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Food Chemistry

Characterization of cooked cheese flavor: Volatile components

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Abstract: The aim of this work was to identify volatiles that contribute to the aroma of cooked cheese, including the role of fat content in their development during cooking. Volatiles and odorants in cooked mature Cheddar were identified using a combination of SPME (solid-phase microextraction)/GC–O (gas chromatography–olfactometry) and SPME/GC–MS (gas chromatography–mass spectrometry). A selection of the odorants was quantitated in six cheeses, uncooked and cooked, (mature Cheddar, high-, medium-, and low-fat mild Cheddar, mozzarella, and Parmesan). Many compounds showed significant differences between cooked and uncooked cheese; Strecker aldehydes, pyrazines, and furanones were all significantly higher in cooked cheeses than in uncooked cheese, while ethyl esters (key odorants in uncooked cheese) were not detected in any of the cooked cheese. Principal component analysis demonstrated that fat concentration in mild Cheddar was positively correlated with the formation of potential odorants (the Strecker aldehydes, methanethiol, 2-methylketones, and fatty acids) upon cooking. Potential lipid precursors for these compounds are discussed.

KEYWORDS
cooked cheese aroma, Cheddar, Phenomenex

Practical Application: This research can be used by the dairy industry to develop better cheeses, especially low- and reduced-fat cheeses, for use in cooked applications such as toppings for pizzas and ready meals. Alternatively, this research describes key volatile compounds in cooked cheese that can be used by the flavoring industry to develop authentic cooked cheese flavorings.

1 | INTRODUCTION

Cheese is an important commodity for the food industry. It is a key ingredient in many cooked dishes, such as toppings to bread, pasta, and pizza, and in melted forms like fondue. The aroma of uncooked cheese has been studied extensively and has been described as a balance between the concentrations of a wide variety of volatile compounds (Avsar et al., 2004; Carunchia Whetstine et al., 2006; Christensen & Reineccius, 1995; Drake et al., 2010; Frank et al., 2004; Suriyaphan et al., 2001; Zehentbauer & Reineccius, 2002). This "component balance theory" (Kilcawley &

O'Sullivan, 2017) states that the differences between cheese varieties can be attributed to the differences in the balance of the cheese odorants.

As a source of protein, sugars, and fats, cheese has the potential to undergo heat-induced flavor and color changes including the Maillard reaction, lipid oxidation, and caramelization. Despite the potential for flavor formation when cheese is cooked, the subject of cooked cheese aroma has received relatively little attention in the literature. Dumont et al. (1976) investigated the volatile compounds formed in gratinated gruyère and reported aldehydes, ketones, and sulfur compounds. They reported that products of protein degradation had a clear role in the aroma of cooked cheese, while the contribution of fat and its breakdown products was less clear. Recently, El-Shamy and Farag (2022) investigated the volatile compounds in heated gouda, emmental, and edam, although the heating conditions they used were mild (5 min at 120°C). They reported lipid-derived compounds (fatty acids, methyl ketones), pyrazines, and sulfur compounds in heated cheese but did not assess the importance of the volatiles to cooked cheese aroma.

Aside from cheese, aroma generation has been studied in other related cooked dairy products. Bertrand et al. (2011) identified 29 odor-active volatiles in cooked processed cheese after heat treatment reaching a maximum temperature of 150°C for 7.5 min. However, the composition (especially the moisture content), structure, and maturity of processed cheese differ substantially from the typical composition of cheese. Therefore, differences may be expected in the volatile compounds formed during the cooking of cheese and processed cheese.

This work is a comparison of the volatile compounds found in six cooked cheeses by headspace solid-phase microextraction (HS-SPME), which has previously been used to investigate the profiles of uncooked and heated cheese (Delgado et al., 2010; El-Shamy & Farag, 2022; Frank et al., 2004; Lecanu et al., 2002; Mondello et al., 2005). The focus was on Cheddar cheese, with three mild Cheddar cheeses with varying fat content (~2%–35%) produced from the same milk to explore the role of fat content in flavor formation in cooked cheese. Additionally, commercially purchased mature Cheddar and two different styles of cheese (mozzarella and Parmesan) were investigated to represent a variety of different cheeses typically used in cooked dishes in the United Kingdom. In a previous study (Sullivan et al., 2023), nonvolatiles decreased in cheese during cooking, including sugars (up to 58% decrease), amino acids (41%–72% decrease), and peptides (43%–69% decrease). It was hypothesized that these changes in nonvolatile precursors would be accompanied by corresponding volatile changes. Furthermore, it was hypothesized that differences in the precursor pool between high-fat (HF) and low-fat (LF) cheese would affect the formation of odorants during cooking.

TABLE 1 Cheeses studied, their abbreviations, and aging periods (McSweeney, 2017).

Cheese	Abbreviation	Aging period
Mozzarella	mozz	<1 months (typical)
Parmesan	parm	>22 months (manufacturer's description on packaging)
Mature Cheddar	ched	9 months (typical)
Mild Cheddar	HF (high fat), MF (medium fat), LF (low fat)	3 months

2 | MATERIALS AND METHODS

2.1 | Materials

The following aroma standards were purchased: trimethylpyrazine and 3-methyl-1,2-cyclopentanedione (IFF); 2-ethyl-3,5-dimethylpyrazine and (*E*)-2-decenal (Oxford Organics); hexanal, 2,3-butanedione, 2-heptanone, 2-methoxyphenol, dimethyl disulfide, dimethyl trisulfide, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, 4-hydroxy-2,5-dimethyl-3-furanone, 2- and 3-methylbutanoic acid, hexanoic acid, methanethiol, 2-methylpropanal, (*Z*)-4-heptenal, octanal, 1-octen-3-one, 2-methyl-3-furanthiol, nonanal, (furan-2-yl)methanethiol, 2-isobutyl-3-methoxypyrazine, (*E*)-2-nonenal, 3-(methylsulfanyl)propanal (methional), 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone, and (*E*,*E*)-2,4-nonadienal (Sigma); 2-acetyl-1-pyrroline (Aroma Lab); and 3-methyl-2-butene-1-thiol (Aroxa). All other chemicals used were obtained from Sigma–Aldrich Ltd. The internal standard was 0.25 mg/L isopropylpyrazine in aqueous saturated sodium chloride. Chemicals used for amino acid analysis were obtained from the EZ:FAAST kit from Phenomenex.

2.2 | Cheeses

Three cheeses were purchased from a supermarket: mature Cheddar (Ched), fresh mozzarella (Mozz), and Parmesan (Parm). Cheddar, mozzarella, and Parmesan represent a range of cheeses that are used in cooked dishes and vary considerably in terms of maturity. Typical aging periods for these cheeses are shown in Table 1. Three mild Cheddar cheeses of differing fat content (LF [2% fat], medium fat [MF; 22% fat], and HF [35% fat]) were made at the University of Reading's pilot plant facility (Reading, UK) as described in Section 2.3 and were included to determine the effect of fat content on the formation of cooked cheese odorants.

2.3 | Cheesemaking

The cheeses were made using a typical Cheddar process from a single batch of milk, which was skimmed to different fat contents to produce LF (2% fat), MF (22% fat), and HF (35% fat) Cheddars. The Cheddars were matured for 3 months at 8°C. For full details of the cheesemaking, see Sullivan et al. (2023).

2.4 | Cheese sample preparation

Grated cheese (50 g) was spread evenly on a glass petri dish (90-mm diameter × 10-mm depth) and baked in a GC Oven (Hewlett Packard 5890 Series II) at 180°C for 20 min. It was then cooled to room temperature, immersed in liquid nitrogen, and ground in a coffee grinder (Quest) to obtain a fine powder. The baking was performed in triplicate.

2.5 | Gas chromatography–mass spectrometry analysis

Powdered cheese (three samples from the uncooked cheese or one sample from each of the three baking triplicates for the cooked cheese) (1.5 ± 0.1 g) was transferred into a 20-mL headspace vial (Supelco) sealed with a screw top lid. Internal standard solution isopropylpyrazine (1.5 mL, 0.25 mg/L in saturated aqueous sodium chloride solution) was added, and the vial was vortexed for 30 s. The samples were incubated (50°C, 10 min) using a CTC 120 autosampler (Agilent). The volatiles in the headspace were extracted (50°C, 20 min) using a Carboxen/DVB/PDMS SPME fiber (Supelco). The fiber was desorbed at 250°C for 20 min in the injection port of a 7890A GC coupled to a 5975C Inert MS detector (both Agilent) fitted with a ZB-5MSi column (30 m, 0.25 mm, 0.25 µm) (Phenomenex). The oven temperature started at 50°C, which was held for 2 min, followed by a 6°C/min ramp to a maximum temperature of 300°C, which was held for 15 min. The carrier gas was helium at a constant flow rate of 1.9 mL/min. Mass spectra were recorded in the electron impact mode at an ionization voltage of 70 eV and source temperature of 250°C.

An alkane standard C5–C25, 10 mg/L in diethyl ether, was extracted and run under the same conditions as the samples and used as a reference for the calculation of the linear retention indices (LRIs). Compounds were identified using the MS mass spectra from the NIST 11 library and confirmed by comparison to LRIs of authentic compounds or using LRIs from the NIST Chemistry WebBook.

2.6 | Gas chromatography–olfactometry analysis

Gas chromatography–olfactometry (GC-O) analysis was performed on the cooked mature Cheddar sample only as this sample appeared from gas chromatography–mass spectrometry (GC–MS) data to have developed the most differences during cooking. Powdered Cheddar was prepared as described in Section 2.5 but without the addition of the internal standard solution. Analysis was conducted on a 7890B GC system (Agilent) fitted with an ODO II olfactory detector from SGE Analytical. An HP-5MS Ui column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies) was fitted. The carrier gas was helium at 2 mL/min. The injection port was maintained at 250°C, the oven was set to 40°C for 2 min, then initially ramped at 4°C/min until 200°C was reached, followed by a ramp of 8°C/min until 300°C was reached. The final temperature was then held for 8 min. The flow from the column was split between a flame ionization detector (FID) and a sniffing port 1:1, followed by two untreated silica-fused capillaries of the same dimensions (1 m, 0.32 mm internal diameter). The flow to the sniffing port was diluted with a moist makeup gas. The FID was kept at 250°C with flow rates of 40 mL/min hydrogen, 400 mL/min air, and 9 mL/min nitrogen. GC–O analysis was conducted in duplicate by two expert sniffers describing the odors in their own words and recording the retention time and the intensity of each odor on a scale of 1–10 (very weak to very strong). An alkane standard C5–C25 (1 mL), 10 mg/L in diethyl ether, was used as a reference for calculating the LRIs. Compounds were identified on the basis of odor (The Good Scents Company website) and verified by comparing the LRIs of authentic compounds and LRIs from the NIST Chemistry WebBook library, as well as the MS in the GC–MS chromatogram. Comparison of LRIs by GC-O on a polar FFAP column (30m x 0.25 mm x 0.25um;Restek, Saunderton, UK) further confirmed the assigment of compounds to the aroma regions. The GC-O on the FFAP column was run under the same conditions as for the HP-5MS Ui except the final hold temperature was 250C.

2.7 | Semiquantitation

The volatiles were semiquantitated according to the equation below:

$$\mathrm{Conc.}\,(A) = (\text{single ion peak area }(A) \times \text{factor}\,(A)) \\ /(\text{single ion peak area }(IS) \times \text{factor}\,(IS)) \\ \times \mathrm{conc.}(IS),$$

where A represents each analyte. Semiquantitation was performed using the peak areas of a single selected ion per analyte from the GC–MS chromatogram, relative to that of the internal standard. The "factor" was used to correct the peak area of the single ion to the peak area of the full scan chromatogram and was calculated from a clean spectrum for each analyte using the following equation:

$$\text{Factor}\,(A) = \text{peak area }(A)\,/\text{single ion peak area }(A)\,.$$

2.8 | Amino acid analysis

The amino acid analysis was reported previously in Sullivan et al. (2023) and was performed using an EZ-FAAST system (Phenomenex).

2.9 | Statistical interpretation

The determination of statistical significance between samples was performed using SPSS (IBM, version 25). The significance of differences between the data was determined using a multivariate linear model and a post hoc test of multiple comparisons for observed means using a Tukey HSD with an alpha of 0.05. Two-way ANOVA (sample × cooking) was carried out across all 12 samples and again with a subset of samples prepared under controlled conditions at the University of Reading (fat content × cooking). The principal component (PC) analysis was performed on the odor-active volatiles using XLSTAT (version 2019.4.2.63912).

3 | RESULTS AND DISCUSSION

3.1 | Identification of odorants in cooked Cheddar

Solid-phase microextraction–gas chromatography–olfactometry (SPME–GC–O) was used to identify odorants in cooked mature Cheddar. This sample was chosen as a typical cheese used in cooked applications in the United Kingdom. Odorants identified in cooked Cheddar are shown in Table 2, while semiquantitative results for these volatiles can be found in Table 3.

Of the 36 odorants identified, eight have not been reported previously in GC–O data from uncooked Cheddar (3-methyl-2-butene-1-thiol, 2-heptanone, (furan-2-yl)methanethiol, 3-methyl-1,2-cyclopentanedione, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2-methyl-3-methyldithiofuran, and (*E*)-2-decenal). These compounds are likely to contribute to the differentiation of cooked Cheddar from uncooked Cheddar aroma. The most intense odorants based on the assessor intensity score were 3-methylbutanal, methylpropanal, 2-methylbutanal, (*Z*)-4-heptenal, methional, (furan-2-yl)methanethiol, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone. The Strecker aldehydes, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 3-methyl-1,2-cyclopentanedione, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone are known products of the Maillard reaction that have been reported in other cooked foods but have not previously been related to aroma in cooked cheese.

3.2 | The effect of cooking

All volatiles were semiquantitated using GC–MS data in all six cheeses (Table 3). Two-way ANOVA showed that for all volatiles, there was a significant difference between cooked and raw cheese, and in general, trends were observed in the behavior of compounds according to their formation pathway. The graphs in Figure 1 outline semiquantitative data for one compound from each formation pathway in the cooked and uncooked cheeses as an example of the broader trends.

Three pyrazines with roasted aromas (trimethyl pyrazine, 3-ethyl-3,5-dimethylpyrazine, and 3-ethyl -2,5-dimethylpyrazine) were identified as odorants in cooked Cheddar, alongside a further six pyrazines that were detected by solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS). Each pyrazine was significantly higher in several cooked cheeses than in their uncooked counterparts. An example of the pyrazine data in cooked cheese is shown for 3-ethyl-3,5-dimethylpyrazine in Figure 1. This agrees with a previous study that reported pyrazines in heated emmental and, to a lesser extent, in heated edam and gouda, but they were not present in unheated cheese (El-Shamy & Farag, 2022).

Pyrazine formation during the Maillard reaction occurs via α-amino carbonyl compounds generated during Strecker degradation (Weenen et al., 1994). Dumont et al. (1976) reported the presence of a number of pyrazines (methylpyrazine, 2,3-dimethylpyrazine, 2,5 dimethylpyrazine, trimethylpyrazine, 2-ethyl-3,6-

TABLE 2 Odorants identified in cooked mature Cheddar by solid-phase microextraction/gas chromatography–olfactometry.

Compound (Code for Figure 2)	Odor descriptor	Intensity	LRI (GC–O) HP-5MS	LRI (GC–O) FFAP	Identity[a]	References[b]
Methanethiol (MSH)	Rotting	5	<600		Odor, lri, ms	EH
2-Methylpropanal (2MP)	Chocolate	8	<600	<800	Odor, LRI, MS	A
2,3-Butanedione (BD)	Butter	6	605	985	Odor, LRI, MS	ABDEFG
3-Methylbutanal (3MB)	Chocolate	10	653	911	Odor, LRI, MS	ADEG
2-Methylbutanal (2MB)	Chocolate	8	664		Odor, LRI, MS	D
Dimethyl disulfide (DMDS)	Savory vegetal	5	718	1047	Odor, LRI, MS	H
Butanoic acid (BA)	Sweaty	7	777	1624	Odor, LRI, MS	ACDEFG
Hexanal	Green	3	804	1077	Odor, LRI, MS	ABFG
3-Methyl-2-butene-1-thiol	Cannabis	6	823		Odor, LRI, MS	
3-Methylbutanoic acid (3MBA)	Sweaty	5	844		Odor, LRI, MS	CE
2-Methyl-3-furanthiol	Meaty	5	867		Odor, LRI, MS	BF
2-Heptanone (HPT)	Fruity, blue cheese	5	898	1178	Odor, LRI, MS	
(*Z*)-4-Heptenal	Lamb fat	8	904		Odor, LRI, MS	BFG
Methional (MET)	Potato	8	909	1450	Odor, LRI, MS	ABCDEFGH
(Furan-2-yl)methanethiol	Coffee	7	913		Odor, LRI, MS	
2-Acetyl-1-pyrroline	Basmati rice	5	926	1333	Odor, LRI, MS	BEG
Hexanoic acid (HA)	Sweaty	3	970	1838	Odor, LRI, MS	DEF
Dimethyl trisulfide (DMTS)	Sulfurous, pungent	6	974	1373	Odor, LRI, MS	ABCEFGH
1-Octen-3-one	Mushroom	4	962	1306	Odor, LRI, MS	ABCDEFG
1,5-Octadien-3-one	Geranium, metallic, earthy	3	987		Odor, lri	AEG
Trimethylpyrazine (TMP)	Pyrazine-like	5	1005		Odor, LRI, MS	H
3-Methyl-1,2-cyclopentanedione (MCPD)	Biscuit	4	1029	1822	Odor, LRI, MS	
Phenylacetaldehyde (PHE)	Floral, honey	6	1047	1639	Odor, LRI, MS	ABCF
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (FRNL)	Candy floss	7	1084	1996	Odor, LRI, MS	ACEFG
4-Methylphenol (4MP)	Fecal	5	1081		Odor, LRI, MS	BC
3-Ethyl-2,5-dimethylpyrazine (EDMP)	Pyrazine-like	3	1083	1443	Odor, LRI, MS	
2-Ethyl-3,5-dimethylpyrazine	Pyrazine-like	5	1088		Odor, lri, ms	
2-Methoxyphenol	Smoky, fire	4	1101		Odor, LRI, MS	C
Nonanal	Fruity, fatty	5	1110		Odor, LRI, MS	AFG
2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone	Sweet, maltol-like	4	1142		Odor, LRI	EG
(*E,E*)-2,6-Nonadienal	Fatty, aldehyde	6	1153		Odor, LRI	BFG
(*E*)-Nonenal	Fatty waxy	4	1165	1527	Odor, LRI	BFG
2-Methyl-3-methyldithiofuran	Meaty	4	1178		Odor, LRI	
2-Isobutyl-3-methoxypyrazine	Pepper	3	1217		Odor, LRI	BCG
(*E,E*)-2,4-Nonadienal	Fatty	4	1221		Odor, LRI	G
(*E*)-2-Decenal	Fatty waxy	4	1255		Odor, LRI	

Note: Aroma intensity was the mean value from both evaluators.

[a] Compounds were identified by verifying odor descriptors with authentic compounds or The Good Scents Company website (odor), comparison of mass spectra with those from authentic standards (MS) or with mass spectra from NIST 11 library (ms), and comparison of linear retention indices (LRIs) with authentic standards on a non-polar HP-5MS Ui column (LRI) or the NIST Chemistry WebBook (lri). LRIs were also confirmed on a polar FFAP column.

[b] Previously reported in: A = cooked processed cheese—Bertrand et al. (2011); B = uncooked Cheddar—Avsar et al., 2004; C = uncooked Cheddar—Suriyaphan et al. (2001); D = uncooked Cheddar—Christensen and Reineccius (1995); E = uncooked Cheddar—Drake et al. (2010); F = uncooked Cheddar—Carunchia Whetstine et al. (2006); G = uncooked Cheddar—Zehentbauer & Reineccius (2002); H = uncooked Cheddar—Frank et al. (2004).

TABLE 3 Semiquantitation of selected volatiles (μg/g) from a range of cooked and uncooked cheeses.

Compound	LRI ZB-5MSi	ID[a]	Cooked Parm[b]	Cooked Ched	Cooked HF	Cooked MF	Cooked LF	Cooked Mozz	Significance Cheese[c]	Significance Cook	Significance Cheese × Cook	Significance Fat × Cook
Strecker aldehydes												
2-Methylpropanal	<600	MS + LRI	5.8 a	5.9 a	3.2 b	2.3 bc	1.5 cd	0.2 d	***	***	***	ns
3-Methylbutanal	657	MS + LRI	13 c	33 ab	46 a	31 ab	17 bc	0.9 c	***	***	***	*
2-Methylbutanal	664	MS + LRI	22 a	16 b	3.4 c	2.0 c	3.1 c	0.5 c	***	***	***	ns
Benzeneacetaldehyde	1055	MS + LRI	2.4 c	3.8 ab	4.1 a	4.6 a	2.6 bc	0.08 d	***	***	***	*
α,β-Unsaturated aldehydes (aldol condensates)												
2-Methyl-2-hexenal	884	ms + lri	0.2 bc	0.3 bc	0.4 bc	0.3 bc	0.5 b	1.7 a	***	***	***	ns
(*E*)-5-methyl-2-isopropyl -2-hexen-1-al	1116	ms + lri	0.6 bc	1 b	0.4 cd	0.4 cd	2 a	–	***	***	***	***
5-Methyl-2-phenyl-2 -hexenal	1510	ms + lri	0.1 bc	0.1 b	0.08 bc	0.1 bc	0.6 a	–	***	***	***	***
Maillard reaction products (CHO)												
2-Furanmethanol	855	MS + LRI	0.4 cd	0.8 b	1.9 a	0.7 bc	0.05 de	0.3 cde	***	***	***	***
2-Acetylfuran	914	MS + LRI	0.2 bc	0.4 ab	0.7 a	0.3 bc	0.07 c	0.05 c	***	***	***	***
3-Methyl-1,2-cyclopentane-dione	1039	MS + LRI	–	0.2 a	0.1 b	0.1 b	0.01 c	0.01 c	***	***	***	***
4-Hydroxy-2,5-dimethyl -3(2H)-furanone	1069	MS + LRI	0.02 cd	0.10 a	0.05 b	0.04 bc	0.01 d	0.01 cd	***	***	***	***
Nitrogen heterocycles (pyrroles and pyrazines)												
Pyrrole	756	MS + LRI	0.2 ab	0.2 a	0.2 ab	0.1 abc	0.07 bc	0.05 c	*	***	*	ns
Methylpyrazine	831	MS + LRI	0.1 bc	0.6 a	0.2 b	0.1 bc	0.03 c	0.2 b	***	***	***	**
2,5/6-Dimethylpyrazine	920	MS + LRI	0.3 d	1.8 a	0.4 cd	0.3 cd	0.1 d	0.8 bc	***	ns	***	***
2-Ethylpyrazine	924	MS + LRI	0 cd	0.1 a	0 b	0.03 bc	0.01 cd	0.04 b	***	***	***	**
2,3-Dimethylpyrazine	929	MS + LRI	0.1 bc	0.2 a	0.1 bc	0.04 bc	0.03 bc	0.06 b	***	***	***	ns
2-Ethyl-6-methylpyrazine	1006	MS + LRI	0.1 b	0.2 a	0.1 b	0.04 bc	0.04 bc	0.04 bc	***	***	***	ns
2-Ethyl-5-methylpyrazine	1011	MS + LRI	0.03 d	0.4 a	0.2 bc	0.2 bc	0.1 cd	0.2 b	***	***	***	ns
Trimethylpyrazine	1013	MS + LRI	0.2 bcd	1.2 a	0.3 b	0.3 b	0.2 bcd	0.3 bc	***	***	***	ns
2-Ethenyl-5-methylpyrazine	1026	MS + LRI	–	0.1 a	–	–	–	0.03 b	***	***	***	
2-Acetylpyrrole	1072	MS + LRI	0.03 cd	0.4 a	0.1 b	0.06 bc	0.01 d	0.01 d	***	***	***	***
3-Ethyl-2,5-dimethylpyrazine	1085	MS + LRI	0.2 b	0.6 a	0.1 b	0.1 b	0.2 b	0.1 bc	***	***	***	

(Continues)

TABLE 3 (Continued)

Compound	LRI ZB-5MSi	ID[a]	Cooked Parm[b]	Cooked Ched	Cooked HF	Cooked MF	Cooked LF	Cooked Mozz	Significance Cheese[c]	Significance Cook	Significance Cheese × Cook	Significance Fat × Cook
Sulfur compounds												
Methanethiol	<600	MS + LRI	0.4 ab	0.6 a	0.3 abc	0.2 bcd	0.08 cd	0.01 d	***	***	***	*
Dimethyl disulfide	750	MS + LRI	1.2 a	0.4 bc	0.6 abc	0.3 bc	1.1 ab	0.2 c	*	***	**	ns
Methional	910	MS + LRI	0.4 c	0.8 a	0.6 b	0.4 c	0.1 d	0.01 d	***	***	***	***
Dimethyl trisulfide	985	MS + LRI	0.7 ab	0.3 bc	0.2 c	0.1 c	0.7 ab	0.1 c	***	**	***	***
Esters												
Ethyl butanoate	801	MS + LRI	–	–	–	–	–	–	***	***	***	***
Butyl acetate	815	MS + LRI	–	–	–	–	–	–	***	***	***	***
Ethyl 2-methylbutanoate	846	MS + LRI	–	–	–	–	–	–	***	***	***	***
Ethyl 3-methylbutanoate	851	MS + LRI	–	–	–	–	–	–	***	***	***	***
3-Methylbutyl acetate	875	MS + LRI	–	–	–	–	–	–	***	***	***	***
Methyl hexanoate	931	MS + LRI	–	–	–	–	–	–	***	***	***	***
Ethyl hexanoate	998	MS + LRI	–	–	–	–	–	–	***	***	***	***
Hexyl acetate	1011	MS + LRI	–	–	–	–	–	–	***	***	***	***
2-Propenyl hexanoate	1080	ms + lri	–	–	–	–	–	–	***	***	***	***
Methyl octanoate	1122	MS + LRI	–	–	–	–	–	–	***	***	***	***
Ethyl octanoate	1164	MS + LRI	–	–	–	–	–	–	***	***	***	***
Ethyl decanoate	1392	MS + LRI	–	–	–	–	–	–	***	***	***	***
Ketones												
2-Heptanone	893	MS + LRI	5.7 b	10.3 a	11.8 a	12.6 a	1.3 c	5.8 b	***	***	***	***
2-Nonanone	1095	MS + LRI	2 b	3.4 a	3.7 a	4.1 a	0.7 cd	1.2 bc	***	***	***	**
2-Undecanone	1298	MS + LRI	0.3 b	0.6 a	0.6 a	0.7 a	0.1 c	0.2 bc	***	***	***	**
2-Tridecanone	1502	MS + LRI	0.1 b	0.2 a	0.3 a	0.3 a	0.05 bc	0.08 bc	***	***	***	***
Lactones												
δ-Decalactone	1507	MS + LRI	–	0.06 a	0.04 bc	0.04 b	–	0.01 de	***	***	***	***
δ-Dodecalactone	1726	MS + LRI	0.1 ab	0.08 a	0.06 ab	0.1 ab	0.01 e	0.02 de	***	***	***	ns
Acids												
Acetic acid	<600	MS + LRI	0.458 a	5.9 b	2.8 c	2.5 cd	0.6 cd	1.1 cd	***	***	ns	ns
Butanoic acid	788	MS + LRI	18 b	4 cd	2.5 def	3 def	0.4 f	0.1 f	***	***	***	*
3-Methylbutanoic acid	835	MS + LRI	0.1 a	0.08 b	0.04 c	0.04 c	0.01 d	–	***	***	***	***
2-Methylbutanoic acid	845	MS + LRI	0.05 a	–	–	–	–	–	***	***	***	***
Pentanoic acid	876	MS + LRI	0.1 b	–	–	1.5 d	–	–	***	***	***	***
Hexanoic acid	975	MS + LRI	12 b	1.5 cdef	0.9 def	–	0.6 ef	0.1 f	***	***	***	***

(Continues)

TABLE 3 (Continued)

Compound	LRI ZB-5MSi	ID[a]	Cooked Parm[b]	Cooked Ched	Cooked HF	Cooked MF	Cooked LF	Cooked Mozz	Significance Cheese[c]	Significance Cook	Significance Cheese × Cook	Significance Fat × Cook
Heptanoic acid	1066	MS + LRI	–	–	–	–	–	–	***	***	***	***
Octanoic acid	1165	MS + LRI	1.4 b	0.2 cd	0.2 cd	0.4 cd	0.3 cd	0.03 d	***	***	***	***
Nonanoic acid	1258	MS + LRI	–	–	–	0.01 b	0.1 a	0.01 b	**	*	**	**
Decanoic acid	1360	MS + LRI	0.3 b	0.07 c	0.07 c	0.2 bc	0.08 c	–	***	***	***	**
Undecanoic acid	1465	MS + LRI	–	–	–	–	–	–	***	***	***	***
Miscellaneous												
4-Methylphenol	1076	MS + LRI	0.01 a	0.012 c	0.003 -	0.002 cde	0.003 cd	0.001 de	***	***	***	ns
2,3-Butanedione	<600	MS + LRI	1.2 b	1.4 b	1.7 b	1.6 b	1.2 b	2.5 a	***	***	***	ns
	LRI expt	ID	Un-cooked Parm	Un-cooked Ched	Un-cooked HF	Un-cooked MF	Uncooked LF	Uncooked Mozz	Significance Cheese	Significance Cook	Significance Cheese × Cook	Significance Fat × Cook
Strecker aldehydes												
2-Methylpropanal	<600	MS + LRI	–	–	–	–	–	–	***	***	***	ns
3-Methylbutanal	657	MS + LRI	0.2 c	0.1 c	0.04 c	0.05 c	0.06 c	–	***	***	***	*
2-Methylbutanal	664	MS + LRI	0.2 c	0.2 c	0.1 c	0.12 c	0.3 c	0.03 c	***	***	***	ns
Benzeneacetaldehyde	1055	MS + LRI	0 d	0.08 d	0.05 d	0.06 d	0.02 d	–	***	***	***	*
α,β-Unsaturated aldehydes (aldol condensates)												
2-Methyl-2-hexenal	884	ms + lri	–	–	–	–	–	–	***	***	***	ns
(*E*)-5-Methyl-2-isopropyl -2-hexen-1-al	1116	ms + lri	–	–	–	–	–	–	***	***	***	***
5-Methyl-2-phenyl-2-hexenal	1510	ms + lri	–	–	–	–	–	–	***	***	***	***
Maillard reaction products (CHO)												
2-Furanmethanol	855	MS + LRI	0.1 de	0.01 e	–	–	–	–	***	***	***	***
2-Acetylfuran	914	MS + LRI	–	–	–	–	–	–	***	***	***	***
3-Methyl-1,2-cyclopentanedione	1039	MS + LRI	–	–	–	–	–	–	***	***	***	***
4-Hydroxy-2,5-dimethyl-3 (2H)-furanone	1069	MS + LRI	–	–	–	–	–	–	***	***	***	***

(Continues)

TABLE 3 (Continued)

	LRI expt	ID	Un-cooked Parm	Un-cooked Ched	Un-cooked HF	Un-cooked MF	Uncooked LF	Uncooked Mozz	Significance Cheese	Significance Cook	Significance Cheese × Cook	Significance Fat × Cook
Nitrogen heterocycles (pyrroles and pyrazines)												
Pyrrole	756	MS + LRI	–	–	–	–	–	–	*	***	*	ns
Methylpyrazine	831	MS + LRI	–	–	–	–	–	–	***	***	***	**
2,5/6-Dimethylpyrazine	920	MS + LRI	0.2 d	0.2 d	2.2 a	1.1 b	0.01 d	–	***	ns	***	***
2-Ethylpyrazine	924	MS + LRI	–	–	–	–	–	–	***	***	***	**
2,3-Dimethylpyrazine	929	MS + LRI	–	–	–	–	–	–	***	***	***	ns
2-Ethyl-6-methylpyrazine	1006	MS + LRI	–	–	–	–	–	–	***	***	***	ns
2-Ethyl-5-methylpyrazine	1011	MS + LRI	–	–	–	–	–	–	***	***	***	ns
Trimethylpyrazine	1013	MS + LRI	–	0.02 cd	–	–	–	–	***	***	***	ns
2-Ethenyl-5-methylpyrazine	1026	MS + LRI	–	–	–	–	–	–	***	***	***	
2-Acetylpyrrole	1072	MS + LRI	–	–	–	–	–	–	***	***	***	***
3-Ethyl-2,5-dimethylpyrazine	1085	MS + LRI	–	–	–	–	–	–	***	***	***	
Sulfur compounds												
Methanethiol	<600	MS + LRI	–	–	–	–	–	–	***	***	***	*
Dimethyl disulfide	750	MS + LRI	0.01 c	–	0.01 c	0.01 c	0.03 c	0.1 c	*	***	**	ns
Methional	910	MS + LRI	0.1 d	0.07 d	0.01 d	0.01 d	0.01 d	–	***	***	***	***
Dimethyl trisulfide	985	MS + LRI	–	–	0.8 a	0.2 c	0.01 c	0.01 c	***	**	***	***
Esters												
Ethyl butanoate	801	MS + LRI	2.2 b	0.8 d	2.6 a	1.7 c	0.2 e	–	***	***	***	***
Butyl acetate	815	MS + LRI	0.1 a	0.03 c	0.07 b	0.06 b	0.01 d	–	***	***	***	***
Ethyl 2-methylbutanoate	846	MS + LRI	0.2 a	0.1 c	0.2 a	0.2 b	0.02 d	–	***	***	***	***
Ethyl 3-methylbutanoate	851	MS + LRI	0.1 a	0.05 c	0.1 a	0.1 b	0.01 d	–	***	***	***	***
3-Methylbutyl acetate	875	MS + LRI	0.7 a	0.2 d	0.6 b	0.5 c	0.05 e	–	***	***	***	***
Methyl hexanoate	931	MS + LRI	0.1 a	–	0.04 b	0.04 b	–	–	***	***	***	***
Ethyl hexanoate	998	MS + LRI	0.9 a	0.02 d	0.7 b	0.4 c	0.01 d	–	***	***	***	***
Hexyl acetate	1011	MS + LRI	0.1 b	0.01 c	0.2 a	0.2 a	–	–	***	***	***	***
2-Propenyl hexanoate	1080	ms + lri	0.02 c	–	0.2 a	0.1 b	–	–	***	***	***	***
Methyl octanoate	1122	MS + LRI	0.03 a	–	0.02 b	0.02 c	–	–	***	***	***	***
Ethyl octanoate	1164	MS + LRI	0.3 a	–	0.06 b	0.04 b	0.01 c	–	***	***	***	***
Ethyl decanoate	1392	MS + LRI	0.06 a	–	0.03 b	0.02 b	0.01 c	–	***	***	***	***

(Continues)

TABLE 3 (Continued)

	LRI expt	ID	Un-cooked Parm	Un-cooked Ched	Un-cooked HF	Un-cooked MF	Uncooked LF	Uncooked Mozz	Significance Cheese	Significance Cook	Significance Cheese × Cook	Significance Fat × Cook
Ketones												
2-Heptanone	893	MS + LRI	1.1 c	0.2 c	0.2 c	0.2 c	0.04 c	0.04 c	***	***	***	***
2-Nonanone	1095	MS + LRI	1.5 c	0.04 d	1.4 bc	0.8 cd	0.02 d	0.02 d	***	***	***	**
2-Undecanone	1298	MS + LRI	0.07 c	0 c	0.08 c	0.03 c	0.01 c	–	***	***	***	**
2-Tridecanone	1502	MS + LRI	0.02 c	–	–	–	–	–	***	***	***	***
Lactones												
δ-Decalactone	1507	MS + LRI	0.04 b	0.03 cd	0.04 ab	0.03 bc	–	–	***	***	***	***
δ-Dodecalactone	1726	MS + LRI	0.06 b	0.04 cd	0.06 ab	0.04 bc	–	–	***	***	***	ns
Acids												
Acetic acid	<600	MS + LRI	9.6 a	3.1 c	1.3 cd	1.4 cd	0.9 cd	0.05 d	***	***	ns	ns
Butanoic acid	788	MS + LRI	40 a	6 c	4.3 cd	3.5 cde	0.3 f	0.7 ef	***	***	***	*
3-Methylbutanoic acid	835	MS + LRI	0.08 b	–	–	–	–	0.01 d	***	***	***	***
2-Methylbutanoic acid	845	MS + LRI	0.04 ab	0.01 d	0.04 b	0.03 c	–	–	***	***	***	***
Pentanoic acid	876	MS + LRI	0.2 a	0.04 c	0 d	–	–	0.001 d	***	***	***	***
Hexanoic acid	975	MS + LRI	51 a	2.8 cd	2.5 cde	3 c	0.4 f	0.9 def	***	***	***	***
Heptanoic acid	1066	MS + LRI	0.4 a	0.01 bc	–	–	–	0.03 b	***	***	***	***
Octanoic acid	1165	MS + LRI	6.6 a	0.3 cd	0.5 cd	0.6 c	0.2 cd	0.7 c	***	***	***	***
Nonanoic acid	1258	MS + LRI	0.04 ab	–	–	0.01 b	0.01 b	0.01 b	**	*	**	**
Decanoic acid	1360	MS + LRI	1.79 a	0.06 c	0.16 bc	0.18 bc	0.05 c	0.17 bc	***	***	***	**
Undecanoic acid	1465	MS + LRI	0.07 a	0 b	0.01 b	0.01 b	–	0.01 b	***	***	***	***
Miscellaneous												
4-Methylphenol	1076	MS + LRI	0.002 cd	–	–	–	–	–	***	***	***	ns
2,3-Butanedione	<600	MS + LRI	–	–	–	–	–	–	***	***	***	ns

Note: En dash (–) denotes not detected; within each row, samples with the same letter are not significantly different (Tukey, $p = 0.05$).

Abbreviations: HF, high fat; LF, low fat; MF, medium fat.

[a] Compounds were identified by verifying odor descriptors with authentic compounds or The Good Scents Company website (odor), comparison of mass spectra with those from authentic standards (MS) or with mass spectra from NIST 11 library (ms), and comparison of LRIs with authentic standards on a non-polar ZB-5MSi column (LRI) or the NIST Chemistry WebBook (lri).

[b] Quantity (µg/g) determined by comparison of the peak area with that of the internal standard with a response factor of 1.

[c] Significance where $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, and ns = not significant.

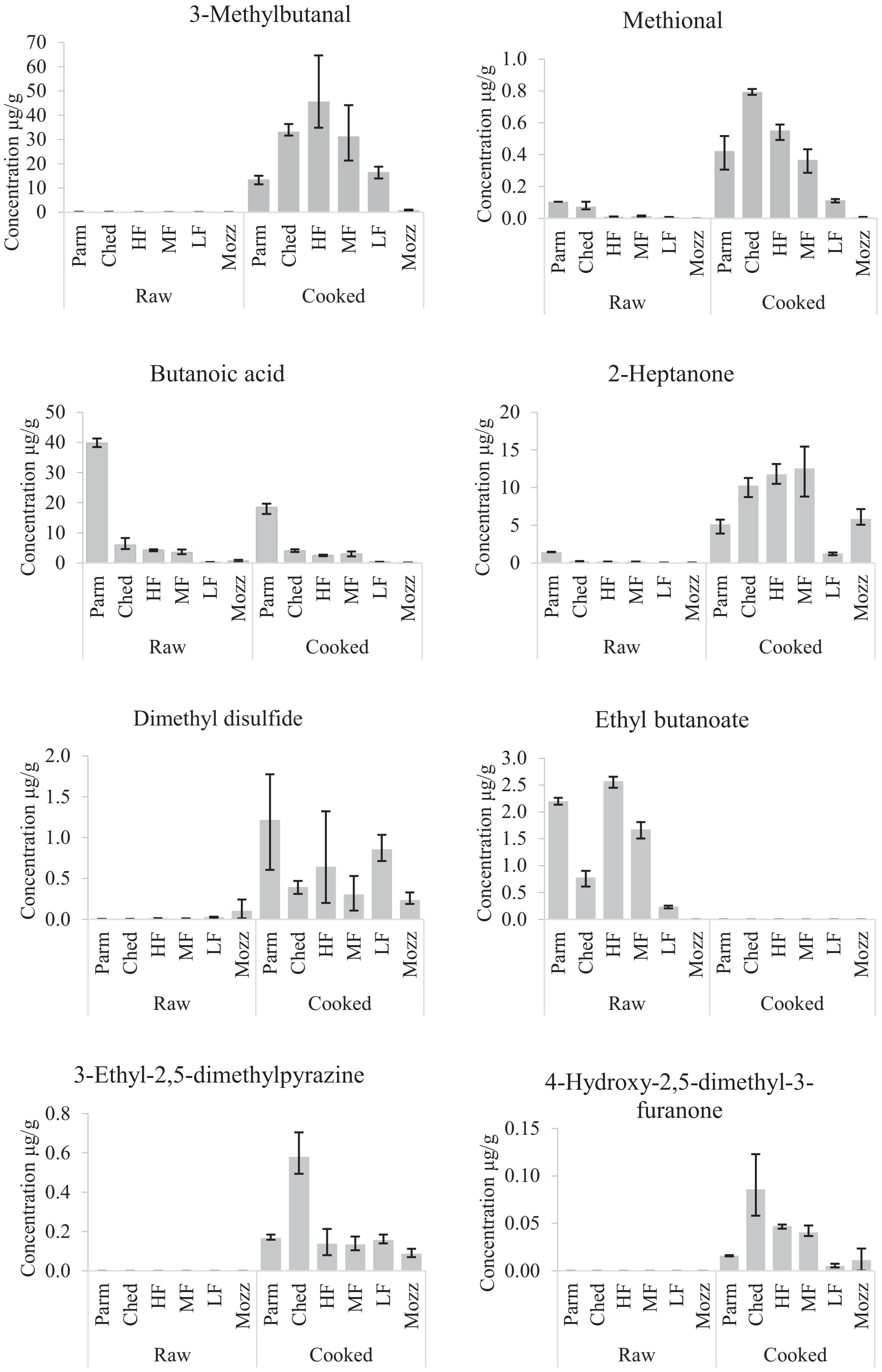

FIGURE 1 Bar graphs of semiquantitative results from a range of compounds derived from different formation pathways in six uncooked and cooked cheeses. Error bars represent the range of the replicates. Abbreviations are as shown in Table 1.

dimethylpyrazine, tetramethylpyrazine, 2-ethyl-3,5,6-trimethylpyrazine, and trimethylpyrazine) in cooked gruyère. Bertrand et al. (2011) also reported the presence of pyrazines in cooked processed cheese, but they were not reported during GC–O. As processed cheese has a much higher moisture content than cheese, and Bertrand et al. used a lower cooking temperature and shorter cooking time (150°C for 7.5 min vs. 180°C for 20 min in the current study), it is likely that the concentration of pyrazines was much lower in the cooked processed cheese than in cooked cheese. Both higher cooking temperatures and lower moisture content are associated with higher pyrazine formation (Ames et al., 2001). Given these findings, pyrazines are likely to be important compounds in cooked cheese aroma.

2-Isobutyl-3-methoxypyrazine was also found to be an odorant in cooked Cheddar; however, it is not formed through the same mechanistic pathway as the other pyrazines reported. 2-Isobutyl-3-methoxypyrazine was not included in the semiquantitative results as it could not be detected by SPME–GC–MS. This compound has been reported to contribute to the earthy flavor of uncooked Cheddar by Suriyaphan et al. (2001). They reported higher concentrations of 2-isobutyl-3-methoxypyrazine near the rind and hypothesized that it may form by the action of cheese molds.

Three odorants (3-methyl-1,2-cyclopentanedione, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone) identified in cooked Cheddar are known to form from the sugar moiety during the Maillard reaction. 3-Methyl-1,2-cyclopentanedione and 4-hydroxy-2,5-dimethyl-3(2H)-furanone were present in high enough concentration to be semiquantitated from SPME–GC–MS data in each of the cooked cheeses. Neither was detected in any of the uncooked cheeses, although 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone have previously been reported as odorants in uncooked Cheddar (see Table 2 for references). Given their previously reported importance in some uncooked Cheddars and their higher concentration in cooked cheeses, all three compounds are likely to be important to the aroma of cooked cheese.

Five Strecker aldehydes (2-methylpropanal, 3-methylbutanal, 2-methylpropanal, methional, and phenylacetaldehyde) were all reported as odorants in cooked Cheddar and were shown to be higher in cooked cheeses than in uncooked by SPME–GC–MS. Figure 1 shows the relative concentration of both 3-methylbutanal and methional in uncooked and cooked cheeses, representing the trend in the formation of Strecker aldehydes during cooking. The formation of Strecker aldehydes is a pathway of the Maillard reaction, in which amino acids are converted into their corresponding aldehydes (2-methylpropanal, 3-methylbutanal, 2-methylbutanal, methional, and phenylacetaldehyde). The corresponding amino acids for each of these Strecker aldehydes were semiquantitated in the uncooked cheeses (Sullivan et al., 2023). Their concentration increased with the typical length of aging of the cheeses (mozzarella < mild Cheddars < mature Cheddar < Parmesan) (see Table 1). The formation of Strecker aldehydes has been reported in cooked gruyère (Dumont et al., 1976) and cooked processed cheese (Bertrand et al., 2011). Although Strecker aldehydes are found in uncooked cheese, their significantly higher concentration in cooked cheeses suggests they are important for flavor development during the cooking of cheese.

Of the eight sulfur compounds identified as odorants during GC–O, only methanethiol, methional, dimethyl disulfide, and dimethyl trisulfide were present in high enough concentration to be semiquantitated by SPME–GC–MS. Each was significantly higher in cooked cheeses than in their uncooked counterparts, as shown for methional and dimethyl disulfide in Figure 1. This agrees with the findings of El-Shamy and Farag (2022) who reported an increase in the concentration of sulfur-containing compounds in heated emmental. Of those odorants too low to quantitate by SPME–GC–MS, 2-methyl-3-furanthiol, (furan-2-yl)methanethiol, and 2-methyl-3-methyldithiofuran have all been reported previously in cooked meat (Mottram, 1998) and can be derived from thiamine breakdown.

Methanethiol, methional, and dimethyl trisulfide have long been considered key odorants in uncooked Cheddar. Methional and dimethyl trisulfide were also reported by Bertrand et al. (2011) as odorants in cooked processed cheese. Methanethiol forms from the breakdown of methional during the Maillard reaction (Belitz et al., 2009), and there was a correlation between the levels of methanethiol and methional in the cooked cheeses. Dimethyl disulfide and dimethyl trisulfide are formed from the oxidation of methanethiol, although there was no correlation between the levels of these sulfides and methanethiol present in the cooked cheese.

2-Heptanone was the only 2-methylketone found to be odor active in cooked Cheddar; however, five other 2-methylketones were also identified by SPME–GC–MS. The 2-methylketones were all found at significantly higher concentrations in the cooked cheeses than their uncooked counterparts, except for LF mild Cheddar. The presence of methyl ketones in heated cheese has been reported previously (El-Shamy & Farag, 2022), although the data from that study do not directly compare the concentrations with those from unheated cheese. 2-Methylketones have been shown to form upon heating of milk fat from esterified β-keto alkanoic acids in glycerides via a hydrolysis and decarboxylation reaction (Calvo & de la Hoz, 1992).

Butanoic acid, 3-methylbutanoic acid, and hexanoic acid were all identified in cooked cheese by GC–O and were quantitated along with several other fatty acids using SPME–GC–MS. They were generally lower, in some cases significantly so, in the cooked cheeses compared to their uncooked counterparts. Figure 1 shows an example of these data for butanoic acid. This suggests that some degradation of short-chain saturated fatty acids occurs when cheese is cooked.

Fatty acids, especially butanoic and hexanoic acids, have been shown to be key odorants in uncooked cheese (Carunchia Whetstine et al., 2006; Christensen & Reineccius, 1995; Drake et al., 2010). Bertrand et al. (2011) also reported butanoic acid to be an odorant in cooked processed cheese. Our findings suggest that fatty acids play a role in cooked cheese aroma, but they were among the few odorants to decrease in concentration during cooking. This suggests they may play a lesser role in cooked cheese aroma than other volatiles, which increased significantly in concentration during cooking.

No esters were detected by GC–O or GC–MS in cooked Cheddar. This is in contrast to previous studies on the aroma of uncooked Cheddar, in which esters such as ethyl butanoate, ethyl hexanoate, and ethyl acetate have been reported as fruity odorants (Avsar et al., 2004; Christensen & Reineccius, 1995; Suriyaphan et al., 2001). Esters were only detected in the uncooked cheeses, as shown for ethyl butanoate in Figure 1. Esters are known to undergo hydrolysis at higher temperatures; furthermore, esters are volatile and have low boiling points. Both of these factors are likely to contribute to the loss of esters during cooking. Unlike other highly volatile compounds in cheese (e.g., 3-methylbutanal, methanethiol), esters are also unlikely to be replaced by the Maillard reaction when lost.

In conclusion, we report a number of differences in the presence of low-odor-threshold odorants in cooked Cheddar compared to those previously reported in uncooked Cheddar. Most notably, these include the presence of 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 3-methyl-1,2-cyclopentanedione and the lack of ethyl esters. Additionally, while the majority of the odorants identified in cooked Cheddar have been previously reported in uncooked Cheddar (see Table 2), the semiquantitative results demonstrate large differences in concentration between the uncooked and cooked cheeses. Strecker aldehydes, sugar-derived compounds such as 3-methyl-1,2-cyclopentanedione and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, sulfur compounds, and 2-methylketones were all substantially higher in cooked cheeses than in their uncooked counterparts, suggesting that these contribute to differentiating the aroma of cooked cheese from that of uncooked cheese.

Component balance theory is often used to describe the variance in flavor of different cheese varieties by their relative balance of a range of volatiles (Kilcawley & O'Sullivan, 2017). Our findings suggest that component balance may also apply to cooked cheeses, as cooking alters the balance of aroma compounds and leads to the formation and loss of odorants.

3.3 | Differences in the composition of cooked cheese by cheese type

Two-way ANOVA showed that for all volatiles, there was a significant difference between cheese types. In most cases, a statistical significance was reported for the interaction between cheese type and cooking. The substantial differences between the cooked products and relatively small and barely significant differences between the raw cheeses (or vice versa for esters and acids) lead to a statistical significance in the interaction term. This tells us that the different cheeses behave differently during cooking, and this is likely to be related to their very different precursor profile (as discussed in Section 3.2).

PC analysis was performed on the compounds in Table 2 across all six cooked cheeses to generate a representation of the data as a smaller set of variables. This allowed further exploration of the balance of volatiles in different varieties of cooked cheese, the clusters within the data, and the relationships between the variables. Note that only one example each of mozzarella, Parmesan, and aged Cheddar has been analyzed; therefore, the results are only indicative of what volatiles might be present in a wider selection of these varieties of cheese. Figure 2 shows the PC plot for the cooked cheeses, along with a plot of the variables. The first two PCs accounted for 48.2% and 27.6% of the variance between the samples, respectively, such that the total variance accounted for was 75.8%. Based on the examination of the rotated component matrix scores, the variables that influenced the separation in each of the PCs were determined. Component 1 (PC 1) was related to a number of Maillard reaction products (pyrazines, 3-methyl-1,2-cyclopentadione, 4-methylphenol, 4-hydroxy-2,5-dimethyl-3-furanone, methanethiol, and the Strecker aldehydes). Component 2 (PC 2) was related to sulfur compounds (dimethyl disulfide and dimethyl trisulfide), 2-heptanone, and 2,3-butanedione.

The cooked Parmesan samples were clustered in the top right of the PC plot, well separated from the other samples by PC 2. Comparison with the variable plot indicates that the separation is driven by higher levels of acids (hexanoic and butanoic acid) and sulfur compounds (dimethyl trisulfide and dimethyl disulfide) in the Parmesan cheese than in the other samples. Both of these differences may be related to the long aging process (typically more than 2 years) used in Parmesan production. A high level of short-chain fatty acids is typical in uncooked Parmesan, in which fatty acids are formed from triglycerides during aging in the process of lipolysis (Fox & McSweeney, 1996). Both dimethyl disulfide and dimethyl trisulfide are formed

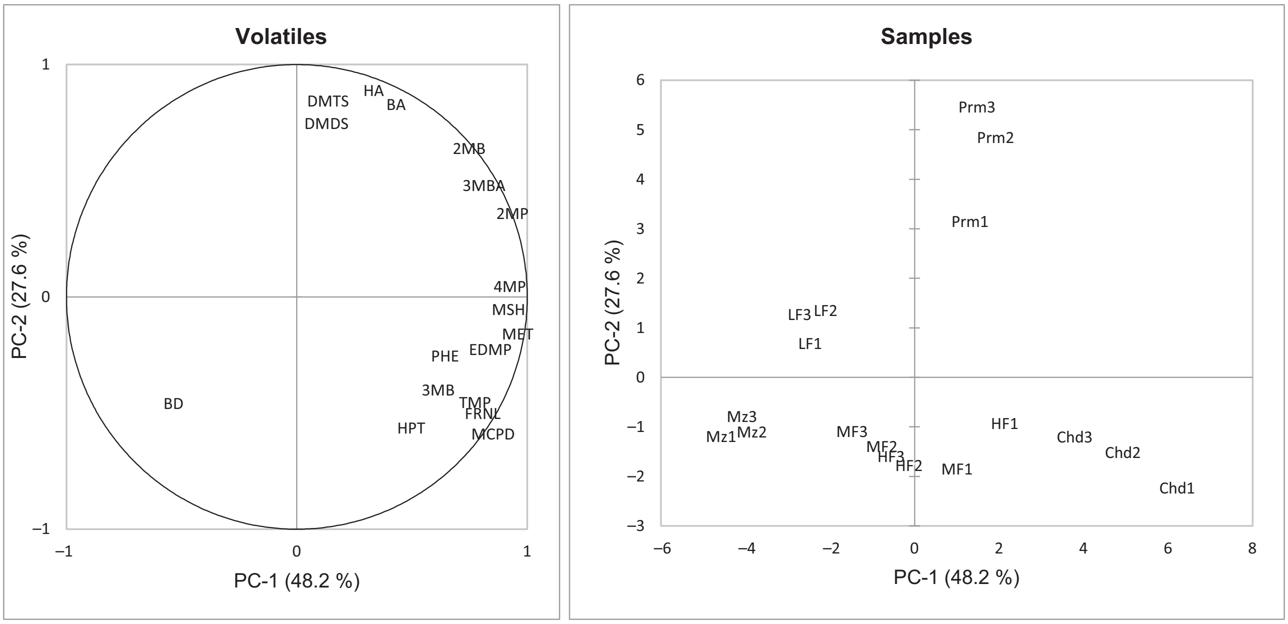

FIGURE 2 Principal component (PC) plot of selected odorants from cooked Cheddar cheese in all six cooked cheeses. Left: variables plot (see Table 2 for codes). Right: observations plot.

in cheese from the amino acid methionine (Belitz et al., 2009). Free amino acids are also produced during the aging process via proteolysis (Fox & McSweeney, 1996). Quantitation of a selection of amino acids in the uncooked cheeses was performed in a previous study (Sullivan et al., 2023). The data confirm that the methionine concentration was significantly higher in Parmesan than in the other cheeses.

Cooked mozzarella is clustered in the bottom left of the PC plot, separated from the Cheddar datapoints by PC 1. This separation is related to the low concentration in mozzarella of many of the cooked cheese odorants, including the Strecker aldehydes and short-chain fatty acids, and the high concentration of 2,3-butanedione compared to the other cooked cheeses. As a fresh cheese, the processes of lipolysis and proteolysis that generate short-chain fatty acids and Strecker amino acids have a limited influence on the composition of mozzarella, explaining the low concentration of fatty acids and Strecker aldehydes when it is cooked. 2,3-Butanedione is a dicarbonyl compound that can form in the early stages of the Maillard reaction from the breakdown of reducing sugars. As a fresh cheese, uncooked mozzarella contains more milk sugars (e.g., lactose, galactose) than aged cheeses (Sullivan et al., 2023).

The Cheddar cheeses form three clusters on the PC plot, separated mostly by PC 1. The mature Cheddar is clustered on the bottom right of the plot. This is related to higher concentrations of Strecker aldehydes, fatty acids, and pyrazines than the other Cheddar samples. As with the Parmesan cheese, the higher Strecker aldehyde and fatty acid concentrations in mature Cheddar may be related to the processes of proteolysis and lipolysis that occur during aging.

However, the pyrazine concentrations were higher in the cooked mature Cheddar than in the cooked Parmesan. Alongside amino acids, the other precursors to pyrazine formation are α-amino carbonyl compounds generated during Strecker degradation (Weenen et al., 1994). A possible theory to explain the low formation of pyrazines in Parmesan compared to Cheddar may be that, due to the extensive aging of Parmesan, reducing sugars and sources of dicarbonyl compounds formed from their breakdown are present at a lower concentration than other low and moderately aged cheeses. As both sugars/dicarbonyls and amino acids are required for the formation of pyrazines, both long and short aging periods may be associated with low levels of pyrazine precursors. Mature Cheddar has a moderate aging period, typically close to 9 months, which may enable high pyrazine formation upon cooking. The theory of low sugar-derived carbonyls in aged cheeses would still be consistent with the high formation of Strecker aldehydes in cooked Parmesan, as lipid precursors may be contributing to their formation. It has been shown that lipid degradation can produce precursors to Strecker aldehydes (Hidalgo & Zamora, 2016, 2019).

Comparison of mozzarella, Parmesan, and Cheddar suggests that the age of a cheese may affect its aroma when cooked, both directly (due to differences in the aroma of uncooked cheese, which are maintained during cooking) and indirectly (by the formation or loss of precursors to aroma compounds during aging, which affects their conversion to aroma active compounds when the cheese is cooked).

3.4 | The effect of fat content on cooked mild Cheddar flavor

The results of the two-way ANOVA are displayed in Table 3, with an additional column showing the interaction between fat content and cooking. The significant interaction term arises because of the relatively small differences between cheeses in the uncooked cheese compared to much more substantial differences between cooked cheese, suggesting that the fat content has a greater influence on the volatile profile of the cooked cheese than on that of the uncooked cheese. A comparison of the three cooked mild Cheddars gives an indication of the role of fat in the development of flavor during cooking. In Figure 2, the mild Cheddars were clustered into two groups distinct from the rest of the cooked cheese data. The LF mild Cheddar differed from the other mild Cheddars, while the MF and HF Cheddars were broadly similar and clustered together. This indicates that moderate reductions in fat concentration in Cheddars do not substantially affect volatile formation during cooking, while larger reductions have a much greater effect.

When comparing matrices of differing compositions using headspace extraction techniques, it is important to consider how flavor release from the differing matrices may affect the results. The hydrophobicity of analytes influences how their release may be affected by differing fat content of the matrix. Highly hydrophobic compounds are likely to be less well released from HF matrices than from matrices of lower fat content, while an opposite trend would be expected from highly hydrophilic compounds. In this study, an internal standard was used to account for some of the matrix differences between the cheese. Nevertheless, it is important to consider that the matrix composition and hydrophobicity of the analytes may have influenced some results. Octanol/water partition coefficient (LogP) values are included in Table 4 and referenced in the discussions below to highlight where the hydrophobicity of an analyte may have affected its quantitation.

The concentration of fatty acids was lower in the LF cooked mild Cheddar than in the HF mild Cheddar. This is a logical result as short-chain fatty acids are formed from triglyceride precursors during cooking (Nawar, 1969). It is in line with the concentrations in the uncooked cheese, which were also lower with lower fat content. Short-chain fatty acids (e.g., butanoic acid) are relatively hydrophilic, while fatty acids become highly hydrophobic as their chain length increases. There is a stronger correlation between fat content and the concentration of short-chain fatty acids than with longer chain fatty acids. It is therefore possible that the difference in the concentration of short-chain fatty acids is inflated by the effect of the matrix on their release during headspace extraction.

TABLE 4 LogP values for selected volatiles.

Compound	Log P
Methional	0.41[b]
Butanoic acid	0.79[a]
Trimethylpyrazine	0.95[a]
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	1.03[a]
3-Methylbutanal	1.23[b]
Dimethyl trisulfide	1.87[b]
Hexanoic acid	1.92[a]
2-Heptanone	1.98[a]
Ethyl hexanoate	2.83[b]

Note: Octanol water partition coefficients (log P) values of selected volatiles from multiple chemical classes.

[a] Data obtained from ChemSpider (experimental).

[b] Data obtained from ChemSpider (estimated).

Significant differences were observed in the concentration of Strecker aldehydes between the mild Cheddars of different fat contents. Concentrations of 2-methylpropanal, 3-methylbutanal, and methional were all highest in the HF cooked mild Cheddar and lowest in the LF version. The difference between LF and HF was significant in each case. The Strecker aldehydes are hydrophobic; therefore, it is unlikely that the higher quantitation of Strecker aldehydes in HF cheeses is caused by matrix differences.

The concentrations of the corresponding amino acids—valine, leucine, and methionine—in the uncooked cheeses (Sullivan et al., 2023) were higher in the uncooked LF mild Cheddar than in the HF variety, although not significantly. This does not correlate with the levels of these Strecker aldehydes in the cooked cheese. The other reactant in the Strecker degradation mechanism is a carbonyl compound (Strecker, 1862), which is typically formed from the breakdown of reducing sugars. A comparison of reducing sugar concentration in the cheeses (Sullivan et al., 2023) showed that the concentration of lactose was significantly higher in the HF than in LF cheese. However, the concentration of dicarbonyl may not have been similarly affected—for example, 2,3-butanedione was not significantly different in the mild Cheddars of differing fat content. The lower concentration of reducing sugars in the LF cheese is likely to contribute to the lower formation of Strecker aldehydes during cooking.

Additionally, lipid-derived reactive carbonyl compounds have also been shown to contribute to the formation of Strecker aldehydes (Hidalgo & Zamora, 2016, 2019). The lower concentration of Strecker aldehydes in cooked LF mild Cheddar compared to higher fat cheeses suggests that lipid-derived carbonyls may be contributing to the formation of Strecker aldehydes in the higher fat mild Cheddars. In the uncooked mild Cheddars, there were no significant differences between the concentrations of Strecker aldehydes by fat content, although in previous literature, methional and phenylacetaldehyde have both been reported as higher in reduced-fat and LF uncooked Cheddars than in HF Cheddar (Drake et al., 2010).

There were few significant differences between the levels of pyrazines in cooked mild Cheddars of differing fat content, or significant interactions between fat content and cooking, suggesting that fat content does not significantly affect the formation of pyrazines during the cooking of cheese. This result is logically consistent with the possible involvement of lipid-derived carbonyls in the formation of Strecker aldehydes, as the hydroxyl amino compounds produced by that mechanism are not precursors to pyrazines; instead, they form 2-alkylpyridines (Hidalgo & Zamora, 2004). However, alkylpyridines were not detected in any of the cooked cheeses. Only 2,5-dimethylpyrazine was detected in the uncooked mild Cheddars. It was significantly higher in the HF uncooked Cheddar than in the MF or LF cheeses.

The levels of 2-methylketones detected in the LF mild Cheddar were significantly lower than the other two mild Cheddars. As 2-methylketones are hydrophobic, this result is not likely to be caused by differences in flavor release from the matrices of differing fat contents. 2-Methylketones have been shown to form from β-keto alkanoic acids esterified in the milk fat glycerides via a hydrolysis and decarboxylation reaction upon heating (Calvo & de la Hoz, 1992; Nawar, 1969); therefore, it is a logical result that their concentration was lower in an LF cheese. The concentrations of 2-methylketones in HF and MF mild Cheddars were similar. The 33% reduced-fat cooked Cheddar (MF) did not contain significantly less 2-methylketones than the HF sample, suggesting that the level of milk fat in reduced-fat cheeses is high enough to still produce high levels of 2-methylketones. 2-Methylketones were also lower in the uncooked LF mild Cheddar than in the uncooked HF mild Cheddar.

The level of methanethiol and methional increased with fat concentration in cooked mild Cheddars. However, their precursor, methionine, had a higher concentration in the LF cheese than in the HF cheese. They are both relatively hydrophilic, so this trend is not likely to be an artifact of matrix release differences. Methanethiol forms from the breakdown of methional during the Maillard reaction (Belitz et al., 2009), and so, the higher concentration of methanethiol in the cooked HF can be attributed to the role of lipids in the formation of Strecker aldehydes (Hidalgo & Zamora, 2016, 2019).

Dimethyl disulfide and dimethyl trisulfide are formed from the oxidation of methanethiol. The levels of both dimethyl disulfide and dimethyl trisulfide were highest in the cooked LF, which was correlated with the concentration of methionine (Sullivan et al., 2023) but not correlated with the concentrations of methional or methanethiol. Both of the sulfides are relatively hydrophobic, so the result is unlikely to be an artifact of matrix release differences. The higher concentration of dimethyl disulfide and dimethyl trisulfide in LF cooked Cheddar may indicate that the Maillard reaction had occurred to a greater extent in the cooked LF than in the MF or HF cheeses. The absence of free fat coating LF cheeses has been shown to promote rapid dehydration and browning in LF cheeses (Rudan et al., 1999). As dimethyl disulfide and dimethyl trisulfide are formed in the later stages of the Maillard degradation of methionine, their high concentration may indicate that these reactions were more advanced in the LF Cheddar.

In the uncooked mild Cheddars, methional, methanethiol, and dimethyl disulfide were all similar in concentration, while dimethyl trisulfide was significantly higher in the uncooked HF and MF Cheddars than in the LF Cheddar. This differs from previous data on dimethyl trisulfide concentration in Cheddars of differing fat levels (Drake et al., 2010), which found significantly higher concentrations of dimethyl trisulfide in reduced-fat and LF uncooked Cheddar than in HF Cheddar.

3-Methyl-1,2-cyclopentanedione and 4-hydroxy-2,5-dimethyl-3(2H)-furanone were both significantly higher in the cooked HF Cheddar than in the cooked LF Cheddar. Both of these compounds are derived from a sugar moiety in the Maillard reaction or from caramelization, and their higher formation in the HF Cheddar relates to the concentration of sugars in the uncooked HF.

The sugar concentration in the HF Cheddar was higher than in the LF Cheddar and also decreased more during cooking (Sullivan et al., 2023). 4-Hydroxy-2,5-dimethyl-3(2H)-furanone is neither strongly hydrophobic nor hydrophilic; therefore, it is possible that this result could be influenced by their release from HF and LF matrices during analysis.

While esters were not detected in any of the cheeses when cooked, there was a positive correlation between fat content in the uncooked mild Cheddars and ester concentration. As ethyl esters are hydrophobic, differences in their release from cheeses of differing fat content would be expected to produce the opposite trend; therefore, we can be confident that there are higher concentrations of ethyl esters in the HF samples compared to the LF samples. Esterification of ethanol and fatty acids in cheese occurs during ripening via acetyl-coenzyme A (Molimard & Spinnler, 1996). A lack of fatty acid precursors in the LF cheese is likely to have contributed to their reduced formation.

In conclusion, fat content influenced the flavor of cooked Cheddar. 2-Methylketones, fatty acids, and Strecker aldehydes had lower concentrations in the lower fat cooked Cheddar. In most cases, the trends observed are inconsistent with those expected if all differences were caused by the effect of matrix composition on flavor release during headspace extraction.

Nevertheless, further confirmatory studies using a solvent extraction technique are underway. Further studies could employ approaches to mitigate the effect of matrix composition on quantitative results, for example, standard addition techniques.

4 | CONCLUSION

The odorants in cooked Cheddar have been determined for the first time. When compared to uncooked Cheddar, the aroma of cooked Cheddar is affected by additional compounds, including Strecker aldehydes, pyrazines, unsaturated aldehydes, 3-methyl-1,2-cyclopentanedione, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Furthermore, esters such as ethyl butanoate and ethyl hexanoate, which have been widely reported as odorants in uncooked Cheddar, were not detected by either GC–O or GC–MS in cooked Cheddar, despite being present in the uncooked samples. The combination of the additional odorants present in cooked Cheddar and the loss of odorants from uncooked Cheddar is likely to affect differences in aroma between uncooked and cooked cheese. Additionally, almost all of the odorants were present at significantly different levels in one or more cooked cheeses than their uncooked counterparts. This suggests that cooking cheese also affects the balance of odorants, contributing to the change in flavor upon cooking.

Cheese type affected the formation of odorants during cooking; in many cases, the formation of aroma was lower in mozzarella than in the other cheeses. This may be related to the low aging time for mozzarella, as the aging processes such as proteolysis affect the concentration of Maillard reaction precursors. Fat content was also related to the concentration of odorants in cooked mild Cheddar, including the Strecker aldehydes, methanethiol, 2-methylketones, and fatty acids. These results suggest that the fat in cheese is involved in flavor formation during cooking, both directly as a precursor and indirectly due to the role of fat in cheese structure and free fat. These results may have relevance for the dairy industry in creating better-performing LF cheeses for cooked applications.

AUTHOR CONTRIBUTIONS

Rosa C. Sullivan: Conceptualization; methodology; data curation; investigation; formal analysis; funding acquisition; writing—original draft; writing—review and editing; resources. **Fiyinfolu Makinwa**: Data curation; investigation; writing—review and editing; formal analysis. **Colette C. Fagan**: Conceptualization; supervision; writing—review and editing. **Jane K. Parker**: Conceptualization; supervision; funding acquisition; writing—review and editing; project administration.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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