols, ketones, esters and monoterpenes. However, more phthalides were detected in line 12 and at a higher average odour intensity. Therefore, although fewer compounds were identified in line 12 M2, it can be hypothesised that this genotype at commercial maturity had a strong celery aroma due to its high phthalide content, whereas line 22 had more odours that are green, grass-like and earthy. Sedanenolide was detected at its highest average odour intensity here and similar to the results reported in Table 1, line 12 reports the highest relative abundance for phthalides when compared to line 22 and is at its highest at M2. Likewise, Kurobayashi et al. (2006) reported sedanenolide, 3-*n*-butylphthalide, *(E)*- and *(Z)*-sedanolides as having the highest flavour dilution factor upon completion of AEDA. Further stating that odour descriptors of these compounds are similar to the expected celery odour and are possibly the more significant contributors to its odour.

Progressing onto M3, line 12 had the highest number of compounds detected here with 77, conversely line 22 had only 31 compounds detected, the lowest number out of all samples analysed. Here, genotypic differences are very apparent, contradicting Fig. 1 whereby M3 showed to have the fewest differences caused by genotype, whereas Table 2 supports the hypothesis that genotype determines how the crop matures. Correspondingly shown in Table 1, the highest number of monoterpenes were identified here and monoterpenoid alcohols such as terpinen-4-ol and (Z)-carveol for line 12. Conversely, these compounds were detected earlier on in maturity in line 22 and not detected at M3, potentially indicating that line 22 was further along maturity that line 12. No odour with an intensity above six was detected for both lines, showing an obvious decline in aroma quality and intensity. L-Carvone was the compound with the highest intensity in M3 line 12 and 22, with herbal, minty and pine odour descriptors.

Only four phthalides were identified with a relatively low odour

intensity and compounds such as 3-*n*-butylphthalide, neocnidilide and *(E)*- ligustilide were not detected at all in line 22 at M3. The absence of these odour active compounds with odour descriptors such as "celery, fresh celery, dried celery" could possibly imply that M3 line 22 did not have the mature celery odour that line 12 may have. On the other hand, line 12 M3 shows an abundance of these phthalides as well as unknown compounds that express a range of celery odour descriptors from cooked, dried and rotten celery. As line 12 was very abundant in these phthalide compounds (Table 1), it could be that phthalide compounds that could not be detected on GC/MS contributed to off-odours and therefore, aroma quality decline.

Within M3, there were compounds present that were not previously detected by the assessors; these include bornyl acetate, β-caryophyllene and carvacrol (line 12). The odour descriptors that were used to describe the compounds present were 'bread', 'woody', 'sweet' and 'starchy'. The sesquiterpene, α-copaene was identified across all maturities for line 22, yet was only detected in line 12 at M3, with odour descriptors including damp, bread and woody, it is possible that this is an indicator for deterioration in line 22. On the other hand, these compounds have been reported in previous investigations (Pino, Rosado & Fuentes, 1997; Marongiu et al., 2013) and identified in GC/MS (Table 1). It could be possible that these compounds with 'starchy' and 'bread' odours could impart a negative odour on the maturity and are synthesised at a higher quantity as the vegetable matures. Due to the nature of GC/O, it is not possible to conclude that these compounds were responsible for off-odours within celery. Using sensory analysis to profile these celery maturities alongside this will help give a better indication of flavour defects within the crop.

Overall, comparing the odours between the three maturity stages and the two genotypes, it was observed that the most odours were identified in line 12 at M3, and a high average odour intensity compared to line 22 and other maturity stages. Despite M2 line 12 expressing a lower number of odours in comparison to M3 line 22, the average odour intensities of these compounds were much higher, particularly for phthalide compounds. From this it can be assumed that at M2 line 12 had a much more distinct odour profile than line 22 and as line 12 matured, it remained aromatic, therefore, having a better field holding capacity and possibly exhibiting a slow bolting trait.

In terms of aroma development, it can be seen that M1 exhibited a high proportion of monoterpenes and alcohols contributing to a fresh, fruity and citrus odour and low intensities of phthalides. The intensity of phthalides increased to M2, whereby a more typical celery odour was observed. Together with monoterpenes, aldehydes, sesquiterpenes and phthalides, the celery odour was present along with subtle floral, woody and herbal notes, whilst remaining fresh and green. As the crop developed beyond commercial maturity these fresh, green notes were at their minimum or not detected. At this stage, the aroma profile was much more herbal and woodier.

Together with 3-*n*-butylphthalide and sedanenolide, neocnidilide could be considered an important compound to the aroma. Although identified in Table 1 at a lower relative abundance, neocnidilide scored a high average odour intensity scored across line 12 in all maturities (Table 2). This is supported by Marongiu et al., (2013), who identified neocnidilide at high abundance across four celery extracts using two varieties grown in Portugal and Spain, extracted using supercritical carbon dioxide extraction as well as hydrodistillation. Despite the two different extraction methods yielding different results, neocnidilide comprised the majority of the aroma profile of both varieties and extraction methods. Furthermore, Shojaei, Ebrahimi, and Salimi (2011) identified *(E)*-3-butylidenephthalide and *(Z)*-ligustilide as key phthalides in wild celery, as reflected correspondingly by the GC/O data, whereby these two compounds were scored at a high intensity for line 12 across all maturities. Ligustilide was only identified in M3 for line 12 but more apparent in line 22 (Table 2).

Interestingly, the compound benzeneacetaldehyde, with a characteristic odour of honey, floral and rose, was found at high abundance in M1 line 22 on the GC/MS data and remained high across maturity. A similar observation was made with line 12, albeit at a lower abundance. Conversely on the GC/O, benzeneacetaldehyde was detected in both genotypes across three maturities, with M1 line 12 exhibiting a stronger average odour intensity. Though not commonly identified in *A. graveolens*, Shojaei et al. (2011) identified benzeneacetaldehyde in three ecotypes of wild celery grown in three regions of Iran (0.13%, 0.03% and 0.08% respectively) using GC/MS on essential oil.

As there have been limited studies investigating the development of celery aroma over maturity and that combine both GC/MS and GC/O analytical techniques to investigate celery aroma, comparison with other datasets is difficult. Therefore, studies that have used GC/O or GC/MS separately have been utilised. Although commonly used, SPME may not be able to extract all the compounds present in the isolate due to the low concentrations of some flavour compounds (Lui, Su & Song, 2018). SAFE, as used by Kurobayashi et al. (2006), combined with GC/O, AEDA and sensory profiling would give a more representative aroma profile. Using a method such as AEDA allows for the detection of further compounds that were identified in GC/MS. Due to the abundance of limonene within celery (Table 1) and the multiple terpene compounds that co-elute with limonene (Table 2), the likelihood of assessors missing or not detecting these compounds are high during GC/O. Although multiple training sessions were completed prior to GC/O, the ability for the assessor to separate and determine these compounds presents difficulties and therefore, only compounds with the lowest odour thresholds are detected. Carrying out various dilutions through AEDA will lead to the detection of compounds with higher odour thresholds that would have been otherwise masked by limonene, building a broadened profile of celery aroma. Furthermore, harvesting vegetable crops at more time points leading up to and after commercial maturity will help to assess the changes in the volatiles profile further. Exploiting different seasons, geographical locations with diverse climates and using different cultivars could help build a better understanding on how celery aroma develops and how is influenced by the various factors.

4. Conclusion

Out of the two genotypes that were used in this experiment, line 12 exhibited a higher abundance for the majority of volatile compounds as well as more odours present when observing the GC/O data. The abundance of these compounds indicated that this genotype may have a more distinctive and complex aroma profile with green, herbal and floral notes along with strong celery notes, contributed from the high abundance of phthalides detected. In contrast, line 22 indicated a more subtle aroma, more similar to cucumber during maturity, but as the crop developed, there was a bigger change in aroma than seen in line 12, with odours developing that suggested a decline in quality. The stability of line 12 in this study shows that genotype could influence field holding capacity.

Monoterpenes contributed to the fresh, piney and earthy notes and were more abundant at prematurity and commercial maturity. The woodier and herbal notes developed as the crop matured and compounds such as sesquiterpenes, monoterpenoid alcohols and most importantly, phthalides were the main contributors to this aroma. Phthalides have been shown in this study, as well as in a plethora of other experiments, to be significant contributors to celery aroma with high relative abundances identified by GC/MS and high average odour intensities from the GC/O; with odour descriptors including 'celery' and 'herbal'.

According to the data presented, the development of the aroma profile of *A. graveolens* changed over time; it commenced as fresh and fruity, progressed to herbal, woody and celery at commercial maturity, and shifted completely away from fresh and fruity towards woody, floral and damp odours at post-maturity. In order to confirm this, the addition of sensory profiling and more sensitive methods of chemical analysis are required. As shown in this study, developmental maturity has a bigger

influence over aroma than genotype. However, genotype determined the way in which the flavour profile developed either through driving the synthesis of new compounds, reducing the synthesis of existing compounds, or driving the degradation of existing compounds.

These insights, especially when combined with future consumer preference studies, will provide celery growers with desirable aroma profile targets that will ensure that the crop is harvested at the optimum developmental stage. Growers should avoid taking a late harvest, even though this may improve yield, since the organoleptic profile of the crop will be compromised as overmature celery exhibit odours of lower intensity and compounds that may distort the flavour profile. This information will be useful to guide breeders to develop varieties that maintain an optimal aroma profile over a longer growing period. Furthermore, celery breeders now have access to biochemical information to assist breeding programmes and develop genotypes with improved field holding capacity which retain desirable aroma profiles.

Declaration of interests

Author FG is employed by the company A.L. Tozer Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. LT is funded by a BBSRC CASE PhD studentship reference BB/M016579/1 in partnership with A. L. Tozer Ltd.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130515.

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Appendix XII – Images of the two genotypes at each time-point of harvest

M1

M2

M3

M1

M2

M3

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Consumer Acceptability and Sensory Profile of Three New Celery (*Apium graveolens*) Hybrids and Their Parental Genotypes

Lucy Turner [1], Carol Wagstaff [1], Frances Gawthrop [2] and Stella Lignou [1,*]

[1] Department of Food and Nutritional Sciences, Harry Nursten Building, University of Reading, Whiteknights, Reading RG6 6DZ, UK; L.Turner@pgr.reading.ac.uk (L.T.); c.wagstaff@reading.ac.uk (C.W.)
[2] A.L. Tozer Ltd., Pyports, Downside Bridge Road, Cobham KT11 3EH, UK; frances.gawthrop@tozerseeds.com
* Correspondence: s.lignou@reading.ac.uk; Tel.: +44-(0)118-378-8717

Abstract: Celery is a stalky green vegetable that is grown and consumed globally and used in many cuisines for its distinctive taste and flavour. Previous investigations identified the aroma composition of celery and profiled its sensory characteristics using a trained panel; however, evaluation of the sensory characteristics of celery combined with a consumer panel, where consumer preferences and acceptability are determined, is novel. In this study, three parental genotypes (12, 22 and 25) and three new hybrids (12x22, 22x12 and 25x12) were presented to a trained sensory panel (n = 12) for profiling and a consumer panel (n = 118), where liking and preference were assessed. Celery samples were analysed by SPME GC–MS and significant differences in aroma composition between all samples were identified, causing significant differences in the sensory profile. Furthermore, significant differences in attributes assessed for liking (appearance, aroma, texture and overall) were identified. Consumer segmentation identified three groups of consumers exhibiting differences in the hedonic reaction to the samples. Sweet and bitter taste along with overall flavour were identified as drivers of liking. Hybrid 25x12 was found to be the hybrid that exhibited high intensities for most of the attributes assessed.

Keywords: celery; volatiles; flavour; sensory perception; consumer liking; postharvest; terpene; phthalides

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1. Introduction

Celery is an aromatic vegetable that is grown and consumed globally in a range of salads, with condiments; in cooking, where it can be boiled, fried, roasted as well as forming the base of many soups, stocks, and sauces [1–3]. Within cuisines, celery is known to form part of the holy trinity or soffritto [3], starring alongside carrots and onions or onions and bell peppers depending on the cuisine. Celery owes its culinary diversity to the distinct aroma and flavour profile, possessing a range of compound groups including terpenes (monoterpenes and sesquiterpenes), alcohols, aldehydes and phthalides contributing to the overall flavour quality of celery [3–8]. The phthalide compounds have been established as the characteristic odorants of celery, with odour descriptors such as 'celery', 'cooked celery' and 'herbal'. Without the presence of these compounds, celery aroma would not be so distinctive [7,9].

Being such a commonly grown and consumed vegetable, research investigating the perception of celery flavour is surprisingly sparse, with only a few sources examining the sensory properties of celery [9–13]. Furthermore, there has been no research conducted that explores the sensory characteristics of celery combined with consumers' perceptions and preferences. Previous research has identified that external characteristics such as product appearance are primary influencers of initial consumer purchase, whilst internal

characteristics that follow consumption (aroma, taste, flavour, texture) influence acceptability and repurchase [14–16]. Without completing sensory and consumer evaluation, the acceptability of celery and the sensory characteristics that consumers find desirable within celery remain unknown and crop breeding programmes are missing key information that should direct their selection processes.

The authors have previously carried out several experiments, where they identified the aroma profile of various celery genotypes and investigated how factors such as genotype, maturity, geographical location, climate, and agronomy influence the aroma profile and the sensory characteristics using a trained panel [9,12,13]. Combining data from instrumental and sensory analysis with multi-site and multi-year investigations that use the same eight genotypes has led to the discovery of three genotypes that consistently performed regardless of influencing environmental or developmental factors; genotypes 12, 22 and 25. Genotype 12 was consistently high in the abundance of volatile compounds with a high percentage of phthalides comprising the aroma profile of celery with a strong, typical celery odour. The trained panel strongly associated this genotype with a grass odour and herbal flavour, including fennel, parsley, and coriander [9,12,13]. On the other hand, genotype 25 exhibited low abundance of phthalides and a high abundance of aldehydes, with the trained panel describing this genotype as having a cucumber flavour. Genotype 22 had similar aroma profile to genotype 12 but with lower abundance and was scored lower by the trained panel for aroma and flavour attributes such as fresh parsley, coriander, and fennel. In terms of mouthfeel, genotype 22 was consistently scored high for a moist and crunchy petiole and low for stringy mouthfeel, opposing genotype 12. Genotype 12 was ribbed, stringy and bitter, genotypes 22 and 25 remained crunchy, moist with minimal stringiness [12,13].

Providing celery growers and breeders with the information gathered from this investigation will aid in the development of new celery hybrids that have been tailor-made according to consumer preference. The aim of this study was to evaluate the sensory characteristics of celery parental genotypes (12, 22 and 25) and their hybrids (12x22, 25x12 and 22x12) using a trained sensory panel and to assess the aroma profile of the same samples using solid-phase microextraction gas chromatography–mass spectrometry (SPME GC–MS) to identify differences and similarities within the aroma profile. Consumer evaluation was also conducted to understand the acceptability, liking and preference of these genotypes and hybrids and to associate sensory and biochemical composition with these desirable characteristics.

2. Results and Discussion

2.1. Volatile Composition of Celery Samples

In total, 100 compounds were identified in the headspace of the six celery samples (Table 1) including 28 monoterpenes, 16 sesquiterpenes, 12 alcohols (five of which are classified as monoterpenoid alcohols), nine aldehydes and five phthalides. Quantitative differences were observed between the genotypes used in this study and one-way ANOVA revealed significant differences in the relative abundance of aroma compounds between the genotypes in most compounds. Compounds such as (*E*)-2-penten-1-ol, (*Z*)-3-hexenol, lavandulyl acetate, δ-3-carene, β-thujone, *p*-1,3,8-menthatriene, fenchol and β-eudesmol expressed no significant difference between genotypes accompanied by several alkanes and unknown compounds.

A large proportion of the aroma profile was comprised of monoterpenes and sesquiterpenes with limonene, β-pinene, myrcene, γ-terpinene and β-caryophyllene exhibiting the highest relative abundance within their compound groups. These compounds are commonly present in celery and have been reported to contribute to odour notes such as woody, herbal, green, waxy, and earthy [3,9]. Monoterpenes have been shown to have the highest proportion of the aroma composition in various studies [3,5,6]. Genotype 12 exhibited the highest abundance of monoterpenes, sesquiterpenes and phthalides, followed by hybrids 22x12 and 12x22, while genotype 25 and hybrid 25x12 had a much lower

abundance of these compounds. However, as reported by the authors, these terpenes are not the characteristic compounds in celery [4].

Sesquiterpenes, whilst at a lower relative abundance to monoterpenes are more typical to the mature celery aroma. Previously reported by the authors [9], during maturation, the celery aroma developed significantly, starting as a fresh, citrus, green aroma due to the high proportion of monoterpenes and lack of sesquiterpene and phthalide compounds. As the celery matured, the abundance of sesquiterpenes and phthalides became much more apparent and thus, a change in the perceived aroma was identified [9]. β-Caryophyllene and β-selinene (Table 1) exhibited the highest relative abundance within all genotypes, and this was most obviously observed in genotype 12 and hybrid 22x12. Ehiabhi et al. [17] reported β-caryophyllene and β-selinene to be major constituents of Nigerian grown celery and Lund, Wagner, and Bryan [18] identified β-selinene to impart a strong celery aroma. Although less abundant in other genotypes, genotype 12 had a high abundance of kessane. Kessane was identified by Philippe, Suvarnalatha, Sankar and Suresh [19] in the essential oil of Indian-grown celery seed, comprising between 2.2 and 7.6% of the volatile profile.

Phthalides have been shown to contribute to strong celery-like odours in addition to being the most odour-active compounds within celery crop. Upon completing aroma extraction dilution analysis (AEDA), Kurobayashi [20] detected phthalide compounds including 3-n-butylphthalide and sedanenolide, also identified within this study, to contribute most to celery odour. This was further confirmed by Lund, Wagner and Bryan [18], whereby sedanenolide, 3-n-butylphthalide and hexahydro-3-n-butylphthalide imparted strong celery odour characteristics. Genotype 12 displayed the highest abundance of phthalide compounds (Table 1) including sedanenolide and 3-n-butylphthalide followed by hybrids 12x22 and 22x12 that also displayed a high abundance of phthalides within their aroma profile. As these compounds consist of strong celery odour notes [8], we can assume these celeries consist of a typical celery flavour.

The maternal inheritance of compounds from parent to hybrid was observed most clearly between genotype 25 and hybrid 25x12, whereby similarities between the presence and absence of compounds within the aroma profile as well as the abundance of compounds was apparent (Table 1). Monoterpene, sesquiterpene and phthalide abundances for these celery samples were the lowest out of the six samples and for example camphor and p-mentha-2,8-diene were both not identified in genotype 25 and 25x12. Furthermore, apart from 3-propylidene phthalide, the relative abundances of phthalide compounds were not significantly different between 25 and 25x12. The influence of the female counterpart of the crop is clear, with 25x12 inheriting more similarities from the female parent, 25 than male parent 12. This is less clearly observed when both parents, 12 and 22, were used in the hybrids 12x22 and 22x12. The relationship of these genotypes is unknown but if there is a close relation, genetically, then this would explain the fewer significant differences observed between these hybrids (Table 1). m-Tolualdehyde was only identified in genotype 22 and hybrid 22x12 and other aldehydes such as (E, E)-2,4-octadienal and hexanal were either only expressed in 12, 12x22 and 22x12 or were expressed in high abundance in these samples. The chemical inheritance of monoterpenes and sesquiterpene compounds appeared to be less clear; however, β-selinene and β-caryophyllene were expressed in a high relative abundance in genotype 12 and hybrid 22x12, displaying a stronger influence from the male parent, 12. Genotype 12 also displayed a high influence over the phthalide content for the hybrids 12x22 and 22x12, where both expressed a higher relative abundance for phthalide compounds than genotype 22.

Table 1. Relative abundance of aroma compounds identified in the headspace of fresh celery samples.

Code	Compound Name	LRI [a]	ID [b]	Relative Abundance (AU) [c]						*p*-Value
				12	22	25	25x12	12x22	22x12	
	Alcohols									
A1	(*E*)-2-penten-1-ol	758	A	nd	0.53 ± 0.74	0.43 ± 0.05	nd	nd	0.83 ± 0.09	ns
A2	pentanol	762	A	nd ^{b}	nd ^{b}	nd ^{b}	0.48 ± 0.14 ^{ab}	0.68 ± 0.33 ^{a}	0.15 ± 0.21 ^{ab}	**
A3	(*Z*)-3-hexenol	849	B [21]	4.1 ± 2.5 ^{a}	4.1 ± 1.7	nd	2.0 ± 0.47	4.3 ± 1.1	1.2 ± 0.18	ns
A4	(*E*)-3-hexenol	852	A	6.2 ± 2.9 ^{a}	3.5 ± 1.8 ^{ab}	1.3 ± 0.26 ^{b}	nd ^{b}	3.7 ± 0.53 ^{ab}	0.69 ± 0.49 ^{b}	*
A5	hexanol	862	A	nd ^{b}	nd ^{b}	0.53 ± 0.03 ^{b}	0.65 ± 0.04 ^{b}	3.0 ± 0.98 ^{a}	3.6 ± 1.1 ^{a}	***
A6	octanol	1072	A	4.9 ± 0.70 ^{ab}	5.3 ± 0.61 ^{a}	1.3 ± 0.13 ^{cd}	nd ^{d}	2.9 ± 1.2 ^{bc}	3.8 ± 0.36 ^{ab}	***
A7	(*Z*)-3-nonenol	1153	B [22]	5.6 ± 2.9	6.1 ± 2.6	1.8 ± 0.81	1.3 ± 0.16	6.9 ± 1.7	5.9 ± 0.98	*
	Aldehydes									
AL1	hexanal	800	A	9.23 ± 0.33 ^{ab}	0.43 ± 0.06 ^{b}	0.15 ± 0.12 ^{b}	0.30 ± 0.05 ^{b}	0.46 ± 0.31 ^{b}	91 ± 18 ^{a}	***
AL2	benzaldehyde	964	A	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}	0.24 ± 0.04 ^{a}	nd ^{b}	***
AL3	octanal	1008	A	7.6 ± 1.4 ^{ab}	9.5 ± 2.4 ^{a}	3.6 ± 0.62 ^{bc}	2.4 ± 0.58 ^{c}	5.3 ± 1.3 ^{abc}	9.4 ± 1.1 ^{a}	**
AL4	benzeneacetaldehyde	1058	A	6.4 ± 1.3 ^{a}	6.5 ± 2.4 ^{a}	1.9 ± 0.25 ^{bc}	0.96 ± 0.43 ^{c}	3.7 ± 1.6 ^{abc}	5.2 ± 0.60 ^{ab}	**
AL5	m-tolualdehyde	1083	B [23]	nd ^{b}	19 ± 2.4 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}	16 ± 1.2 ^{a}	***
AL6	(*E*,*E*)-2,4-octadienal	1116	A	2.0 ± 1.1 ^{b}	nd ^{b}	nd ^{b}	nd ^{b}	1.6 ± 0.57 ^{b}	4.2 ± 0.72 ^{a}	***
AL7	(*E*,*E*)-2,6-nonadienal	1155	A	2.3 ± 1.6	nd	nd	0.39 ± 0.55	nd	nd	*
AL8	(*E*)-2-nonenal	1171	A	3.2 ± 0.44 ^{a}	2.7 ± 0.46 ^{a}	0.69 ± 0.09 ^{b}	0.89 ± 0.14 ^{b}	0.69 ± 0.97 ^{b}	1.8 ± 0.07 ^{ab}	***
AL9	undecanal	1306		nd ^{c}	nd ^{c}	0.93 ± 0.28 ^{bc}	1.4 ± 0.35 ^{bc}	1.6 ± 0.44 ^{b}	3.8 ± 0.79 ^{a}	***
	Esters									
E1	allyl hexanoate	1080	A	3.9 ± 0.62 ^{ab}	nd ^{c}	2.0 ± 0.43 ^{bc}	1.2 ± 0.92 ^{bc}	3.1 ± 0.96 ^{ab}	6.0 ± 1.5 ^{a}	***
E2	(*E*,*Z*)-3,6 nonadienol acetate	1174	B [24]	4.4 ± 0.45 ^{a}	2.2 ± 0.49 ^{bc}	1.0 ± 0.12 ^{c}	1.5 ± 0.15 ^{c}	2.2 ± 0.41 ^{bc}	3.3 ± 0.48 ^{ab}	***
E3	(*Z*)-3-hexenyl butanoate	1185	A	2.5 ± 0.23 ^{b}	2.6 ± 0.10 ^{b}	nd ^{d}	nd ^{d}	1.3 ± 0.45 ^{c}	4.5 ± 0.54 ^{a}	***
E4	lavandulyl acetate	1285	B [25]	0.34 ± 0.48	0.72 ± 0.20	0.15 ± 0.22	0.64 ± 0.14	0.15 ± 0.22	1.1 ± 0.79	ns
	Ketones									
K1	acetophenone	1077	A	8.4 ± 1.1 ^{a}	nd ^{b}	1.8 ± 0.26 ^{b}	0.68 ± 0.35 ^{b}	8.2 ± 0.86 ^{a}	14 ± 1.5 ^{a}	***
K2	(*Z*)-jasmone	1405	A	2.3 ± 0.38 ^{a}	0.24 ± 0.33 ^{c}	0.48 ± 0.04 ^{bc}	0.10 ± 0.15 ^{c}	nd ^{c}	0.99 ± 0.05 ^{b}	***
	Alkanes									
AK1	nonane	897	A	17 ± 2.8 ^{b}	46 ± 1.9 ^{a}	8.4 ± 1.5 ^{b}	19 ± 1.1 ^{b}	21 ± 1.6 ^{b}	52 ± 11 ^{a}	***
AK2	decane	998	A	nd ^{c}	10 ± 3.5 ^{ab}	4.9 ± 0.93 ^{bc}	5.0 ± 0.93 ^{bc}	6.3 ± 3.2 ^{bc}	14 ± 1.3 ^{a}	***
AK3	undecane	1097	A	27 ± 9.6	23 ± 11.2	10 ± 2.1	9.3 ± 1.9	12 ± 4.1	22 ± 5.1	ns
AK4	dodecane	1197	A	14 ± 9.6	6.3 ± 3.6	1.5 ± 0.65	2.9 ± 0.85	4.5 ± 1.2	6.8 ± 0.60	ns
AK5	tridecane	1297	A	18 ± 1.2	4.0 ± 3.8	1.1 ± 0.20	1.1 ± 0.92	1.7 ± 1.3	1.9 ± 1.2	ns
AK6	tetradecane	1397	A	40 ± 1.5	9.5 ± 7.9	3.2 ± 1.8	2.7 ± 2.0	4.6 ± 3.5	5.5 ± 2.8	ns

Table 1. *Cont.*

Code	Compound Name	LRI [a]	ID [b]	Relative Abundance (AU) [c]						*p*-Value
				12	22	25	25x12	12x22	22x12	
AK7	pentadecane	1498	A	35 ± 9.1	9.3 ± 6.1	3.3 ± 0.84	3.3 ± 1.9	6.0 ± 3.9	3.2 ± 2.3	ns
AK8	hexadecane	1599	A	17 ± 11	4.6 ± 2.2	1.7 ± 0.71	1.8 ± 0.84	3.4 ± 1.8	4.0 ± 1.3	ns
AK9	heptadecane	1699	A	8.2 ± 2.6 ^{a}	2.3 ± 0.49 ^{b}	0.99 ± 0.08 ^{b}	1.0 ± 0.20 ^{b}	2.2 ± 1.1 ^{b}	2.8 ± 0.13 ^{b}	***
AK10	octadecane	1800	A	nd	0.76 ± 0.20	0.13 ± 0.19	0.25 ± 0.19	0.32 ± 0.45	0.75 ± 0.17	*
	Monoterpenes									
M1	α-thujene	932	B [26]	10 ± 1.8 ^{a}	4.8 ± 0.42 ^{b}	2.7 ± 0.39 ^{b}	3.7 ± 0.49 ^{b}	4.2 ± 0.49 ^{b}	5.0 ± 0.45 ^{b}	***
M2	α-pinene	941	A	22 ± 2.9 ^{a}	24 ± 2.1 ^{a}	6.2 ± 0.97 ^{b}	8.5 ± 0.80 ^{b}	19 ± 1.8 ^{a}	20 ± 2.8 ^{a}	***
M3	camphene	958	A	5.6 ± 0.59 ^{a}	6.0 ± 1.3 ^{a}	2.0 ± 0.13 ^{b}	2.5 ± 0.25 ^{b}	4.3 ± 0.46 ^{ab}	5.4 ± 0.81 ^{a}	***
M4	sabinene	980	A	34 ± 5.5 ^{a}	18 ± 5.9 ^{b}	5.8 ± 1.1 ^{b}	8.7 ± 1.3 ^{b}	12 ± 1.1 ^{b}	19 ± 6.8	**
M5	β-pinene	987	A	110 ± 15 ^{ab}	122 ± 23 ^{ab}	70 ± 12 ^{b}	86 ± 12 ^{b}	120 ± 8.2 ^{ab}	145 ± 23 ^{a}	**
M6	myrcene	990	A	799 ± 67 ^{a}	100 ± 9.0 ^{bcd}	42 ± 4.4 ^{d}	59 ± 7.7 ^{cd}	149 ± 24 ^{bc}	173 ± 25 ^{b}	***
M7	*p*-mentha-2,8-diene	1005	B [27]	2.5 ± 1.1	5.2 ± 0.89	nd	nd	3.3 ± 1.1	4.3 ± 0.64	*
M8	α-phellandrene	1013	A	19 ± 2.6 ^{a}	14 ± 2.6 ^{ab}	6.3 ± 0.87 ^{c}	5.5 ± 1.1 ^{c}	9.6 ± 2.1 ^{bc}	17 ± 0.80 ^{a}	***
M9	δ-3-carene	1019	A	1.2 ± 1.6	nd	nd	0.82 ± 0.19	nd	nd	ns
M10	α-terpinene	1024	A	30 ± 5.6 ^{a}	14 ± 1.9 ^{b}	8.0 ± 0.89 ^{b}	11 ± 3.0 ^{b}	8.1 ± 2.7 ^{b}	14 ± 2.4 ^{b}	***
M11	*o*-cymene	1030	A	469 ± 11 ^{a}	190 ± 22 ^{de}	128 ± 20 ^{e}	213 ± 0.16 ^{cd}	299 ± 37 ^{b}	267 ± 14 ^{bc}	***
M12	limonene	1037	A	6524 ± 207 ^{a}	3259 ± 236 ^{b}	1188 ± 89 ^{d}	1285 ± 84 ^{d}	2371 ± 246 ^{c}	3638 ± 441 ^{b}	***
M13	β-(*E*)-ocimene	1048	B [28]	54 ± 6.2 ^{a}	63 ± 2.3 ^{a}	13 ± 0.89 ^{c}	5.1 ± 0.95 ^{c}	34 ± 8.6 ^{b}	45 ± 7.2 ^{ab}	***
M14	γ-terpinene	1065	A	1455 ± 112 ^{a}	732 ± 127 ^{b}	329 ± 39 ^{c}	539 ± 96 ^{bc}	389 ± 89 ^{bc}	689 ± 179 ^{bc}	***
M15	*p*-cymenene	1095	A	nd ^{b}	19 ± 2.6 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}	7.0 ± 9.9 ^{ab}	**
M16	terpinolene	1096	A	38 ± 4.6 ^{a}	nd ^{c}	7.0 ± 0.48 ^{bc}	6.5 ± 1.0 ^{bc}	14 ± 3.9 ^{b}	11 ± 7.6 ^{bc}	***
M17	β-thujone	1119	A	1.9 ± 1.3	0.58 ± 0.82	0.45 ± 0.32	0.13 ± 0.18	nd	nd	ns
M18	allo-ocimene	1130	B [29]	150 ± 16 ^{ab}	177 ± 13 ^{a}	30 ± 3.2 ^{c}	9.2 ± 0.74 ^{c}	106 ± 20 ^{b}	144 ± 17 ^{ab}	***
M19	*p*-1,3,8 menthatriene	1134	B [30]	6.2 ± 8.7	11 ± 7.7	2.4 ± 1.7	1.2 ± 0.05	13 ± 2.0	8.7 ± 6.1	ns
M20	trans-allo-ocimene	1144	B [31]	81 ± 5.9 ^{a}	79 ± 8.6 ^{a}	20 ± 2.3 ^{bc}	12 ± 2.9 ^{c}	42 ± 11 ^{b}	78 ± 11 ^{a}	***
M21	camphor	1157	A	nd ^{c}	2.2 ± 0.16 ^{b}	nd ^{c}	nd ^{c}	1.9 ± 0.39 ^{b}	3.2 ± 0.28 ^{a}	***
M22	pentylcyclohexa-1,3-diene	1161	B [32]	3.3 ± 0.64 ^{b}	5.4 ± 1.2 ^{b}	16 ± 1.1 ^{ab}	17 ± 2.0 ^{ab}	56 ± 13 ^{a}	25 ± 7.1 ^{ab}	*
M23	*trans*-dihydrocarvone	1206	A	4.1 ± 0.95 ^{a}	1.9 ± 0.41 ^{b}	1.3 ± 0.86 ^{b}	0.91 ± 0.19 ^{b}	1.9 ± 0.34 ^{b}	2.7 ± 0.32 ^{ab}	**
M24	safranal	1215	A	11 ± 2.6 ^{a}	4.6 ± 0.69 ^{bc}	1.5 ± 0.63 ^{c}	2.5 ± 0.68 ^{c}	2.7 ± 0.98 ^{c}	7.9 ± 0.44 ^{ab}	***
M25	β-cyclocitral	1235	A	3.6 ± 0.79 ^{a}	1.9 ± 0.50 ^{ab}	0.73 ± 0.19 ^{b}	1.0 ± 0.29 ^{b}	0.81 ± 0.61 ^{b}	3.5 ± 0.35 ^{a}	***
M26	L-carvone	1251	A	2.5 ± 0.86 ^{ab}	2.1 ± 0.57 ^{ab}	nd ^{c}	0.89 ± 0.18 ^{bc}	1.5 ± 0.39 abc	2.9 ± 0.64 ^{a}	***
M27	D-carvone	1259	A	3.5 ± 0.31	2.9 ± 1.2	1.5 ± 0.51	1.4 ± 0.23	1.7 ± 0.39	3.4 ± 0.77	*
M28	carvacrol	1318	A	nd ^{b}	nd ^{b}	0.12 ± 0.17 ^{b}	0.42 ± 0.09 ^{b}	0.51 ± 0.39 ^{ab}	1.1 ± 0.15 ^{a}	**

Table 1. *Cont.*

Code	Compound Name	LRI [a]	ID [b]	Relative Abundance (AU) [c]						*p*-Value
				12	22	25	25x12	12x22	22x12	
	Monoterpenoid Alcohols									
MA1	(+)-*cis*-*p*-mentha-2,8-dien-1-ol	1124	A	5.0 ± 1.1 ^{a}	5.5 ± 0.35 ^{a}	0.95 ± 0.17 ^{b}	0.15 ± 0.21 ^{b}	4.7 ± 0.97 ^{a}	4.0 ± 0.15 ^{a}	***
MA2	fenchol	1127	A	0.55 ± 0.76	nd	nd	0.14 ± 0.19	nd	0.87 ± 0.64	ns
MA3	*trans*-carveol	1225	B [33]	9.8 ± 4.5 ^{a}	1.9 ± 0.18 ^{c}	0.99 ± 0.10 ^{d}	1.4 ± 0.10 ^{cd}	1.7 ± 0.13 ^{c}	3.0 ± 0.26 ^{b}	***
MA4	*cis*-carveol	1238	A	3.3 ± 0.10 ^{a}	2.3 ± 0.18 ^{a}	0.63 ± 0.48 ^{b}	0.63 ± 0.18 ^{b}	0.45 ± 0.63 ^{b}	2.6 ± 0.16 ^{a}	***
MA5	(*Z*)-8-hydroxy linalool	1346	B [34]	2.7 ± 0.43 ^{a}	0.76 ± 0.08 ^{c}	0.27 ± 0.19 ^{c}	0.59 ± 0.14 ^{c}	0.50 ± 0.37 ^{c}	1.7 ± 0.12 ^{b}	***
	Sesquiterpenes									
S1	α-ylangene	1387	B [35]	3.1 ± 1.1 ^{a}	3.0 ± 0.65 ^{a}	1.7 ± 0.16 ^{ab}	0.69 ± 0.09 ^{b}	1.1 ± 0.39 ^{b}	1.8 ± 0.17 ^{ab}	**
S2	α-copaene	1392	A	nd ^{e}	9.2 ± 0.11 ^{a}	6.2 ± 0.18 ^{b}	2.0 ± 0.18 ^{d}	1.8 ± 0.30 ^{d}	4.5 ± 0.43 ^{c}	***
S3	(*E*)-β-caryophyllene	1427	B [31]	2.2 ± 0.42 ^{a}	0.25 ± 0.35 ^{b}	0.49 ± 0.05 ^{b}	0.33 ± 0.07 ^{b}	nd ^{b}	0.87 ± 0.68 ^{b}	**
S4	β-caryophyllene	1442	A	217 ± 9.8 ^{a}	71 ± 1.3 ^{c}	60 ± 1.2 ^{cd}	46 ± 4.5 ^{d}	44 ± 8.4 ^{d}	97 ± 11 ^{b}	***
S5	(+)-aromadend rene	1461	A	2.2 ± 0.10 ^{ab}	1.2 ± 0.38 ^{cd}	2.7 ± 0.42 ^{a}	0.21 ± 0.30 ^{d}	0.98 ± 0.32 ^{cd}	1.5 ± 0.14 ^{bc}	***
S6	curcumene	1470	B [36]	3.3 ± 0.15 ^{a}	nd ^{b}	0.78 ± 0.11 ^{b}	0.72 ± 0.13 ^{b}	nd ^{b}	0.59 ± 0.83 ^{b}	***
S7	α-humulene	1477	A	19 ± 1.2 ^{a}	12 ± 0.69 ^{b}	4.5 ± 0.10 ^{c}	6.3 ± 0.66 ^{c}	6.1 ± 1.3 ^{c}	11 ± 0.89 ^{b}	***
S8	γ-himachalene	1493	B [33]	2.8 ± 0.33 ^{a}	2.1 ± 0.16 ^{ab}	1.1 ± 0.05 ^{c}	0.92 ± 0.14 ^{c}	1.3 ± 0.35 ^{bc}	2.3 ± 0.19 ^{a}	***
S9	β-selinene	1511	B [33]	192 ± 14 ^{a}	31 ± 0.93 ^{c}	24 ± 0.82 ^{c}	24 ± 1.9 ^{c}	29 ± 4.7 ^{c}	59 ± 4.9 ^{b}	***
S10	valencene	1515	A	261 ± 31 ^{a}	3.5 ± 1.5 ^{b}	3.6 ± 0.16 ^{b}	1.6 ± 0.16 ^{b}	34 ± 4.4 ^{b}	33 ± 2.4 ^{b}	***
S11	α-selinene	1519	B [32]	22 ± 1.3 ^{a}	5.4 ± 0.16 ^{bc}	3.7 ± 0.19 ^{c}	3.2 ± 0.27 ^{c}	3.8 ± 0.64 ^{c}	7.4 ± 0.71 ^{b}	***
S12	(*E*)-nerolidol	1540	B [37]	nd ^{d}	2.3 ± 0.19 ^{a}	1.7 ± 0.05 ^{b}	0.91 ± 0.21 ^{c}	0.21 ± 0.29 ^{d}	1.2 ± 0.11 ^{bc}	***
S13	kessane	1555	B [32]	200 ± 39 ^{a}	2.3 ± 0.30 ^{b}	0.51 ± 0.04 ^{b}	0.51 ± 0.09 ^{b}	26 ± 3.1 ^{b}	27 ± 1.9 ^{b}	***
S14	liguloxide[$]	1561	B [38]	5.2 ± 0.89 ^{a}	nd ^{b}	nd ^{b}	nd ^{b}	0.67 ± 0.11 ^{b}	0.66 ± 0.47 ^{b}	***
S15	rosifoliol	1588	B [39]	nd ^{c}	0.45 ± 0.32 ^{abc}	0.16 ± 0.23 ^{bc}	0.70 ± 0.09 ^{ab}	0.41 ± 0.29 ^{abc}	0.99 ± 0.04 ^{a}	**
S16	β-eudesmol	1633	B [40]	nd	nd	nd	0.29 ± 0.19	0.65 ± 0.92	nd	ns
	Oxides									
O1	caryophyllene oxide	1608	A	2.0 ± 0.26 ^{a}	0.30 ± 0.23 ^{d}	0.39 ± 0.05 ^{d}	0.59 ± 0.08 ^{cd}	1.2 ± 0.02 ^{bc}	1.7 ± 0.23 ^{ab}	***
	Phthalides									
P1	3-propylidene phthalide	1603	A	7.7 ± 0.91 ^{a}	0.87 ± 0.37 ^{b}	0.54 ± 0.03 ^{b}	nd ^{b}	0.46 ± 0.33 ^{b}	nd ^{b}	***
P2	3-n-butylphthalide	1675	B [9,12,13]	18 ± 7.8 ^{a}	8.7 ± 2.9 ^{ab}	3.8 ± 1.3 ^{b}	3.4 ± 0.70 ^{b}	13 ± 1.4 ^{ab}	13 ± 1.7 ^{ab}	*
P3	sedanenolide	1747	B [9,12,13]	58 ± 4.0 ^{a}	16 ± 2.9 ^{c}	5.2 ± 0.50 ^{d}	4.5 ± 0.35 ^{d}	25 ± 3.4 ^{b}	21 ± 2.2 ^{bc}	***
P4	*trans*-neocnidilide	1754	B [32]	2.7 ± 0.24 ^{a}	2.8 ± 0.33 ^{a}	1.3 ± 0.12 ^{b}	1.8 ± 0.08 ^{b}	2.7 ± 0.05 ^{a}	2.9 ± 0.19 ^{a}	***
P5	(*Z*)-ligustilide	1763	B [9,12,13]	4.0 ± 0.49 ^{a}	0.41 ± 0.08 ^{b}	0.21 ± 0.08 ^{b}	0.24 ± 0.04 ^{b}	1.0 ± 0.79 ^{b}	0.77 ± 0.10 ^{b}	***

Table 1. *Cont.*

Code	Compound Name	LRI [a]	ID [b]	Relative Abundance (AU) [c]						*p*-Value
				12	22	25	25x12	12x22	22x12	
	Unknowns									
U1	unknown 1	840		2.6 ± 0.79	nd	3.1 ± 0.71	2.0 ± 0.23	nd	4.5 ± 3.5	ns
U2	unknown 2	1076		nd [b]	19 ± 5.5 [a]	nd [b]	nd [b]	nd [b]	nd [b]	***
U3	unknown 3	1084		15 ± 2.0 [a]	nd [b]	nd [b]	2.7 ± 0.54 [b]	11 ± 3.3 [a]	nd [b]	***
U4	unknown 4	1141		2.2 ± 0.38 [a]	1.4 ± 0.98 [ab]	nd [b]	0.30 ± 0.25 [ab]	1.6 ± 0.35 [ab]	1.4 ± 0.98 [ab]	*
U5	unknown 5	1189		1.2 ± 1.7	0.62 ± 0.88	1.2 ± 1.7	0.15 ± 0.21	0.35 ± 0.49	nd	ns
U6	unknown 6	1243		2.4 ± 0.16	2.0 ± 1.1	0.93 ± 0.12	1.2 ± 0.23	2.0 ± 0.37	3.4 ± 1.3	ns
U7	unknown 7	1276		7.3 ± 1.5 [a]	4.1 ± 2.1 [ab]	1.0 ± 0.29 [b]	0.66 ± 0.09 [b]	2.2 ± 0.88 [b]	3.2 ± 0.71 [b]	**
U8	unknown 8	1450		12 ± 3.8 [a]	3.3 ± 0.53 [b]	nd [b]	2.0 ± 0.34 [b]	1.9 ± 0.48 [b]	4.3 ± 0.50 [b]	***
U9	unknown 9	1543		2.0 ± 1.7	0.38 ± 0.53	nd	0.22 ± 0.31	0.36 ± 0.50	nd	ns
U10	unknown 10	1652		5.5 ± 0.70 [a]	1.3 ± 0.35 [bc]	3.2 ± 0.62 [b]	1.2 ± 0.86 [c]	1.3 ± 0.31 [bc]	1.7 ± 0.17 [bc]	***
U11	unknown 11	1710		2.0 ± 0.50 [a]	nd [b]	nd [b]	nd [b]	nd [b]	nd [b]	***
U12	unknown 12	1758		2.1 ± 1.2 [a]	0.27 ± 0.20 [b]	0.18 ± 0.06 [b]	0.19 ± 0.08 [b]	0.87 ± 0.38 [ab]	0.44 ± 0.31 [ab]	*
U13	unknown 13	1842		1.4 ± 0.07 [a]	0.69 ± 0.10 [b]	0.11 ± 0.16 [c]	nd [c]	0.55 ± 0.10 [b]	nd [c]	***

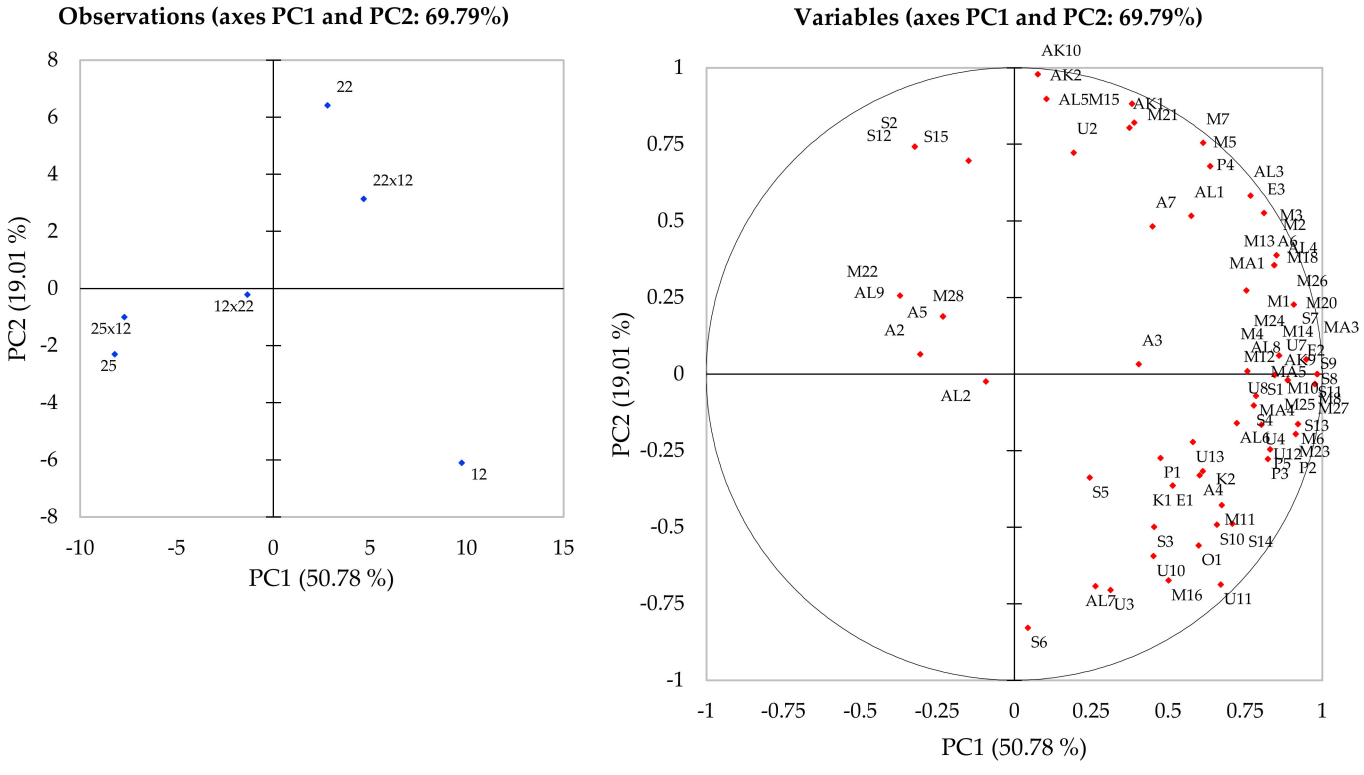
[a] Linear retention index on a DB-5 column. [b] A, mass spectrum and LRI agree with those of authentic compounds; B, mass spectrum (spectral quality value > 80 was used) and LRI agrees with reference spectrum in the NIST/EPA/NIH mass spectra database and LRI agree with those in the literature cited; $ tentatively identified, spectral quality value of 70 was used for this compound. [c] Estimated quantities (mg) collected in the headspace of celery samples containing 0.5 mL of saturated calcium chloride and filled up to 5 mL with HPLC-grade water, calculated by comparison with of 100 µg/mL propyl propanoate used as internal standard; internal standard was used to normalise chromatograms; means of three replicate samples are shown; means not labelled with the same letters are significantly different ($p < 0.05$) according to genotype and Tukey's HSD multiple pairwise comparison; nd—not detected; ns—not significant probability obtained by ANOVA; * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

Principal component analysis was used to visualise graphically the differences in the volatile composition of three parental genotypes and their hybrids and to examine any correlations occurring between genotypes (Figure 1). Using only the significant compounds according to the one-way ANOVA, a separation between genotypes was observed. Principal components one (PC1) and two (PC2) explained 69.79% of the total variation present within the data. Samples 12, 25, 25x12 and 12x22 were separated across F1, whereas samples 12, 22 and 22x12 along F2, respectively. The observation plot confirmed the findings presented in Table 2, where samples 12 and 22x12 expressed a strong association with many volatile compounds due to the high abundance identified. Conversely, samples 25 and 25x12, observed on the opposite side of the observation plot, displayed little or weak association with all volatile compounds (Figure 1). Due to the low abundance of volatile compounds, we can assume that these genotypes would be perceived as less aromatic when compared to the other genotypes. The hybrid 12x22 was positioned in the middle of the observation plot, displaying a stronger association with volatile compounds than genotype 25 and its hybrid 25x12; however, the relative abundance expressed within this hybrid remains consistently lower than 22x12 in all compound groups, except for phthalides. Thus, we could assume that this hybrid (12x22) was less aromatic than 22x12 but still had the typical, distinctive celery aroma. Comparing the aroma profile between the three parental genotypes and the hybrid lines, genotype 12 and hybrid 22x12 expressed the highest relative abundance of volatile compounds and it can be hypothesised that these will be more aromatic genotypes in comparison to the other samples. The current results (Table 1) confirmed previous work [12,13] where genotype 12 was shown to be very aromatic with strong flavour associations but low scoring in mouthfeel attributes such as crunchy and moist yet scored high for stringiness. Genotype 25 was reported to be less aromatic with a distinct cucumber flavour but was profiled as very crunchy, moist and with a firm first bite. The volatile content of genotype 22 was not significantly higher to genotype 12 or lower than 25 [12,13].

Overall, genotype 25 and hybrid 25x12 displayed clear maternal inheritance within the volatile content in terms of the compounds identified and their relative abundance. The high abundance of volatile compounds identified in genotype 12 appeared to have been inherited by hybrids 22x12 and 12x22 (Table 1). This relationship is also clear in the observation plot (Figure 1), where genotypes 12 and 22 with 22x12 and 12x22 expressing strong associations with all volatile compounds identified. We hypothesised that the parental genotypes would perform as previously [12,13] and maternal and paternal inheritance patterns become clearer upon sensory assessment, identifying phenotypic similarities between the parents and hybrids. Therefore, sensory evaluation was performed using a trained panel to further investigate these assumptions.

2.2. Sensory Evaluation of Celery Samples

The sensory profile of the three parental genotypes and hybrids was generated by a trained panel who came to the consensus of 28 terms for the quantitative assessment of celery samples and mean panel scores for these attributes are presented in Table 2. Out of the 28 attributes that were profiled, 15 of these were identified to be significantly different between genotypes. Few significant assessor x sample interactions were identified, suggesting that the panellists scored the samples in a consistent manner [41].



(**A**) (**B**)

A2	pentanol	M22	pentyl cyclohexa-1,3-diene
A3	(Z)-3-hexenol	M23	trans-dihydrocarvone
A4	(E)-3-hexenol	M24	safranal
A5	hexanol	M25	β-cyclocitral
A6	octanol	M26	L-carvone
A7	(Z)-3-nonenol	M27	D-carvone
AL1	hexanal	M28	carvacrol
AL2	benzaldehyde	MA1	(+)-cis-p-mentha-2,8-dien-1-ol
AL3	octanal	MA3	trans-carveol
AL4	benzeneacetaldehyde	MA4	cis-carveol
AL5	m-tolualdehyde	MA5	(Z)-8-hydroxy linalool
AL6	(*E*,*E*)-2,4-octadienal	S1	α-ylangene
AL7	(*E*,*E*)-2,6-nonadienal	S2	α-copaene
AL8	(E)-2-nonenal	S3	(E)-β-caryophyllene
AL9	undecanal	S4	β-caryophyllene
E1	allyl hexanoate	S5	(+)-aromadendrene
E2	(E,Z)-3,6 nonadienol acetate	S6	curcumene
E3	(Z)-3-hexenyl butanoate	S7	α-humulene
K1	acetophenone	S8	γ-himachalene
K2	(Z)-jasmone	S9	β-selinene
AK1	nonane	S10	valencene
AK2	decane	S11	α-selinene
AK9	heptadecane	S12	(E)-nerolidol
AK10	octadecane	S13	kessane
M1	α-thujene	S14	liguloxide
M2	α-pinene	S15	rosifoliol
M3	camphene	O1	caryophyllene oxide
M4	sabinene	P1	3-propylidene phthalide
M5	β-pinene	P2	3-n-butylphthalide
M6	myrcene	P3	sedanenolide
M7	p-mentha-2,8-diene	P4	trans-neocnidlide
M8	α-phellandrene	P5	(Z)-ligustilide
M10	α-terpinene	U2	unknown 2
M11	ortho-cymene	U3	unknown 3
M12	limonene	U4	unknown 4
M13	β-(E)-ocimene	U7	unknown 7
M14	γ-terpinene	U8	unknown 8
M15	p-cymenene	U10	unknown 10
M16	terpinolene	U11	unknown 11
M18	allo-ocimene	U12	unknown 12
M20	trans-allo-ocimene	U13	unknown 13
M21	camphor		

(**C**)

Figure 1. Principal component analysis of six celery samples showing correlations with volatile compounds: (**A**) projection of the samples; (**B**) distribution of variables; (**C**) compound codes as appear in plot (**B**).

Table 2. Mean panel scores for sensory attributes of six celery samples.

Code	Attribute	Scores [A]						*p*-Value [B]
		12	25	22	25x12	22x12	12x22	
				Appearance				
CA	Colour	66.9 ^{a}	31.1 ^{d}	62.9 ^{ab}	51.1 ^{c}	59.6 ^{abc}	55.6 ^{bc}	***
STA	Stalk thickness (depth of cross-section)	25.2 ^{c}	61.2 ^{a}	60.0 ^{a}	58.4 ^{a}	45.4 ^{b}	49.3 ^{ab}	***
RA	Ribbed (well-defined ribs)	77.3 ^{a}	52.5 ^{d}	61.1 ^{bc}	58.5 ^{cd}	65.1 ^{bc}	68.9 ^{b}	***
				Aroma				
FFA	Fresh fennel	16.3	14.2	18	15.9	13.1	20	ns
GGA	Grassy/green	34.5 ^{a}	19.9 ^{b}	31.3 ^{ab}	28.9 ^{ab}	29.5 ^{ab}	32.9 ^{a}	**
FPA	Fresh parsley	23.7 ^{a}	12.3 ^{b}	22.3 ^{ab}	13.1 ^{ab}	23.4 ^{ab}	16.8 ^{ab}	**
FCA	Fresh coriander	14.5	10.5	16.9	16.7	13.2	14.2	ns
				Taste/flavour				
BT	Bitter	44.5 ^{a}	26.0 ^{c}	36.1 ^{ab}	28.6 ^{bc}	32.1 ^{bc}	34.1 ^{bc}	***
ST	Sweet	3.4 ^{b}	11.7 ^{a}	7.9 ^{ab}	7.5 ^{ab}	8.9 ^{ab}	9.1 ^{ab}	*
SAT	Salt	19.1	14.9	17.6	17.3	17.9	17.6	ns
UT	Umami	2.7	4	2.9	3.7	3.3	3.6	ns
FFF	Fresh fennel	15.8	12	20.3	15.7	15.7	23.5	ns
RF	Rocket	4.8	1.1	2.5	3.9	3.4	2.9	ns
FCF	Fresh coriander	16.1	14.5	18.9	18.7	13	16.8	ns
FPF	Fresh parsley	25.9 ^{a}	9.8 ^{b}	20.9 ^{ab}	16.3 ^{ab}	20.7 ^{ab}	16.5 ^{ab}	*
SF	Soapy	18.6	10.5	13.4	16.8	15.3	15.9	ns
GGF	Grassy/green	28.4	26.5	26.5	24.4	24.4	30	ns
				Mouthfeel				
CM	Crunchy	54.7 ^{a}	55.4 ^{a}	63.8 ^{a}	65.7 ^{a}	59.3 ^{a}	63.2 ^{a}	*
SM	Stringy	68.1 ^{a}	45.2 ^{b}	44.5 ^{b}	55.3 ^{ab}	54.4 ^{b}	55.5 ^{ab}	***
MM	Moist	42.6 ^{c}	70.7 ^{a}	67.5 ^{a}	66.1 ^{a}	53.6 ^{b}	61.3 ^{ab}	***
FM	Firmness of first bite	50.5 ^{b}	54.5 ^{ab}	62.3 ^{ab}	62.2 ^{ab}	54.4 ^{ab}	65.2 ^{a}	**
				After-effects				
CAE	Celery residue in the mouth	40.4 ^{a}	29.9 ^{b}	29.8 ^{b}	31.9 ^{b}	30.5 ^{b}	34.5 ^{ab}	***
NAE	Numbness	21.7 ^{a}	10.3 ^{b}	17.6 ^{ab}	16.4 ^{ab}	16.2 ^{ab}	15.4 ^{ab}	**
BAE	Bitter	31.9 ^{a}	16.8 ^{b}	23.9 ^{ab}	22.9 ^{b}	21.2 ^{b}	22.3 ^{b}	***
UAE	Umami	3.2	3.3	3.1	1.4	3.2	3.5	ns
SAE	Salty	13.5	11.7	11.8	12.9	12.6	13.4	ns
SOAE	Soapy	11.7	9.3	9.5	13.3	12.3	12.5	ns
GGAE	Grassy/green	27.1	21.2	21.9	20.8	21.5	24	ns

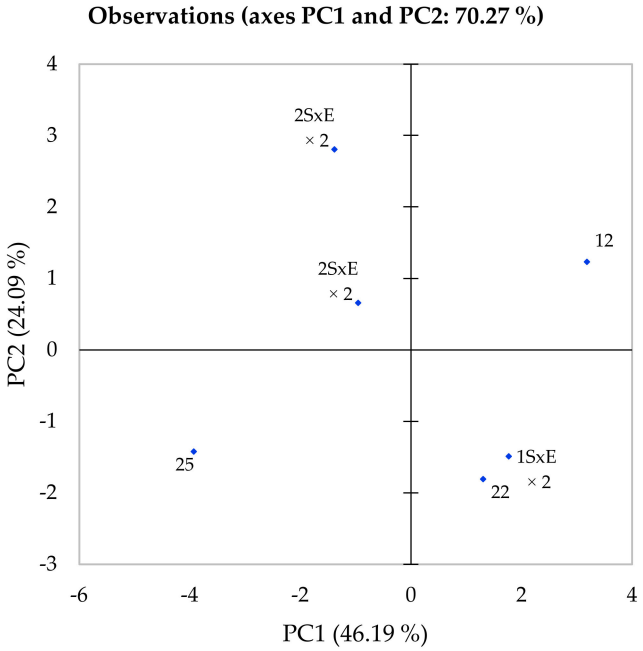
[A] Means are from two replicate samples; differing small letters $^{(a,b,c,d)}$ represent sample significance from multiple comparisons and means not labelled with the same letters are significantly different ($p < 0.05$); nd, not detected. [B] Probability obtained by ANOVA that there is a difference between means; ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

Appearance and mouthfeel attributes expressed the highest number of significant differences between genotypes. The appearance of the celery samples can be found in Table 9. Genotype 12 was scored high for appearance attributes (CA, RA) and hybrids descended from this genotype appear to have inherited these phenotypic characteristics, as high scores for both colour and ribbed were apparent. Their resemblance is also clear as shown in Table 9. Hybrid 22x12 displayed less prominent ribs and the scoring of this attribute was further decreased for 25x12 hybrid. Clearly, genotype 25 had a stronger influence on 25x12, where lower scores were observed for appearance. In terms of mouthfeel attributes, genotype 12 was shown to be the least crunchy, most stringy, with the driest petiole with a soft first bite. The genetic crosses appear to have these altered mouthfeel attributes, expressing higher scores for crunchiness, stringiness, and moistness. Hybrids 12x22 and 25x12 exhibited higher mean moistness and lower mean stringiness scores when compared to genotype 12. The data provide evidence of the influence of the female counterpart (the first number expressed in the hybrid cross) upon the appearance outcome of the offspring but when the male counterpart used displayed less prominent ribs (22 and 25), the ribbed appearance is reduced in the hybrids accordingly (Table 2).

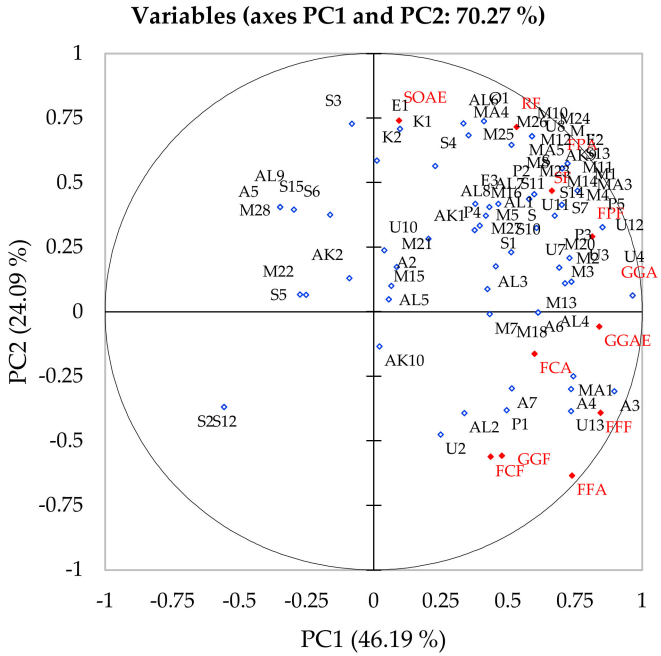
Seven out of the ten odour and flavour attributes evaluated showed no significant differences between genotypes apart from grass odour and fresh parsley odour and flavour. Genotype 12 was scored significantly higher for grass and fresh parsley odour and flavour followed by genotype 22. The resemblance in scoring is reflected by the volatile content between these parents, whereby fewer significant differences were observed (Table 1). Although the genetic code of these genotypes was not revealed, it is possible that these parents are closely related as they share several characteristics. Investigating their hybrids, 12x22 displayed a high score for grass odour, like genotype 12, whereas 22x12 was scored high for fresh parsley odour and flavour as genotype 22. The parental genotype is closely associated with the descendent hybrid, with the hybrids expressing similar appearance, odour, and flavour characteristics (Table 2).

PCA was used to visualise the sensory and chemical differences observed across the genotypes and hybrids with the volatile compounds identified (Table 1) and odour and flavour attributes (Table 2) used as variables (Figure 2). Principal components one (PC1) and two (PC2) explained 70.27% of the total variation present within the dataset where the first axis separated genotypes 22, 25 and 12x22 and the second axis separated genotypes 12, 22 and 12x22, respectively. Genotypes 12 and 25 were displayed as opposites with genotype 12 expressing associations with many aroma compounds due to the high relative abundance identified and genotype 25 displayed no association with any flavour attribute due to its low relative abundance (Table 1). The profiling of genotypes 12 and 25 reflects previous studies, whereby both 12 and 25 were profiled as high and low extremes when grown in different geographical locations and across multiple years [12,13]. Throughout these experiments, these genotypes have represented the most significantly different genotypes for all sensory attributes as well as behaved consistently in terms of their volatile profile when grown in different geographical locations and across multiple years. For this reason, they were recommended as "stable" genotypes for fresh produce growers [9,12,13]. Genotypes 12, 22 and 12x22 were mostly associated with flavour and odour attributes including fresh fennel, coriander, and parsley and with most of the volatile compounds. Hybrid 25x12 expressed lower associations with these flavour attributes due to its lower relative abundance of monoterpenes, sesquiterpenes and phthalides and low scoring by the trained panel (Tables 1 and 2).

The grass odour observed in the hybrid 12x22 was inherited from its female parent genotype 12, both expressing high relative abundance in (*Z*)- and (*E*)-3-hexenol, (*Z*)-3-hexenyl butanoate and (*E*,*Z*)-3,6-nonadienol acetate, compounds observed to express a fresh, grass-like odour. Whereas the fresh parsley odour observed in hybrid 22x12 was inherited from the female parent genotype 22, both expressing a high relative abundance of monoterpene compounds also identified in fresh parsley including α-pinene, camphene, *p*-mentha-2,8-diene and β-pinene [5,42] (Table 2). Along with this, genotype 12 was positively correlated with soapy flavour and the associations to flavour and odour attributes, combined with the high abundance of many volatile compounds (Table 1) confirms that genotype 12 is very aromatic. On the other hand, genotype 25 expresses no close association with any of the flavour and odour attributes confirming the previous statement that this genotype is not aromatic compared to genotype 12 or 22. Similar odour and flavour characteristics of genotype 25 were displayed in hybrid 25x12 (Figure 2, Table 2).



(**A**)



(**B**)

A2	pentanol	M22	pentyl cyclohexa-1,3-diene
A3	(Z)-3-hexenol	M23	trans-dihydrocarvone
A4	(E)-3-hexenol	M24	safranal
A5	hexanol	M25	β-cyclocitral
A6	octanol	M26	L-carvone
A7	(Z)-3-nonenol	M27	D-carvone
AL1	hexanal	M28	carvacrol
AL2	benzaldehyde	MA1	(+)-cis-p-mentha-2,8-dien-1-ol
AL3	octanal	MA3	trans-carveol
AL4	benzeneacetaldehyde	MA4	cis-carveol
AL5	m-tolualdehyde	MA5	(Z)-8-hydroxy linalool
AL6	(*E*,*E*)-2,4-octadienal	S1	α-ylangene
AL7	(*E*,*E*)-2,6-nonadienal	S2	α-copaene
AL8	(E)-2-nonenal	S3	(E)-β-caryophyllene
AL9	undecanal	S4	β-caryophyllene
E1	allyl hexanoate	S5	(+)-aromadendrene
E2	(E,Z)-3,6 nonadienol acetate	S6	curcumene
E3	(Z)-3-hexenyl butanoate	S7	α-humulene
K1	acetophenone	S8	γ-himachalene
K2	(Z)-jasmone	S9	β-selinene
AK1	nonane	S10	valencene
AK2	decane	S11	α-selinene
AK9	heptadecane	S12	(E)-nerolidol
AK10	octadecane	S13	kessane
M1	α-thujene	S14	liguloxide
M2	α-pinene	S15	rosifoliol
M3	camphene	O1	caryophyllene oxide
M4	sabinene	P1	3-propylidene phthalide
M5	β-pinene	P2	3-n-butylphthalide
M6	myrcene	P3	sedanenolide
M7	p-mentha-2,8-diene	P4	trans-neocnidlide
M8	α-phellandrene	P5	(Z)-ligustilide
M10	α-terpinene	U2	unknown 2
M11	ortho-cymene	U3	unknown 3
M12	limonene	U4	unknown 4
M13	β-(E)-ocimene	U7	unknown 7
M14	γ-terpinene	U8	unknown 8
M15	p-cymenene	U10	unknown 10
M16	terpinolene	U11	unknown 11
M18	allo-ocimene	U12	unknown 12
M20	trans-allo-ocimene	U13	unknown 13
M21	camphor		

(**C**)

Figure 2. Principal component analysis of six celery samples showing correlations with volatile compounds and sensory profiling: (**A**) projection of the samples; (**B**) distribution of variables, sensory attributes are highlighted in red; (**C**) compound codes as appear in plot (**B**).

In terms of the sensory attributes, grass odour and flavour and parsley flavour were positively correlated with genotype 12, 22 and their hybrids. Alcohols (A3, A4), monoterpenes (M6, M11), sesquiterpenes (S13, S14) and phthalides (P3, P4) also displayed positive correlation with these samples and attributes. Fresh parsley odour and flavour that was scored highly in genotype 22 and hybrid 22x12 expressed a positive relationship with each other accompanied by; esters (E1, E2), monoterpenes (M1-M4, M6, M8, M10, M12, M14, M20, M23–27), sesquiterpenes (S7–S9, S11, S13) and phthalides (P2, P3) (Figure 2). Many compounds displayed a positive correlation with fresh parsley which was expected due to similarities between the celery and parsley aroma composition. Genotype 25 and hybrid 25x12 displayed the lowest scores of fresh parsley aroma and flavour due to the lower relative abundance of these compounds that were identified (Table 1).

The results presented in Tables 1 and 2 showed significant differences in the aroma composition and sensory characteristics between the parental genotypes and hybrids and inherited characteristics were observed between parents and their offspring. Whether these celery hybrids meet the desires of the consumer, if there is a more preferred hybrid and what are the drivers of preference in celery was determined through the completion of a consumer trial, whereby the consumer acceptability of these hybrids and parental genotypes was investigated.

2.3. Consumer Evaluation of Celery Samples

One hundred and eighteen consumers evaluated the celery samples, and the demographic data are summarised in Table 3. A higher proportion of the consumers were female (63.6%), and the mean and median ages were 34.9 and 30, respectively. Close to half of the consumers were working (48.3%) and 47.5% were students. In total, 43.2% of consumers related to the food and nutrition department at the University of Reading. The largest ethnic group was White (English, Welsh, Scottish, Northern Irish or British), making up 42.4% of the sample population. Most consumers taking part stated that they liked celery (70.3%) and the most frequent consumption was less than once a month (45.8%).

The mean liking scores of the celery samples are presented in Table 4. The results demonstrated a significant difference in appearance, aroma, texture, and overall liking for all the samples that were tested, with results ranging from dislike slightly to like slightly. No significant difference was identified in taste liking for all samples and all samples were scored with an average score of 5; 'neither like nor dislike'. While consumers did not like the celery samples extremely, the attributes of the hybrids, particularly 25x12 and 12x22, were scored higher for appearance, aroma and texture liking than the parental genotypes. Genotype 12 was scored the lowest for overall liking. When consumers were asked to rank the hybrids from the most liked (1) to least liked (3), no significant difference was observed; samples were scored at approximately 2, which demonstrated no significant preference.

Consumers were also asked to rank a list of six attributes that they found most important when consuming celery. The list that was presented to them contained attributes that are common in celery and in some cases, were very prominent in the samples such as the smooth exterior (not stringy). The attribute 'crunchy' was ranked as the most important followed by sweet taste, whereas the attribute bitter taste ranked as the least important when consuming celery (Table 5). Although ranked as least important, bitterness should still be considered an important characteristic to celery taste as the compounds that inflict bitterness and astringency often possess multiple health benefits upon consumption including antioxidant, anti-inflammatory, and anticancer properties [43–45]. These are predominately from non-volatile compounds such as phenolic acids and flavonoids [43–45].

Table 3. Consumer demographics and characteristics of the consumer panel.

Consumers	Number	Percentage (%)
Total number of volunteers	118	
Age		
mean	34.9	
median	30	
min	19	
max	71	
Gender		
male	42	35.6
female	75	63.6
prefer not to say	1	0.84
Working Status		
working	57	48.3
unemployed	3	2.5
student	56	47.5
other	2	1.7
working in food/nutrition/sensory sector	51	43.2
Ethnic group		
White	73	61.9
Mixed or Multiple ethnic groups	2	1.7
Asian or Asian British	21	17.8
Black, African, Caribbean or Black British	15	12.7
other ethnic group	7	5.9
Celery liking		
yes	83	70.3
no	35	29.7
Consumption Frequency		
less than once a month	54	45.8
once a month	19	16.1
2 to 3 times per month	19	16.1
once a week	13	11
2 to 4 times per week	9	7.6
once a day	4	3.4
Purchase Frequency		
once a month	80	67.8
once a week	17	14.4
never	21	17.8
Method of consumption		
I do not eat celery	15	12.7
raw (on its own)	25	21.2
raw (with condiments)	49	41.5
raw (in salads)	42	35.6
cooked (boiled, roasted, fried, on its own)	47	39.8
cooked (in soups, stocks or sauces)	68	57.6
other	6	5.1

Agglomerative Hierarchical Cluster Analysis of Consumer Data and Internal Preference Mapping

Agglomerative hierarchical cluster (AHC) analysis was completed to identify relatively homogeneous groups of consumers based on their overall liking scores. Three clusters of consumers were identified and the mean liking scores of the clusters are presented in Table 6. Consumers in cluster 1 (43.2%) neither liked or disliked hybrids 25x12 and 22x12 and expressed a moderate dislike for genotype 12. Cluster 2 (38.9%) behaved in a similar manner to cluster 1, liking slightly genotypes 25, 22 and 25x12 and neither liked or disliked genotype 12 and hybrid 22x12. Opposing clusters 1 and 2, consumers in cluster 3 (17.8%) liked slightly genotype 12 and moderately disliked 25x12 due to its strong flavour attributes.

Table 4. Liking scores and preference ranking for celery samples.

Samples	Liking [A]					Ranking [B]
	Appearance	Aroma	Taste	Texture	Overall	
12	5.7 [bc]	6.2 [a]	5.0	4.7 [c]	4.7 [b]	-
25	5.0 [c]	5.5 [b]	5.3	6.0 [ab]	5.5 [a]	-
22	6.3 [ab]	6.1 [a]	5.3	6.6 [a]	5.5 [a]	-
25x12	6.1 [b]	6.1 [ab]	5.4	6.1 [ab]	5.6 [a]	2.0
22x12	6.3 [ab]	6.1 [ab]	5.4	5.8 [b]	5.4 [ab]	2.0
12x22	6.8 [a]	6.2 [ab]	5.4	6.1 [ab]	5.6 [a]	2.1
p-value [C]	***	*	ns	***	**	ns

[A] Means not labelled with the same letters [(a,b,c)] are significantly different ($p < 0.05$); means are from 118 consumers on a 9-point hedonic scale (from dislike extremely to like extremely). [B] Mean rank (1: most preferred to 3: least preferred). [C] ns, no significant difference between means ($p > 0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

Table 5. Consumers' ranking for important attributes when consuming celery.

Attributes	Ranking [A]
Crunchy texture	2.3 [a]
Sweet taste	2.8 [ab]
Moist texture	3.8 [c]
Smooth exterior (not stringy)	3.4 [bc]
Strong aroma	4.1 [d]
Bitter taste	4.6 [cd]

[A] Mean rank (1: most important to 6: least important). Means not labelled with the same letters [(a,b,c,d)] are significantly different ($p < 0.05$).

Table 6. Overall liking of the celery samples for the cluster of consumers obtained from agglomerative hierarchical clustering.

Cluster/Percentage of Consumers	Samples [1]						*p* Value [2]	Overall Liking per Cluster [3]
	12	25	22	25x12	22x12	12x22		
1 (43.2%)	3.5 [c,AB]	4.6 [ab,ABCD]	4.5 [b,ABC]	5.5 [a,CDEFGH]	5.2 [ab,CDEF]	5.0 [ab,CDE]	***	4.7 [c]
2 (38.9%)	5.4 [b,CDEFG]	6.8 [a,H]	6.8 [a,H]	6.7 [a,GH]	5.7 [b,CDEFGH]	6.1 [ab,EFGH]	***	6.2 [a]
3 (17.8%)	6.5 [a,FGH]	4.8 [bc,BCDE]	5.2 [ab,CDEF]	3.3 [c,A]	5.1 [ab,CDEF]	6.0 [ab,DEFGH]	***	5.1 [b]
Overall liking per sample [4]	4.7 [b]	5.5 [a]	5.5 [a]	5.6 [a]	5.4 [ab]	5.6 [a]		

[1] Significant differences for the means per cluster ($p < 0.05$) within a row are denoted by differing small letters [(a,b,c)]; means are from 51 consumers for cluster 1, 46 consumers for cluster 2 and 21 consumers for cluster 3, respectively; significant differences from the interaction (sample x cluster) are denoted by differing capital letters [(A,B,C,D,E,F,G,H)]. [2] ***, significant at 0.1% level. [3] Mean for overall liking per each cluster was significantly different with $p < 0.0001$. [4] The mean for overall liking per sample is from 118 consumers and it was significantly different with $p = 0.0004$. Significant interaction between sample x cluster was observed as calculated by two-way ANOVA ($p < 0.0001$).

Labelling each participant present within each cluster as a liker or non-liker, 60.8, 82.6 and 57.1% were celery likers in clusters 1, 2 and 3. Interestingly, cluster 3 contained the highest proportion of celery non-likers and they liked the most genotype 12, a genotype that expressed a high abundance of volatile compounds and profiled as very aromatic with a strong bitter taste, whereas 25x12 was the least liked and profiled as less aromatic (Table 2). On the other hand, hybrid 25x12 was the most liked of the hybrids according to clusters 1 and 2. One reason might be the high score of crunchiness and moist mouthfeel by the trained panel (Table 2); both attributes ranked as important according to consumers (Table 5). There was also significant interaction between sample x cluster for overall liking confirming that consumers scored differently the samples in each cluster (Table 6).

Sensory attributes assessed by the trained panel (Table 2) and mean liking scores of each cluster were regressed onto the first two principal components of the consumer overall

liking data to form an internal preference map (Figure 3). Principal components one (PC1) and two (PC2) explained 47.63% of the variation in the data with hybrids and genotype 22 separated from genotypes 12 and 25 across PC1, driven by sweet taste (ST), moist mouthfeel (MM) and stalk thickness (STA) attributes. Genotypes 12 and 25 were separated across PC2 with genotype 12 being positively correlated with grass/green flavour (GGF), bitter taste (BT) and stringy mouthfeel (SM) attributes.

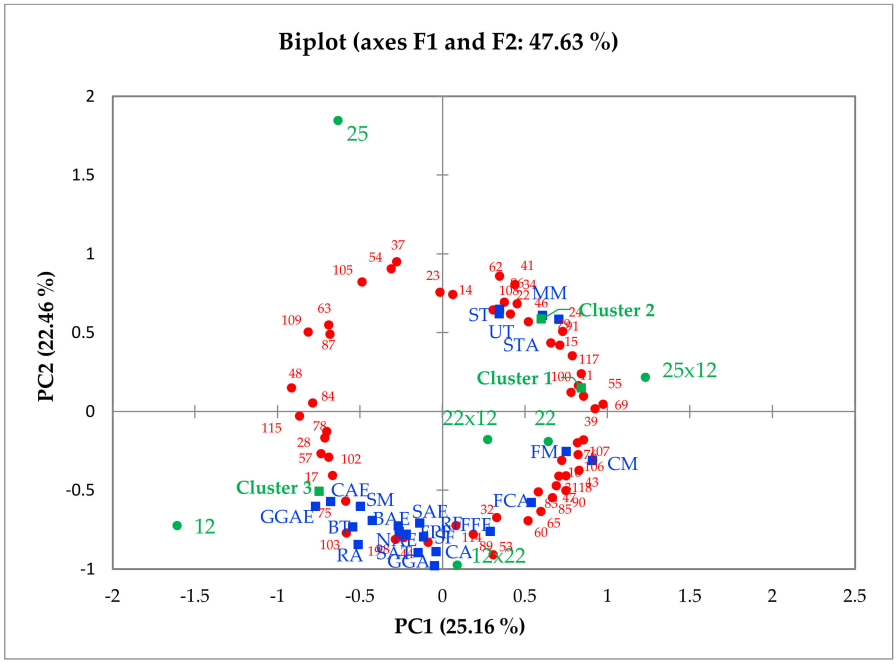


Figure 3. Internal preference map of six celery samples. Sensory attributes and consumer cluster means were regressed onto the consumer preference matrix generated by PCA. Blue squares—sensory attributes, codes correspond to those in Table 2. Green squares—clusters 1, 2, 3, mean liking positions of three clusters from AHC (Table 6). Red circles: overall liking scores of each consumer.

Cluster 1 displayed no significant relationship with any sensory characteristics (Figure 3), therefore, confirming that celery not possessing a strong aroma such as hybrids 22x12 and 25x12 (Tables 1 and 2), were more liked. Genotypes 25 and 22 and hybrid 25x12 were scored highly for stalk thickness (STA), moist mouthfeel (MM) and had a firm first bite (FM) with a sweet taste (ST) as discussed during sensory profiling (Table 2) and these attributes were closely associated to the most liked genotypes within cluster 2. Both clusters expressed no significant correlation with any flavour or odour attributes and preferred the celery that expressed low relative abundance of the volatile compounds (Table 1). For this reason, genotype 12 was the most disliked celery sample for clusters 1 and 2. Genotype 12 expressed a high relative abundance of volatile compounds (Table 1) in addition to scoring significantly higher in grass/green flavour (Table 2). Ribbed appearance (RA), grass/green aroma (GGA), bitter taste (BT) and fresh parsley aroma and flavour (FPA and FPF) were attributes positively correlated with this genotype.

Clusters 1 and 2 displayed similar overall liking scores in comparison to cluster 3. However, observed in the bottom right quadrant there appears to be a 'gap' where none of the clusters are placed (Figure 3) yet genotype 22 and hybrids 22x12 and 12x22 are positioned there. Although no cluster were associated with these hybrids, the consumers that are situated there displayed preference to celery that expressed a fresh fennel flavour and aroma accompanied by a soapy aftertaste. Hybrid 25x12 was the closest match to the highest proportion of consumers that were grouped into clusters 1 and 2. However, the hybrid requires further development with particular focus on the moist mouthfeel, stalk thickness and sweet taste attributes. These attributes are the drivers of liking for 82% of

the consumers in this study. On the other hand, the drivers of liking for those consumers placed in cluster 3 (18%) were grassy flavour and bitter taste.

Penalty analysis was used to relate Just-About-Right (JAR) data to liking scores and explain drivers of overall liking in relation to aroma, sweetness, bitterness, flavour and stringiness intensity and the results are presented in Table 7.

Table 7. Mean Just-About-Right ratings and penalty analysis showing the influence on overall liking ratings.

Samples	Overall [A]	Significance of Sample (p-Value) [B]	Penalty Analysis			
			Too Little		Too Much	
			Mean Drop	Frequency (%)	Mean Drop	Frequency (%)
	JAR Aroma					
12	2.9 [a]		0.69	24.6	1.15	17.0
25	2.5 [b]		0.49	48.3	3.30	7.6
22	2.8 [a]	**	0.70	29.7	1.54	11.9
25x12	2.7 [ab]		0.39	31.1	1.32	13.6
22x12	2.8 [a]		0.61	30.5	1.62	13.6
12x22	2.9 [a]		0.74	28.0	1.55	15.3
	JAR Bitterness					
12	3.4 [a]		1.15	15.3	2.09 *	45.8
25	2.9 [b]		0.72	28.0	2.17 *	22.9
22	3.3 [a]	**	1.45	14.4	2.09 *	40.7
25x12	3.1 [ab]		0.60 *	21.2	1.98 *	30.5
22x12	3.2 [ab]		0.52	21.2	1.56 *	33.9
12x22	3.2 [ab]		0.51	21.2	2.22 *	30.5
	JAR Sweetness					
12	2.2		1.18 *	66.1	0.53	1.7
25	2.5		1.545 *	50.9	0.06	4.2
22	2.4	ns	1.31 *	52.5	-	0.0
25x12	2.4		1.69 *	50.9	0.41	2.0
22x12	2.4		1.73 *	54.2	2.36	0.9
12x22	2.4		1.76 *	46.6	1.44	0.9
	JAR Flavour					
12	3.3 [a]		1.11	17.8	2.26 *	41.5
25	2.8 [b]		1.37 *	38.1	2.75	15.3
22	3.0 [ab]	***	1.26 *	23.7	2.28 *	40.7
25x12	3.1 [ab]		1.10 *	24.6	2.39 *	28.8
22x12	3.0 [ab]		1.16 *	22.9	1.96 *	25.4
12x22	3.1 [ab]		1.26 *	22.0	2.39 *	30.5
	JAR Stringiness					
12	4.0 [a]		1.76	5.1	1.33 *	70.3
25	3.2 [cd]		0.71	19.5	0.60	30.5
22	3.0 [d]	***	−0.57	22.9	0.59	22.0
25x12	3.4 [bc]		0.24	15.3	0.88 *	42.4
22x12	3.5 [b]		−0.19	14.4	0.90 *	49.2
12x22	3.3 [bcd]		0.62	11.9	1.64 *	35.6

[A] Means not labelled with the same letters [(a,b,c,d)] are significantly different ($p < 0.05$). [B] Represents a significant difference ($p < 0.05$) within a sample in overall liking compared with mean liking rating when the sample was considered Just-About-Right; * significant at the 5% level; ** significant at the 1% level; *** significant at 0.1% level.

When the attributes are not at the optimum intensity for a consumer this may influence the overall liking. Sweetness was ranked by the consumers as the second most important characteristic, and this was reflected in Table 7, whereby for all genotypes and hybrids, there was a negative impact on the overall liking when the sweetness of the samples was considered too low. This agreed with over 50% of the consumers in all samples. On the other hand, there was a significant drop in the liking of all samples when the bitter taste intensity was "too much" by the consumers with the genotypes 12 and 22 perceived the most bitter and genotype 25 the least bitter. Hybrid samples were scored in between the

parent genotypes. Interestingly, regarding the flavour intensity attribute, it can be observed that there was a significant drop in the liking for almost all samples when the flavour intensity of the samples was considered either "too little" or "too much". Where significant drops were observed for flavour intensity attribute, no significant drop in overall liking was observed for aroma intensity, too little or too much, displaying that consuming celery is more important for deciding preference than just smelling the sample. Stringiness, which expressed a negative correlation with crunchy texture by the sensory panel (Table 2), displayed significant drops in overall liking if samples were considered to be "too much" in genotype 12 and all the hybrids. Genotype 12 and hybrid 12x22 were considered to be the most stringy, and a mean drop of 1.3 and 0.9 in the overall liking occurred, respectively. Although scored lower, the stringiness scored by the panel of 12x22 was like genotype 12 (Table 2). The maternal inheritance of the ribbed appearance is clearly demonstrated from genotype 12 in 12x22. As texture was scored as an important attribute for consumers (Table 5), we would recommend to breeders to use a female parent that expresses the desirable appearance and textural attributes as a strong maternal inheritance has been observed in this study.

Additional comments on the samples provided by the participants contained both positive and negative points and these are shown in Table 8. Although bitter and sweet taste have been identified as drivers of disliking and liking, the results from the consumer evaluation of celery samples demonstrated that consumers could not identify differences in taste (Table 4) whereas the trained panel clearly identified significant differences between all samples in sweetness and bitterness (Table 2).

Table 8. Examples of participants' comments (three positive and three negative comments) relating to the celery samples used in this study.

Sample	Comments and Participants Details
12	Very different from any other celery I had before. This is very yummy (IP12). Flavours were balanced and texture and appearance were good and appealing (IP120). It is very good fresh smell (IP63). Would not be pleased if I had bought this Did not finish it (IP3). I was unable to break it in two due to the fibres. It was excessively stringy, and the flavour was too strong too (IP32). It was very stringy. The aroma and taste was herbal (IP62)
25	Had a slight salty taste which I liked (IP117). This one is very juicy (IP65). Good texture and light overall flavour (IP19). Looked very pale. Bland flavour (IP51). Too pale in colour (IP112). I would not buy this because of the colour (IP88).
22	Very juicy in texture (IP14). This sample will be a good quality celery that I'm expecting when buying one (IP31). what I would expect from a good celery stick (IP49). No distinct flavour (IP59). Unpleasant after taste (IP110). Really bitter and salty (IP77)
25x12	Beautiful sample of celery (IP52). Overall good celery to taste and flavour (IP30). Crunchy and juicy (IP96). Very sweet and aromatic. Too stringy (IP116). Too stringy and rather boring overall (IP28). Too bitter, unpleasant (IP98).
22x12	Attractive celery, good cross section, and colour. Good crunch and mouthfeel not as stringy as many (IP09). I enjoyed this one was quite good and not as stringy as some of the other flavour was good and have a nice crunch (IP70). It looks more appealing (IP21). Flavour too strong and too stringy (IP7). This sample is stringy for me. Some fibres are left in mouth (IP40). This one is too stringy and bitter (IP75).
12x22	Very strong aroma and flavour. Texture and lack of strings was good. Nice colour (IP11). Really liked this sample, tastes of what celery to me should taste like (IP28). Good texture and flavour. My favourite (IP122). The intense taste bothered me. It tasted bitter at the first bite (IP83). Tasted very chemical-like (IP44). Very bitter aftertaste (IP36).

Overall, there was no hybrid that was significantly preferred by the consumer with all hybrids scoring between 2.0 and 2.1 (Table 4). Both 25x12 and 22x12 were scored in a similar manner in preference ranking (Table 4) as well as in sensory analysis; however, upon combining the data collected from liking (Table 4), attribute ranking (Table 5), cluster analysis (Table 6) and JAR (Table 7), with further developing, 25x12 holds the potential to be a new hybrid that matches most of the consumers' desire. Expressing characteristics including a crunchy and moist mouthfeel, low stringiness and an odour and flavour that was not scored too highly by the panel (Tables 1 and 2, Figures 1 and 2). Contrastingly, hybrid 12x22 expressed high abundance of volatile compounds (Table 1) and was scored accordingly by the panel, with strong associations to fresh parsley flavour (Figures 2 and 3). The maternal inheritance was clear in both 12x22 and 25x12, with the characteristics of both female parents displayed within the hybrids. This was less apparent in hybrid 22x12, whereby the possibility of these genotypes being closely related causes difficulties with matching parental characteristics. The overall liking score for genotype 12 was the lowest (Table 4), possibly due to the sample expressing a stringy and dry mouthfeel attributes yet high scoring flavour attributes such as soapy, fresh parsley and grass (Table 2). This genotype was also scored as the most bitter and least sweet. Bitterness was an attribute ranked as least important and sweetness was ranked as second most important for consumers, when considering their most desirable characteristics for a celery (Table 5). 25x12 was the only hybrid that expressed a mean drop in liking if an increase or decrease in bitterness occurred (Table 7) possibly indicating that the bitter intensity of this crop is at an acceptable level for 21% of consumers. This hybrid contains genetic material from both genotypes 25 and 12, the most sweet and bitter parental genotypes, and we can clearly see that the favourable attributes of both genotypes have been passed on; the preferred mouthfeel attributes of genotype 25 combined with the distinct flavour of genotype 12 without being overpowering. The taste characteristics have been combined to produce a less bitter hybrid.

3. Materials and Methods

3.1. Celery Material and MIAPAE Standard

3.1.1. Sample Information

The three parental genotypes used in this experiment were chosen due to their differences in physical and chemical attributes and the original genetic crosses of the hybrid were carried out in 2018 at Tozer Seeds Ltd. (Pyports, UK). Although commercial confidentiality precludes revealing the exact genetic identity of each genotype used in this paper, the origins of the parental breeding lines and their image postharvest are presented in Table 9.

Table 9. Images of the petioles of the six celery samples used in this study.

	Samples					
Line	**12**	**22**	**25**	**12x22**	**22x12**	**25x12**
Origin	UK	USA	EU	-	-	-
Appearance						

3.1.2. Timing, Location and Environment

Celery seed (*Apium graveolens*) of eight parental genotypes supplied by Tozer Seeds Ltd. (Cobham, UK) were grown in commercial conditions and harvested in El Albujon, Murcia, Spain 2021 (37°43′05.5″ N 1°03′24.3″ W). Plugs were transplanted after 56 days growing in a nursery and then harvested 113 days later. Plants were lifted, packed, and despatched on the same day. Average daily air temperature was 17.7 °C, with 1.0 mm average daily rainfall; average relative humidity was 81.5%, with an average daily wind speed of 6.3 m/s.

3.1.3. Raw Material Collection, Processing Storage

The celery was grown in three randomised blocks in the centre of the field to reduce any influence from edge effects at a density of 10 plants per m^2 and three replicates were harvested from each block using a celery knife. Celery petioles were cut to 20 cm, discarding outer petioles, the base, leaves and any knuckles and sealed in labelled freezer bags with freezer blocks for transportation to the UK. Samples arrived in the UK within two days postharvest. Celery samples used for sensory and consumer evaluation were refrigerated for two further days. Samples for aroma analysis were refrigerated for two days before analysis. Panel and consumer tasting occurred on the same day as aroma analysis (P + 4).

3.2. Chemical Reagents

For GC–MS analysis, calcium chloride and the alkane standard C_6–C_{25} (100 µg/mL) in diethyl ether were obtained from Merck (Poole, UK).

3.3. Volatile Analysis Using SPME GC–MS

Prior to analysis, the fresh celery sample was macerated, and a 2 g sample was combined with 0.5 mL of saturated calcium chloride solution and filled up to 5 mL with HPLC-grade water in a 15 mL SPME vial fitted with a screw cap lid. After equilibration at 37 °C for 10 min, a 75 µm DVB/CAR/PDMS fibre (Supelco, Bellefonte, PA, USA) was exposed to the headspace above the samples for 30 min. Throughout equilibration and fibre exposure, the sample was constantly agitated at a rate of 500 rpm. Samples were analysed by automated headspace SPME using an Agilent 110 PAL injection system and Agilent 7890 gas chromatograph with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA) with a DB5 column (30 m × 0.25 mm × 0.25 µm) from Agilent (Palo Alto, CA, USA) and the identification of volatile compounds was conducted as described by Turner et al. [9].

3.4. Sensory Profiling

Sensory evaluation was carried out using quantitative descriptive analysis (QDA™) to determine the sensory characteristics of the celery samples and the characteristics were estimated quantitatively as suggested by Stone, Sidel, Oliver, Woolsey and Singleton [46]. The trained sensory panel at the Sensory Science Centre (University of Reading, n = 12; 11 female and 1 male) was used to develop a consensus vocabulary to describe the sensory characteristics of the three celery genotypes and three celery hybrids. During the development of the sensory profile, the panellists were asked to describe the appearance, odour, taste, flavour, mouthfeel and aftereffects of the samples in order to produce as many descriptive terms as seemed appropriate. References were used to help confirm the characteristics of certain attributes including fresh and dried fennel, salad rocket, flat leaf parsley and fresh coriander. The terms were discussed by the panellists as a group, with the help of the panel leader, and this led to a consensus of 28 attributes. Due to the COVID-19 pandemic restrictions, the trained panel assessed the samples from home. Vocabulary refreshment and training sessions occurred prior to scoring virtually on the Teams platform. Samples were prepared and were sent out to panellists using chilled transport couriers. The panellists completed their scoring simultaneously using Compusense Cloud software (Version 21.0.7713.26683, Compusense, Guelph, ON, Canada) whilst on video on Teams. Celery petioles presented to the panellists were chosen to be as uniform as possible. The

first outer petioles were removed and discarded. The next ring of petioles was used, and these were washed with filtered water and cut to 15 cm petiole length. The panellists scored in duplicate for each sample in separate sessions. Samples, coded with three-digit random numbers, were provided in a monadic balanced order, with sample sets randomly allocated to panellists. The panellists were asked to assess the appearance first; to break the petiole in half to assess the odour; to bite from the middle for taste, flavour and mouthfeel; and then after 30 s delay to assess the aftereffects. The intensity of each attribute for each sample was recorded on a 100-point unstructured line scale. Between samples, the panellists cleansed their palate with water and crackers.

3.5. Consumer Evaluation

One hundred and eighteen volunteers were recruited across the University of Reading (male and female, aged 18 years and above, non-smokers and without allergies or intolerances to wheat, gluten and/or celery). This study was performed as an at-home study due to ongoing COVID-19 restrictions, complying with social distancing and COVID-19 guidelines, as well as risk assessments in place. This study was fully explained to the volunteers and their informed written consent was obtained prior to participation. Participants collected their samples from the Sensory Science Centre (University of Reading) along with palate cleanser (crackers) and other information regarding how to access this study online. Participants were asked to complete this study within 24 h and keep the samples refrigerated until ready to begin the test. Participants were asked, after observing the samples, to rate their liking (appearance, aroma, taste, texture and overall) on a 9-point hedonic scale (where 1: dislike extremely, 5: neither like nor dislike, 9: like extremely) for all samples. They also indicated the appropriateness of attribute level on a 5-point Just-About-Right (JAR) scale for the following attributes: aroma intensity, bitterness, sweetness, flavour intensity and stringiness (where 1: much too low, 3: JAR and 5: much too strong). Participants were asked to indicate their preference for the hybrid genotypes only (25x12, 22x12 and 12x22) and rank various celery characteristics such as smooth exterior, moist texture, crunchy texture, sweet taste, bitter taste, and strong aroma (from most important to least important). Finally, participants were asked a series of demographic questions, purchase intent and celery consumption and were given the opportunity to leave additional comments after evaluating each sample if they wanted to. In total, six samples were evaluated (three parental genotypes and three celery hybrids in one session). Samples were presented to participants in a monadic balanced order using William's design, with sample sets randomly assigned to consumers. Data were collected using Compusense Cloud Software (Version 21.0.7713.26683, Compusense, Guelph, ON, Canada). The School of Chemistry, Food and Pharmacy Research Ethics Committee (SREC) provided a favourable opinion for conduct (SREC 11/2021) and this study was conducted in March 2021.

3.6. Statistical Analysis

Quantitative data for all compounds identified in the SPME GC–MS analysis were analysed by one-way analysis of variance (ANOVA) and principal component analysis (PCA) using XLSTAT Version 2020.1.3 (Addinsoft, Paris, France). For those compounds exhibiting significant difference in the one-way ANOVA, Tukey's Honest Significant Difference post hoc test was applied to determine which sample means differed significantly ($p < 0.05$) between the celery genotypes. Only those compounds exhibiting significant differences between genotype were included in the principal component analysis.

SENPAQ version 6.3 (Qi Statistics, Kent, UK) was used to carry out ANOVA of sensory panel data, where the main effects (sample and assessor) were tested against the sample by assessor interaction with sample as a fixed effect and assessor as a random effect. The means from sensory data were taken over assessors and correlated with the relative abundance means from the instrumental data via PCA using XLSTAT (Version 2020.1.3 (Addinsoft, Paris, France)). Internal preference mapping was used to relate sensory characteristics of celery samples to consumer liking data. XLSTAT was used to carry out

the following analyses: (i) PCA of the volatile and sensory panel data, (ii) one-way ANOVA for the aroma analysis and consumer liking, (iii) analysis of the preference (ranking) data using Friedman's test, (iv) agglomerative hierarchical clustering (AHC) for overall liking, (v) penalty analysis of the JAR data and (vi) internal preference mapping. In more detail, for the AHC, dissimilarity of responses was determined by Euclidean distance, and agglomeration using Ward's method (set to automatic truncation). Sample by cluster interactions were also tested by two-way ANOVA. For the penalty analysis, the influence of consumer perception of appropriateness of attribute level rating (JAR) on consumer liking was evaluated by calculating the mean drop in liking rating (scale 1–9) compared with mean liking of consumers that rated the attribute as JAR (JAR 3 on a 1–5 scale), determining whether this drop in liking score was significant.

4. Conclusions

The present study aimed to explore the sensory characteristics of new celery hybrids and their parental genotypes, identifying similarities and differences between the parents and offspring, and to evaluate consumer liking and perceptions of celery hybrids. Significant differences between parental genotypes and hybrids were observed in the aroma composition, sensory profiling, and consumer liking. In addition, non-significant differences were observed in parent genotypes and their hybrid off-spring highlighting the potential for maternal and paternal inheritance of phenotypic characteristics.

The hybrids in this study were grown in Spain (2021) and before we can confirm with confidence that we have developed a celery variety that meets the consumer demands, these hybrids must be grown in different scenarios and investigate any variation occurring within the aroma composition and changes in the sensory characteristics. Growing these hybrids in different geographical locations and over multiple years will identify the stability of these hybrid lines and examine how variables including air temperature, soil type, water composition and different agronomical techniques might influence the aroma profile. Following this up with sensory profiling will identify the impact of these variables upon the aroma composition and consumer preference for the hybrids.

The findings from this study combined with previous studies completed by the authors will contribute to further understanding how changes in the aroma and sensory profile may influence consumer acceptability and preference. This work provides knowledge and pinpoints the importance of attributes that drive consumer preference which in turn is useful to fresh produce growers and breeders. Furthermore, the information on the maternal inheritance of characteristics in celery has been displayed in this paper will aid breeders in the understanding of inheritance in celery, ultimately leading to the production of new celery hybrid lines that are consumer preference-driven based on their metabolite and sensory profile.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the School of Chemistry, Food and Pharmacy Research Ethics Committee of University of Reading (study number: SREC 11/2021 and date of approval: 2 March 2021).

Informed Consent Statement: Informed consent was obtained from all participants involved in this study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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Conflicts of Interest: The funders discussed the design of this study with the research team, but they were not involved in the collection or analysis of data. Frances Gawthrop is a co-author on this paper; she reviewed the manuscript and agreed to publish the results. The other authors declare no conflict of interest.

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