University of Reading

Valorisation of melon seed (*Cucumis melo L.*)

Thesis submitted for degree of Doctor of Philosophy

Department of Food and Nutritional Sciences

Guoqiang Zhang

July 2023

Declaration

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

Guoqiang Zhang

Abstract

Melon seeds (*Cucumis melo L.*), accounting for 5% - 10% of the total melon weight, are regarded as a low value by-product within the melon supply chain, and are often disposed as a waste. However, this by-product holds potential as a source of nutritionally valuable compounds for food applications. To date, there is still insufficient information about valorisation strategies of melon seeds. Therefore, the aim of this study was to assess melon seed potential value and develop valorisation strategies for food applications.

Compositional analysis showed that melon seed contains considerable levels of protein (28.4 - 30.4%, w/w), oil (43.5 - 48.3%, w/w), fibre (15.9 - 16.6%, w/w), as well as minerals, especially potassium (988.3 - 1076.6 mg/100 g DW) and magnesium (514.3 - 541.0 mg/100 g DW). Investigating and developing processing technologies are very important as it can reduce food waste and could result to products of good nutritional quality, suitable for human consumption. Melon seeds were processed via three processing methods namely, soaking, boiling, and roasting, to assess its effect on melon seeds nutritional quality. Soaking and boiling methods showed positive effect on reducing tannins content (13% - 20%, 10% - 26%, respectively), whereas roasting showed adverse effects, including increase in tannins (40% - 114%) and phytic acid content (3% - 5%), and decrease in linoleic acid content (approximately 8%).

Melon seed oil extraction was investigated using solvent extraction (SE), cold-pressed extraction (CPE), and aqueous enzymatic extraction (AEE) methods. Melon seed oil

was identified as a rich source of linoleic acid (C18:2; 53.6% - 70.8%), β-sitosterol (119.5 – 291.9 mg/100 g), and squalene (101.1 - 164.7 mg/100 g). It was seen that the choice of oil extraction technology influenced melon seed oil quality; oil obtained by aqueous enzymatic extraction (AEE) exhibited higher tocopherol content and better oxidative stability as compared to SE and CPE.

After oil extraction, the defatted melon seed, as a major by-product of melon seed oil extraction (often referred to as meal/residue), contained considerable as amounts of protein (34.1% w/w) and fibre (35.1% w/w). Subsequently, defatted melon seed was used as ingredient in bread formulations as partial substitute to wheat flour. The addition of defatted melon seed reduced dough strength, made dough softer and weaker, reduced bread specific volume and increased bread hardness. This could be attributed to gluten dilution and increased fibre content which induces greater disruption of the gluten network. In contrast, from a nutritional point, defatted melon seed addition at 10% (w/w) comprehensively improved the nutritional quality of bread that can be labelled as ‘source of fibre’ and could contribute to an increased dietary intake in fibre.

Acknowledgement

I would like to express my sincere thanks to my supervisors, Prof. Dimitris Charalampopoulos and Dr Afroditi Chatzifragkou, for their patience, guidance, supporting, supervision, and encouragement throughout my PhD study.

I would like to express my gratitude to Dr Tiffany Lau, for her supporting and valuable assistance in my first year PhD research. Many thanks to all staff of department of Food and Nutritional Sciences during my PhD research, for their valuable assistance. Special thanks to Dr J. Stephen Elmore, Dr Julia Rodriguez Garcia, Dr Sameer Khalil Ghawi, Dr Graham Bardbury, and Dr Raymond Lau.

Also, I would like to thank my friends, lab mates, and PhD office colleagues, we had wonderful time during PhD study. I also wish to thank Miss Yuhan Su who help me lots during melon seed collection.

Last but not least, I would like to express my appreciation and special thanks to my parents, my entire family, my grandfather (Shengchang Zhu), and my grandmother (Guizhen Zhang), for their full supporting, understanding, and encouragement on my PhD and entire life.

Table of contents

List of figures

List of Tables

List of Abbreviations

AA	Ascorbic acid
AEE	Aqueous enzymatic extraction
BP	Bound phenolics fraction
CE	Catechin
CO_2	Carbon dioxide
CP	Conjugated phenolics fraction
CPE	Cold-pressed extraction
DAD	Diode-Array Detection
DDT	Dough developing time
DMSR	Defatted melon seed residue
DPPH	2,2-diphenyl-1-picrylhydrazyl
DST	Dough stability time
E	Extensibility
EC	Emulsify capacity
FAME	Fatty acid methyl esters
FC	Foaming capacity
FID	Flame Ionization Detector
FP	Free phenolics fraction
FRAP	Ferric reducing antioxidant capacity
FS	Foaming stability
GABA	γ-Aminobutyric acid
GAE	Gallic acid
GC	Gas chromatography
H_2SO_4	Sulfuric acid
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
I_2	Iodine
KOH	Potassium hydroxide

MUFA	Monounsaturated fatty acid
MTI	Mixing tolerance index
N_2	Nitrogen
NaOH	Sodium hydroxide
ND	Not detected
OAC	Oil absorption capacity
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
R/E	Resistance to Extension
SE	Soxhlet extraction
SFA	Saturated fatty acid
TE	Trolox equivalent
TPC	Total phenolic content
TPTZ	2,4,6-Tripyridyl-S-triazine
WA	Water absorption
WAC	Water absorption capacity
WL	Weight loss

Chapter 1. General Introduction

1.1. Introduction

Food waste is currently a significant issue worldwide and has resulted in negative environmental, economic, and social impact (Jin et al., 2018). For this reason, most countries and organisations have developed strategies based on more circular food systems that are focused on capturing value and minimizing waste, aiming to reduce food waste by 50% by 2030 (Ellen MacArthur Foundation, 2019; European Commission, 2015).

Melon seed, as a major by-product from melon (*Cucumis melo L.*) supply chain, is scarcely utilised, and is usually discarded into landfill (Frankowska et al., 2019; Fundo et al., 2018; Miller et al., 2020; Rabadán et al., 2020; Wang et al., 2019). Literature shows that melon seeds contain nutritionally valuable compounds, such as protein, oil, fibre, and minerals (Miller et al., 2018; Rolim et al., 2020; Silva et al., 2020). In addition, melon seed oil contains considerable levels of unsaturated fatty acid, with linoleic acid (C18:2) representing the dominant fatty acid, and holds value as an alternative vegetable oil source (Mallek-Ayadi et al., 2018; Rezig et al., 2019). Despite their rich nutrient composition, melon seeds are underutilised as a food ingredient. Their consumption is only encountered in a few countries in Middle East countries, India, Indonesia, and China. In those countries, melon seeds are consumed either raw or processed (e.g. soaked, boiled, or roasted). According to Mallek-ayadi et al. (2019) and Wang et al. (2019), melon seeds have been used as a snack after roasting in the Middle

East, and then as sauce or drink after boiling and soaking in India and Indonesia. Overall, there is lack of knowledge on efficient ways to utilise melon seed as a source of food or as food ingredient. Therefore, the development of valorsation routes for melon seed is necessary, to establish this by-product as ingredient into various sectors, such as food, cosmetics, and pharmaceutical industries, reduce food waste and stimulate sustainable food production and circular economy.

This project aims to develop valorisation strategies for melon seeds targeting the recovery of high value-added compounds, and investigate potential food applications as a valuable ingredient.

1.2. Research aims and objectives:

The overall aim of this project was to develop valorisation strategies for melon seeds, targeting the recovery of high value-added compounds, and investigate potential food applications as a valuable ingredient. In order to achieve this aim, the following objectives were identified:

1) Evaluate the effect of different processing technologies on melon seed macronutrients and anti-nutritional compounds.

2) Evaluate the effect of extraction technologies on the quality of melon seed oil.

3) Evaluate the nutrition value and functional properties of defatted melon seed as food ingredient.

4) Investigate the effect of partial substitution of wheat flour with defatted melon seed,

on dough properties and bread quality.

1.3. Contents of the thesis

Chapter 2 is a literature review covering the botanical characteristics, origin and species of melon, the physicochemical composition of melon seeds, processing technologies, bioactivities of key molecules in melon seeds, as well as well as current melon seed applications in food.

Chapter 3 presents the results from investigating the impact of three processing technologies (soaking, boiling, and roasting) on the physicochemical components of melon seeds, including proximate composition, minerals, fatty acid composition, anti-nutritional compounds, and amino acid profile.

Chapter 4 presents the results from evaluating the effect of extraction methods (Soxhlet extraction, cold-pressed, and aqueous enzymatic extractions) on the physicochemical properties, bioactive compounds, and oxidative stability of melon seed oil.

Chapter 5 presents the results from evaluating the defatted melon seed as functional food ingredient, analysis of their nutritional value, functional properties from three varieties of defatted melon seed and defatted pumpkin seed (as control group).

Chapter 6 presents the results from reformulating bread by incorporating defatted melon seed as partial substitution of wheat flour, and subsequent analysis including dough development and bread quality.

Chapter 7 is a general discussion for this thesis, presenting key findings, limitations, and future work.

Chapter 2. Literature review

Abstract

Melon (*Cucumis melo L.*) is a commercial fruit planted worldwide in large quantities; meanwhile, substantial amounts of melon seeds as a by-product are generated with in the food chain supply. Studies have shown that melon seed is an excellent natural source of nutrients (oil, protein, minerals, and bioactive compounds), and could be considered as a novel ingredient in food development. In addition, melon seed extracts have been associated with positive effects on human health, such as antioxidant capacity and lowering the risk of diabetes and cardiovascular conditions. Currently, there has been extensive attention to valorisation strategies of melon seeds, driven by circular economy strategies and UN sustainable development goals agenda. Therefore, the aim of this literature review is to highlight melon seed nutritional composition, biological activities of individual components, and provide state- of-the-art applications of melon seeds as ingredients in food product development. Focus is also given to promising green extraction technologies for maximising recovery value from melon seeds. Ultimately, a better understanding of melon seed properties could enable their efficient utilisation and promote viable valorisation routes.

2.1. Introduction

The Food and Agriculture Organisation (FAO) of the United Nations reported in 2015 that around 1.3 billion tons per year of globally food produced are lost or wasted across the food supply chain (FAO, 2015). Food waste is currently a significant issue worldwide and has resulted in negative environmental, economic, and social impact (Jin et al., 2018). For this reason, most countries and organisations have been focusing on the development of circular food systems that are based on capturing value and minimizing waste, aiming to reduce food waste by 50% by 2030 (Ellen MacArthur Foundation, 2019; European Commission, 2020; FAO, 2015). To this end, a variety of food waste management practices have been proposed: (i) recovery and reuse can contribute to food waste reduction and promote an efficient use of natural resources; (ii) development and design of green technologies and circular economy systems towards more sustainable patterns of production, to minimize the adverse effect of chemical and waste on human health and the environment; (iii) raise public's awareness for sustainable development and promote lifestyles in harmony with nature (Ellen MacArthur Foundation, 2019; European Commission, 2015, 2020; Ritchie et al., 2018). Based on this background, the food biorefinery concept has been put forward to advance circular economy across the food supply chain (Ekman et al., 2013; Jin et al., 2018; Teigiserova et al., 2019). The food biorefinery concept considers the recovery of components from food supply chains, including waste or by-products and develops novel valorisation strategies as an extension or addition to established strategies (e.g. animal feed and composting), leading to the production of

medium/high value products, such as functional ingredients, chemicals and biomaterials (Lin et al., 2013).

Melon (*Cucumis melon L.*) is an important commercial horticultural crop in the world. It belongs to the Cucurbitaceae family and is cultivated in several warm parts of the world, including Europe, Asia and Africa (Silva et al., 2020). Due to its sweet and juicy flesh as well as attractive aroma, melon appeals to a large consumer base and is also processed by the food industry into a variety of food products (e.g. juices, fruit salads, desserts, canned fruit, and ice cream) (Górnaś et al., 2015). Data from FAOSTAT (Food and Agriculture Organization of the United Nations Statistical Analysis) in 2021 showed that the annual melon worldwide production was approximately 29 million tons, with the largest production in Asia (77%), followed by America (11.5%) and Europe (6.9%). As a seasonal fruit, roughly 70% of melon is directly consumed as fresh fruit to households and the rest is processed to food products; in addition, about 49% of total melon weight is generally considered as non-edible part (seeds and peels), therefore classified as by-product and usually ends into landfill (Frankowska et al., 2019; Fundo et al., 2018; Miller et al., 2020; Rabadán et al., 2020; Wang et al., 2019). This disposal method reflects the inefficient use of natural resources, leading into economic value loss, and has negative impact on the environment [generating greenhouse gas (GHG) emissions when decompose in landfills, and soil/water contamination] (Osorio et al., 2021; Socas-Rodríguez et al., 2021).

Melon seeds (accounting for 5% - 10% of the melon total weight, about 1.5 to 2.9 million tons) contain high amounts of lipids, proteins, minerals, and carbohydrates and

could be therefore considered as a renewable resource to produce medium to high value products (Miller et al., 2018; Rolim et al., 2020; Silva et al., 2020). Wang et al. (2019) reported that only the USA generates as much as 595 tons of melon seeds annually by household consumption, which can create over 6 million US dollars value. On the other hand, although melon seeds are rarely used as food ingredient in most countries, they are regarded as an edible ingredient in some countries' traditional cuisine, including Middle East, India, Pacific Islands, and China (BBC, 2022; Cecily Dignan et al., 2004; Mallek-Ayadi et al., 2019; Rabadán et al., 2020). Specifically, melon seeds have been used as a ready-to-eat snack after roasting, or they are crushed and used into desserts, drinks, curries, and stews (BBC, 2022; Mallek-Ayadi et al., 2019; Rabadán et al., 2020). Nevertheless, in most countries, melon seed is regarded as an uncommon ingredient and has hardly been used in food product development. In line with the content of the United Nations Sustainable Development Goals (SDG), such as SDG 2 (zero hunger) and SDG 12 (sustainable consumption and production), melon seed has recently attracted attention as a potential food ingredient, aiming to improve the sustainability of the food system. Therefore, it is important to carry out research investigating the valorisation strategies for melon seeds; such approach would reduce food waste and environmental issues, and stimulate circular economy.

2.2. Botany, origin and cultivation of *Cucumis melo*

Melon (*Cucumis melo*) is one of the most ancient and important crops in the world and has been cultivated for several thousand years. The origin of melon is under

debate, but several areas have been proposed such as Asia, Africa and Australia (Endl et al., 2018). According to the geographical distribution and the most comprehensive phylogenetic analysis, it seems that the initially domesticated melon (*Cucumis melo*) originated in Asia (Laghetti et al., 2008; Paris et al., 2012). In addition, the highly diversified melon varieties in India and China further support this view (Thakur et al., 2019).

Melon (*Cucumis melo L.*) is a eudicot diploid plant species and belongs to the Cucurbitaceae family which includes watermelon, squash, cucumber and pumpkin (Garcia-Mas et al., 2012). As a result of variety, morphological, and physiological diversity, two subspecies were put forward to distinguish melon, *C. melo* subsp. *melo* and *C. melo* subsp. *agrestis* (Nee & Kirkbride, 1994). In addition, melon is divided further into 16 botanical species, with 11 botanical group classifications in the *C. melo* subsp. *melo* and 5 botanical group classifications in the *C. melo* subsp. *agresits* (Burger et al., 2010; Pitrat, 2008). Some of these groups are major commercial melon varieties due to their great taste and high productivity; the most commercially important groups and their representing varieties are listed in **Table 2.1** and **Figure 2.1**. In the UK, the most consumed melon varieties are Galia, Honeydew, Cantaloupe, Piel de Sapo, and Matice, mainly from Spain, Brazil, Honduras, and Costa Rica (Frankowska et al., 2019).

Table 2.1. The most commercially important cultivar groups of melon and their characteristics *.

Number	Group name	Representing variety	Description
(a)	Reticulatus (subsp. melo)	Netted muskmelon	Round, netted, having a green or orange flesh, climacteric
(b)	Cantalupensis (subsp. melo)	Cantaloupe melon	Round, smooth or warted, flesh green or orange, non-netted, climacteric
(c)	Inodorus (subsp. melo)	Honeydew melon	Smooth-skinned, having a sweet, juicy, and light green to white flesh, non-climacteric
(d)	Flexuosus (subsp. melo)	Serpent melon	Long to very long (cucumber-like), wrinkled or smooth, flesh white and not sweet or with slightly acidic, climacteric
(e)	Conomon (subsp. agrestis)	Oriental pickling melon	Smooth, green or white peel, with white flesh, sweet or bland, not aromatic, non-climacteric
(f)	Chito * (subsp. melo)	Garden melon	Round, smooth, small lemon-size or orange-size, with bland white flesh
(g)	Dudaim (subsp. melo)	Queen Anne's pocket melon	Small, striped orange or brown, white and bland flesh, highly aromatic, climacteric
(h)	Chinensis (subsp. agrestis)	Songwhan Charmi melon	Pyriform, green with spots, medium sweet with green or orange flesh, no aromatic
(i)	Makuwa (subsp. agrestis)	Oriental melon	Oblate, smooth, light or yellow peel, sweet white flesh with quite aromatic, climacteric

*Data compiled from: Burger et al., 2010; Moing et al., 2020; Monforte et al., 2014;

Swamy, 2017. Climacteric/non-climacteric: information not found in 'Chito' group.

Figure 2.1. Images of representative melon cultivar varieties (Monforte et al., 2014; Swamy, 2017).

2.3. Chemical composition of melon seeds

Melon seed have an oval shape, smooth hard surface with yellow colour, and a white-yellow inner kernel (**Figure 2.2**). It has been reported that melon seeds are a rich source of oil, protein, and minerals (Mallek-Ayadi et al., 2018; Silva et al., 2020). Their composition is summarised in **Table 2.2** and is affected by multiple factors, including variety, region, climate, and growing conditions among others (Mallek-Ayadi et al., 2019; Mian-Hao & Yansong, 2007; Petkova & Antova, 2015; Rabadán et al., 2020; Yanty

et al., 2008).

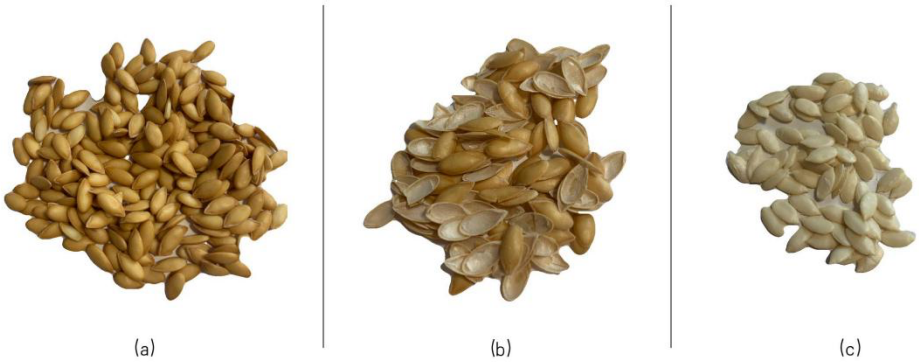

Figure 2.2. The morphology of the different parts of melon seeds (*Cucumis melo L.*): (a) the whole melon seeds; (b) the shell of melon seeds; (c) the kernel of melon seeds.

Table 2.2. Proximate composition of melon seeds *.

Component	**Content** (% w/w dry weight)
Moisture	4.9 - 7.8
Protein	14.9 - 39.8
Ash	2.8 - 4.8
Oil	25.0 - 51.4
Carbohydrate	5.9 - 30.0
Fibre	19.0 - 34.1
Minerals	**Content** (mg/100 g of dry weight)
Potassium	509.8 - 1148.8
Magnesium	101.7 - 1062.2
Calcium	55.4 - 506.1
Sodium	41.2 - 336.5
Iron	2.7 - 23.1
Zinc	2.3 - 9.4
Copper	0.8 -64.9

*Data compiled from: Bouazzaoui & Mulengi, 2018; De Melo et al., 2000; Mallek-Ayadi et al., 2018; Mian-Hao & Yansong, 2007; Petkova & Antova, 2015; Rezig et al., 2019; Sahin et al., 2022; Yanty et al., 2008.

2.3.1. Oil

The oil content of melon seeds ranges from 25% to 51.4% w/w (**Table 2.2**). Compared with other conventional oilseeds such as sunflower seed (approximately 40% w/w) (Pérez-Vich et al., 1998), peanut (approximately 50% w/w) (Wang et al., 2012), and soybean (approximately 15% w/w) (Medic et al., 2014), the oil content in melon seed is generally similar to sunflower seed and peanut and higher than soybean. Due to the expansion of markets and the increased requirement for food and non-food applications of vegetable oils (e.g. biodiesel), the development of new oil sources has been important for many oilseed-importing countries (Zanetti et al., 2013). Compared with other novel sources, melon seeds have higher quantities of oil than mango seeds (7.0% w/w), strawberry seeds (7.6% w/w), grape seed (9.7% w/w) and kumquat (33.5% w/w) (da Silva & Jorge, 2017), indicating that melon seeds could be used as a novel source of oil.

2.3.1.1. Fatty acid composition of melon seed oil

The fatty acid composition of melon seed oil is shown in **Table 2.3**. It is mainly constituted of linoleic acid (50.7% - 69.0%), followed by oleic acid (12.1% - 34.0%), palmitic acid (7.1% - 23.9%), and stearic acid (4.6 - 7.4%) (Bouazzaoui & Mulengi, 2018; de Melo et al., 2000; Mallek-Ayadi et al., 2018; Mian-Hao & Yansong, 2007; Petkova & Antova, 2015; Rezig et al., 2019; Yanty et al., 2008). The linoleic acid content of melon seed oil is similar to apple seed oil (58.9%), sunflower oil (62.2%), soybean oil (53.2%), and grape seed oil (74.7%) (da Silva & Jorge, 2017; Orsavova et al., 2015; Zhang et al.,

2021). According to Marangoni et al. (2020), linoleic acid is inversely correlated with cardiovascular disease risk. Additionally, Wang et al. (2019) detected conjugated linolenic acid (CLnA) in melon seed (1.97 - 2.16 mg CLnA/g seed), which was lower than cherry seed (12.79 CLnA/g seed), but higher than apple seed (0.38 mg CLnA/g seed) and pear seed (0.05 mg CLnA/g seed). CLnA is a positional and geometric isomer of linolenic acid (C18:3), which has been suggested to have potentially positive effects for cardiovascular health (Dhar Dubey et al., 2019; Fontes et al., 2017).

Table 2.3. Fatty acid composition of melon seed oil *.

Fatty acid compositions	**Content** (%)
C4:0 (Butanoic acid)	Trace
C6:0 (Hexanoic acid)	Trace
C8:0 (Octanoic acid)	Trace
C10:0 (Decanoic acid)	Trace
C12:0 (Dodecanoic acid)	Trace
C13:0 (Tridecanoic acid)	0.0 - 0.4
C14:0 (Tetradecanoic acid)	0.0 - 0.3
C15:0 (Pendadecanoic acid)	Trace
C16:0 (Hexadecanoic acid)	7.1 - 23.9
C17:0 (Heptadecanoic acid)	0.0 - 0.2
C18:0 (stearic acid)	4.6 - 7.4
C20:0 (Eicosanoic acid)	0.2 - 0.3
C21:0 (Heneicosanoic acid)	Trace
C22:0 (Docosanoic acid)	Trace
C23:0 (Tricosanoic acid)	0.0 - 0.2
C24:0 (Tetracosanoic acid)	0.0 - 1.5
Saturated fatty acids	11.9 - 34.2
C15:1 (10-Pentadecenoic acid)	0.0 - 0.3
C16:1 (9-Hexadecenoic acid)	0.0 - 0.2
C17:1 (1-Heptadecenoic acid)	Trace
C18:1 (Oleic acid)	12.1 - 34.0
C20:1 (Eicosenoic acid)	0.0 - 0.2
C22:1 (Erucic acid)	0.0 - 0.4
C24:1 (Nervonic acid)	Trace
Monounsaturated fatty acids	12.1 - 35.1
C18:2 (Linoleic acid)	50.7 - 69.0
C18:3 (Linolenic acid)	0.2 - 0.9
C20:2 (8,11-Eicosadienoic acid)	Trace
Polyunsaturated fatty acids	50.9 - 69.9

*Data compiled from: da Cunha et al., 2020; De Melo et al., 2000; Mallek-Ayadi et al., 2018; Manohar & Murthy, 2014; Mian-Hao & Yansong, 2007; Rabadán et al., 2020; Rezig et al., 2019; Yanty et al., 2008.

2.3.1.2. Physicochemical characteristics of melon seed oil

The physicochemical characteristics of the oil indicate its quality. **Table 2.4** lists the various chemical and physical parameters of melon seed oil which highlight its potential as edible oil. The acid value is normally positively correlated with the free fatty acid content, whereas the peroxide value relates to the degree of lipid oxidation (He et al., 2016; Huang et al., 2023). These two parameters indicate oil stability during storage because high acid and peroxide values characterize oils that are more susceptible to oxidation (Nehdi et al., 2013). Melon seed oil has a relatively good oxidative stability, as shown by its low peroxide and acid values (**Table 2.4**) (Bora et al., 2000; Nyam et al., 2009; Rabadán et al., 2020; Zhang et al., 2022). The iodine value reflects the level of unsaturated content in oil (Nehdi et al., 2013). The high iodine value of melon seed oil indicates a high level of unsaturated fatty acid composition as also confirmed by its high content of oleic acid (18: 1) and linoleic acid (18:2) (Mallek-Ayadi et al., 2018; Manohar & Murthy, 2014; Rezig et al., 2019; Zhang et al., 2022). The saponification value indicates the content of high molecular weight triacylglycerols (Nyam et al., 2009). The saponification value of melon seed oil is slightly higher than other crop oils (e.g. sunflower oil, pumpkin oil, and kenaf seed oil) and this indicates a high content of high molecular weight triacylglycerols (Nehdi et al., 2013; Nyam et al., 2009; Rezig et al., 2012). Generally, the iodine and saponification values can be used as indicators of the potential nutritional value of the oil (Huang et al., 2023; Kaur et al., 2022). Therefore, and considering the above, it can be suggested that melon seed oil could be considered as a novel edible oil with commercial potential.

Table 2.4. Physicochemical parameters of melon seed oil *.

Parameters	Melon seed oil
Acid value (mg KOH/g)	1.2 - 9.6
Iodine value (g I_2/100 g)	89.5 - 128.4
Peroxide value (meq O_2/kg)	0.0 - 7.4
Saponification value (mg KOH/g)	178.3 - 226.8

*Date compiled from: Bora et al., 2000; Mian-Hao & Yansong, 2007; Petkova & Antova, 2015; Rabadán et al., 2020; Rezig et al., 2019.

2.3.1.3. Sterols and tocopherols in melon seed oil

Sterols have similar chemical structures and biological properties to cholesterol, and are associated LDL-cholesterol reduction in humans (Jew et al., 2015; Shahzad et al., 2017). The sterols content in melon seed oil is shown in **Table 2.5**. Total sterol content is reported higher than some conventional oils, such as sunflower (192 mg/100 g of oil), pumpkin (91.3 mg/100 g of oil), and soybean (164.9 mg/100 g of oil), but lower than grape seed (300.5 mg/100 g of oil), tomato seed (169.8 mg/100 g of oil) and passion fruit seed oil (244.4 mg/100g of oil) (da Silva & Jorge, 2014; Fine et al., 2016; Mallek-Ayadi et al., 2018). Petkova & Antova (2015) analysed the sterol composition from three melon seed varieties and reported that β-sitosterol was the main component (50% - 64%), followed by Δ5-avenasterol (19.7% - 42.7%) and stigmasterol (2.7% - 4.8%).

Table 2.5. Sterols and tocopherol content in melon seed oil*.

Sterol	**Content** (mg/100 g of oil)
Campesterol	0.9 - 39.0
Stigmasterol	1.2 - 19.0
β-Sitosterol	58.0 - 206.4
Δ5-avenasterol	2.2 - 153.3
Cholesterol	0.3 - 3.3
Total phytosterol	62.6 - 421.0
Tocopherols	**Content** (mg/100 g of oil)
α-tocopherol	1.2 - 20.5
β-tocopherol	0.0 - 2.7
γ-tocopherol	25.0 - 63.3
δ-tocopherol	0.0 - 1.9
Total tocopherols	26.2 – 88.4

*Data compiled from: da Silva & Jorge, 2014; Górnaś et al., 2015; Mallek-Ayadi et al., 2018; Rabadán et al., 2020; Rezig et al., 2012; Zhang et al., 2022.

Tocopherols, major forms of vitamin E, are natural lipid-soluble antioxidants, which include homologues of α, β, γ, and δ (**Figure 2.3**). γ-Tocopherol is predominant in melon seed oil (Górnaś et al., 2015) and is considered a highly effective antioxidant that can inhibit oil oxidation and increase the oxidative stability by scavenging the peroxy radicals (Choe & Min, 2006). Hashemi et al. (2017) investigated the oxidative stability of several seed oils under microwave heating conditions; melon seed oil was more stable than watermelon oil, due to the tocopherol content of the former (125 mg/kg) compared to watermelon oil (111 mg/kg).

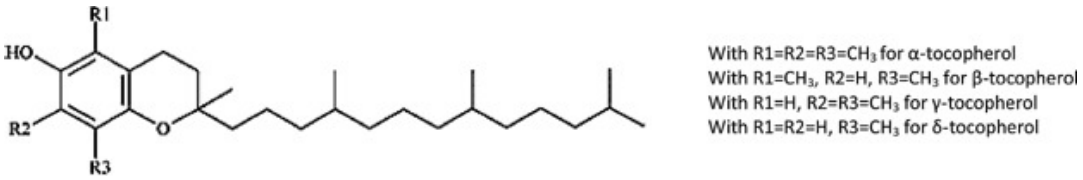

Figure 2.3. Chemical structure of tocopherol (Fine et al., 2016).

2.3.2. Protein

The protein content of melon seed ranges from 14.9% to 39.8% (w/w) (**Table 2.2**) and is comparable to other high oil seeds in protein content, including flaxseed (20.3%, w/w), rapeseed (19%, w/w), sesame seed (15.7%, w/w), sunflower seed (24.2%, w/w) (Kotecka-Majchrzak et al., 2020). Glutelin (38.7% w/w of protein), albumin (34.5% w/w of protein), and globulin (24.1% w/w of protein) are the predominant protein fractions, whereas prolamin is usually present in low levels (2.8% w/w of protein). In terms of protein solubility, it is a typical U curve and the minimum solubility at pH 4 (Pasrija & Sogi, 2022; Siddeeg et al., 2015). With regards to amino acid profile, the major amino acids of melon seed protein are glutamic acid (17.5% - 20% of protein), arginine (13.4% - 17% of protein), leucine (7.4% - 7.5% of protein), and aspartic (9.0% - 10% of protein), whereas lysine (2.8% - 3.5% of protein) and methionine (0.8% - 2.2% of protein) are present in low levels (De Mello et al., 2001; Mallek-Ayadi et al., 2019; Mian-Hao & Yansong, 2007). Several studies have shown that melon seed protein contains all 10 essential amino acids but is limited in cysteine (non-essential amino acid) (Mallek-Ayadi et al., 2019; Mian-Hao & Yansong, 2007; Zhang et al., 2021).

2.3.3. Fibre

Dietary fibre is important to human gut health and can increase satiety to reduce food intake (Hua et al., 2019). The dietary fibre in melon seed ranges from 19% to 34% w/w (**Table 2.2**). In addition, dietary fibre can be divided into soluble (SDF) (e.g. pectin, oligosaccharides, and soluble hemicelluloses) and insoluble (IDF) (e.g. cellulose, insoluble hemicelluloses, and lignin) (Hua et al., 2019). Studies conducted by Mallek-Ayadi et al. (2018) reported that SDF and IDF in melon seeds of Maazoun variety were 3.14% and 22.18% w/w, respectively, indicating that IDF is the major dietary fibre in melon seed. However, detailed information on the carbohydrate composition of melon seed is still scarce; further research is needed to provide more comprehensive understanding of melon seed carbohydrates, their nutritional profile and any potential benefits for human health.

2.3.4. Minerals

The mineral content of melon seeds is detailed in **Table 2.2**, with the most prominent ones being potassium, magnesium, calcium, and sodium. It is important to note that the potassium content can reach 1148 mg/100 g in melon seed. According to the new guidance on dietary potassium from WHO, potassium-rich foods include bean and peas (~1300 mg/100 g), nuts (~ 600 mg/100 g), spinach (~ 550 mg/100 g), and banana (~350 mg/100 g) (WHO, 2013). Compared with these potassium-rich foods, it can be concluded that melon seeds have high content of potassium and could be considered as a new source of potassium. High sodium dietary intake is a major public health issue in both developed and developing countries and is linked with increased risk of hypertension and cardiovascular diseases. WHO suggests increased potassium intake

and decreased sodium intake to reduce the risk of heart disease (Cepanec et al., 2017; Du et al., 2020). Besides, melon seed can also be regarded as a source of calcium (506 mg/100 g) when compared with plain low-fat yogurt (189 mg/100 g), tofu (183 mg/100 g), and whole milk (116 mg/100 g) (Titchenal & Dobbs, 2007).

2.3.5. Polyphenols

Polyphenols can be found in various plant sources, which through their antioxidant activities could offer benefits on human health, through their anti-inflammatory role and protection against chronic diseases (Yannone et al., 2012). The total phenolic content and antioxidant capacity in melon seeds ranges from 22.9 to 111.7 mg GAE (gallic acid equivalent)/100 g, which is similar to potato (92 mg GAE/100 g), rambutan seed (120 mg GAE/100 g), but lower than mango seed (895 mg GAE/100 g), passion fruit seed (440 mg GAE/100 g) (Fundo et al., 2018; Nguyen et al., 2019; Rolim et al., 2018; Xu et al., 2009; Zeb, 2016). It would suggest that melon seeds could be considered as a relatively good source of antioxidants. However, *in vitro* antioxidant assays cannot accurately evaluate real antioxidant activity *in vivo* (Granato et al., 2018; Martinelli et al., 2021). Besides, these chemical assays also do not consider the relevant parameters within a biological environment (e.g. bioavailability, distribution, and absorption) as well as other confounding factors (e.g. age and gender) (Apak, 2019; Martinelli et al., 2021). Therefore, further research is required to establish reliable protocols to determine antioxidant activity of polyphenols *in vivo* (Granato et al., 2018). Moreover, studies conducted by Mallek-Ayadi et al. (2019) and Zeb (2016) investigated the phenolic profile of melon seed extracts and indicated that their antioxidant

capacity could be associated with the presence of various phenolic compounds including gallic acid (4.2 - 6.7 mg/100 g), caffeic acid (66.0 mg/100 g), vanillic acid (1.6 - 3.9 mg/100 g), catechin (4.3 mg/100 g), ellagic acid (6.5 mg/100 g), resveratrol (2.9 mg/100 g), and 4-hydroxybenzoic acid (3.3 mg/100 g).

2.4. Extraction technology

Based on their composition, melon seeds hold potential as a source of value-added compounds, such as lipids, proteins, carbohydrates, minerals, and polyphenols, which can have a variety of applications in many sectors, such as food, pharmaceutical, and cosmetics. Moreover, they could be used as a resource for bioenergy production through bioconversion processes (e.g. enzymatic and fermentation processes). Recovery of these value-added compounds can maximise value from melon seed. Therefore, appropriate extraction technologies are key for scale-up studies and commercial applications (More et al., 2022). In the following sections, extraction technologies are discussed in detail, including conventional and novel green technologies, targeting the recovery of value-added compounds from melon seed. Additionally, the advantages and disadvantages for conventional and novel green technologies (supercritical fluid extraction, enzyme assisted extraction, and ultrasonic assisted extraction) are listed in **Table 2.6**.

Table 2.6. Summary of advantages and disadvantages of conventional and novel green technologies *.

Method	Advantage	Disadvantage
Conventional solvent extraction (CSE)	Simplicity of process Cost effectiveness Modulation of selectivity by solvent choice	Solvent residue in final products Use of large amount of toxic solvents Energy intensive Flammable risks
Supercritical fluid extraction (SFE)	Low temperature Less or no solvent usage Fast extraction rate Easy solvent recovery avoiding expensive post-processing	Expensive and complex equipment High operation cost Low polarity of supercritical CO_2
Enzyme assisted extraction (EAE)	Enzyme reusability Mild reaction conditions Low energy consumption and operational costs High selectivity Ease of operation	Long incubation time Complicated drying process after enzymatic treatment Lack of long-term stability of enzymes Enzyme cost
Ultrasonic assisted extraction (UAE)	Simplicity of process High yield Fast extraction rate Less solvent usage	Non-uniformity distribution of ultrasound energy Degradation of active compounds from plant matrices due to oxidative pyrolysis caused by hydroxyl (OH-) radicals during cavitation

* Data compiled from: AlYammahi et al., 2023; Ameer et al., 2017; Garavand et al., 2019; Geow et al., 2021; Gligor et al., 2019; Renard, 2018; Wu et al., 2017.

2.4.1. Conventional solvent extraction (CSE)

Solvent extraction is a conventional method for the recovery of high value-added compounds, such as protein, oil, polysaccharides, and polyphenols. Solvent extraction usually includes solid-liquid and liquid-liquid extraction. The process involves mixing the material with a suitable non-polar solvent (e.g. hexane and petroleum ether for oil extraction) or polar solvent (e.g. ethanol and methanol for polyphenols extraction) to make the compound solubilise into the extraction solvent where it can be recovered from (Alexandre et al., 2018; Jha & Sit, 2022; More et al., 2022). However, some of the organic solvents used, such as n-hexane and tetrahydrofuran could be potentially harmful and toxic for humans, animals and/or the environment (Chemat et al., 2020). Besides, conventional solvent extraction has many drawbacks, including long operation times, poor extraction efficiency, solvent residue in final products and flammable risks (Garavand et al., 2019; Wu et al., 2017). Therefore, novel, environmentally friendly extraction methods are needed to improve on these drawbacks.

2.4.2. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is an environmentally friendly extraction method that mainly uses carbon dioxide (CO_2; typical non-polar solvent; non-toxic and green) as supercritical fluid. CO_2 at its supercritical fluid state, has high solvation power and can diffuse like a gas in solid matrices (Rai et al., 2016; Sagar et al., 2018; Sharif et al., 2014). In addition, ethanol or methanol, as a polar co-solvent, can be added into

supercritical CO_2 to target the extraction polar compounds (**Figure 2.4**) (Chemat et al., 2020). Supercritical fluid extraction has many advantages compared with conventional solvent extraction including a faster, cleaner, and easier separation of the solute from the solvent. Maran & Priya (2015) used SFE to extract oil from melon seeds. They reported that the fatty acid composition of melon seed oil was not different between SFE and hexane extraction, but the total yield of oil (48.11%) using SFE (extraction at 44 MPa, 49 ℃, 0.64 g/min of flow, and 81 mins of extraction time) was slightly higher than hexane Soxhlet extraction (46.83%). Ekinci & Gürü (2019) reported that the highest yields of extracted oil, β-sitosterol and stigmasterol by SFE were 36.8 g/100 g, 304 mg/kg, and 121 mg/kg, respectively, whereas the optimal conditions were 33 ℃, 200 bar, and 11 g CO_2/min.

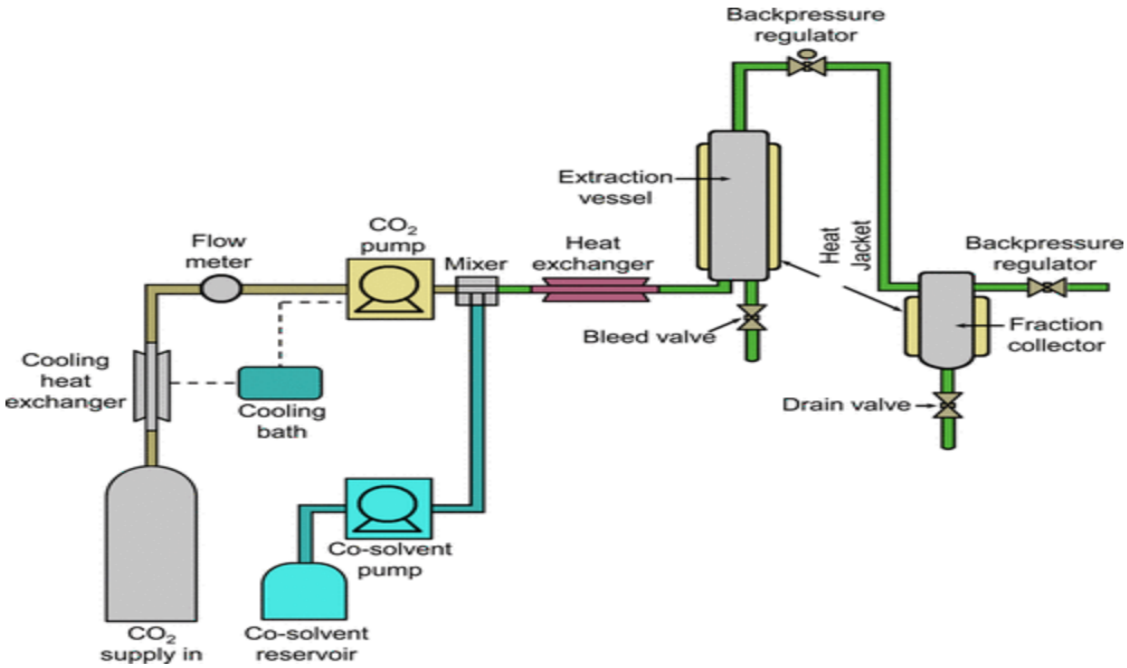

Figure 2.4. Schematic representation of supercritical CO_2 system coupled with co-solvent (Koubaa et al., 2015).

2.4.3. Enzyme assisted extraction (EAE)

Enzyme assisted extraction (EAE) is an environmentally friendly extraction method, because enzymes replace traditional solvents (Wen et al., 2020). The principle of enzyme assisted extraction is that specific enzymes (such as cellulases, pectinases and hemicellulases) are selected to disrupt or hydrolyse the cell wall structure, enhance cell permeability and thus increase the extraction yield of targeted components (e.g. oil and polyphenols) (Alexandre et al., 2018; Marić et al., 2018; Tang et al., 2022). It is important to note that many parameters should be considered to achieve optimal extraction conditions in the enzyme assisted processing, including pH, temperature, time, enzyme concentration, agitation, as well as the ratio between enzyme and substrate (Alexandre et al., 2018; Garavand et al., 2019; Wen et al., 2020). EAE has demonstrated its potential for oil extraction, and the schematic representation of enzyme assisted oil extraction is presented in **Figure 2.5**. For example, Ribeiro et al. (2016) compared oil extraction from sesame oil by EAE (using pectinase and alcalase) and conventional solvent extraction; it showed that the quantity of the oil extracted by EAE (36.65%) was lower than solvent extraction (SE) (59.97%), while the quality of the EAE extracted sesame oil was better than that of conventionally extracted in terms of antioxidant capacity, total phytosterols content, and total polyunsaturated fatty acid content. However, in practical oil extraction, EAE could leads into the formation of an emulsion, which in turn needs de-emulsification processing to further improve the oil extraction yield.

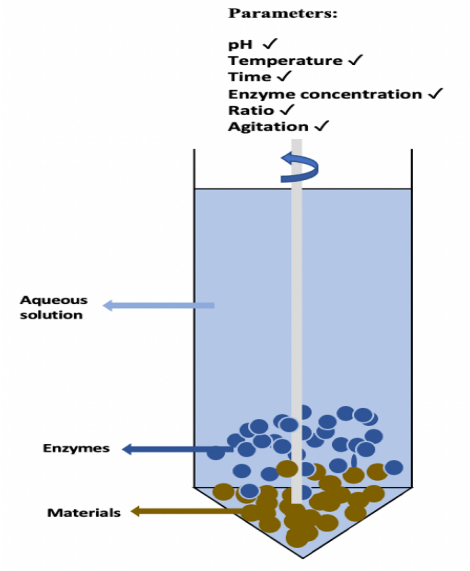

Figure 2.5. Schematic representation of enzyme assisted oil extraction.

2.4.4. Ultrasonic assisted extraction (UAE)

The principle of ultrasonic assisted extraction (UAE) is based on the energy change of the expansion of sound waves in a liquid (**Figure 2.6**) (Jha & Sit, 2022). In UAE processing, the transmission of ultrasound through the medium generates cavitation bubbles which grow further and collapse, resulting in vibration, mixing, and pulverization; these in turn, enhance the molecular vibration frequency, disrupt the cell wall, and accelerate the rate of heat or mass transfer (Alexandre et al., 2018; Chemat et al., 2020; Mwaurah et al., 2020). Therefore, extraction yield is increased and extraction time is decreased (Mwaurah et al., 2020). Process parameters such as type of solvent, extraction time and temperature, as well as frequency of ultrasound wave have been mentioned as keys variables for the effectiveness and efficiency of UAE process (Bruno Siewe et al., 2021). Besides, UAE can be combined with other

extraction methods to facilitate cell wall disruption and overcome some limitations of other extraction methods (e.g. considerable long extraction time and low extraction efficiency) (Wen et al., 2020). For example, it can be combined with enzymes, leading to an ultrasonic-assisted enzymatic extraction (UAEE) method (Alexandre et al., 2018). Tang et al. (2022) and Kumar et al. (2022) used UAEE method to extract dietary fibre and oil in bamboo shoot by-products and sea buckthorn berry, respectively. The potential advantage that UAEE could offer is the acceleration of extraction and enzymatic hydrolysis process through cavitation, thereby improving the enzymatic extraction efficiency (Gao et al., 2022).

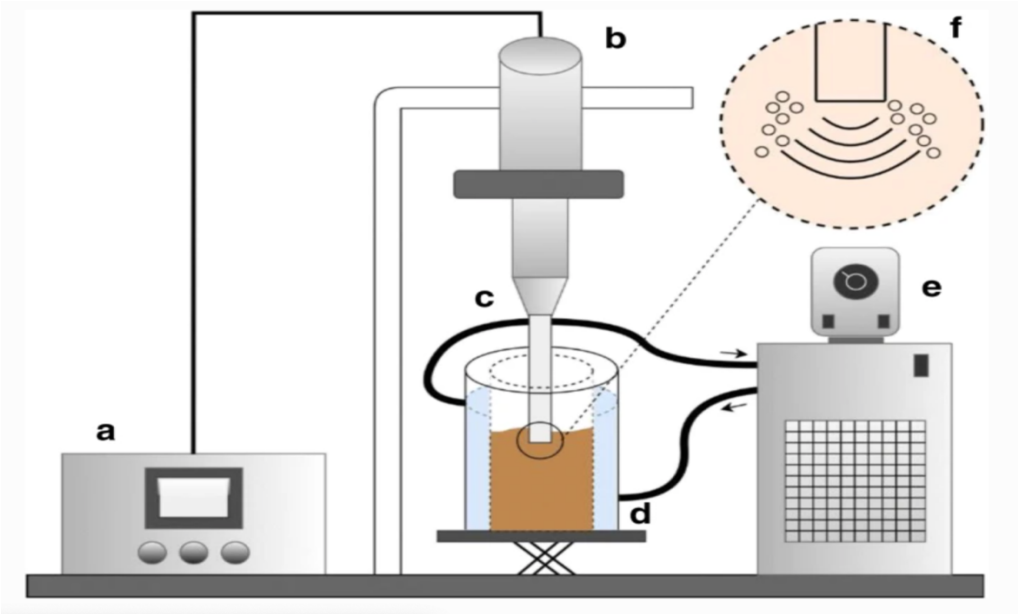

Figure 2.6. Schematic representation of Ultrasonic assisted extraction: (a) the ultrasound controller; (b) transducer; (c) the ultrasound probe; (d) the jacketed beaker with sample-solvent mixture inside; (e) water bath system; (f) cavitation bubbles phenomena (Wen et al., 2021).

2.5. Utilisation of melon seeds in food product development

The conversion of food by-products as ingredients in food formulations is one of the most attractive strategies to reduce food loss and capture value. Recently, the utilisation of melon seed and their subsequent components in food formulation has been explored and has demonstrated their potential as ingredient. The following section presents current advances in the application of melon seeds and their components in various food matrices.

Karakaya et al. (1995) developed a melon seed beverage which was made of melon seeds, sugar, and water through blending, and demonstrated that the beverage was a good source of mineral (iron and magnesium) and protein, and gave very good results in hedonic test as 'liked very much' (4.9 on a 5-point hedonic scale). In addition, Zungur Bastıoğlu et al. (2016) produced a plant-based milk from melon seed spray dried powder as alternative to vegans, vegetarians, and lactose-intolerant. Melon seed spray-dried powder showed good solubility (92.0% - 99.2%) and storage stability (moisture content and water activity are 2.1% - 2.4 % and 0.260 to 0.310, respectively), but exhibited poor flowability (50.44 - 53.23 Carr index value) and tended to form lumps. In terms of sensory evaluation, melon seed milk showed very good visual attributes, particularly a creamy, white colour appearance.

da Cunha et al. (2020) used melon seeds in confectionary products. Specifically, melon seed flour was used as raw material to partly replace wheat flour in three inclusions: 10% melon seed flour, 30% melon seed flour, and 50% melon seed flour; the control was 100% wheat flour. The results demonstrated that all cakes containing melon seed flour were well accepted by the panel, as did the control group (100% wheat flour), in

terms of their flavour (sweetness) attribute, the score was slightly reduced with increasing the ratio of melon seed flour. The 10% melon seed flour cake had the highest acceptance.

Tarjuelo et al. (2022) reformulated dried sausage (fuet) using melon seed oil (extracted by hydraulic press) as a pork fat substitute, at 50%, 75%, and 100% (w/w). Reformulated fuet sausages had higher linoleic acid content as compared to control fuet sausages (100% pork fat). However, in the sensory evaluation, the sensory score for fuet sausages made with melon seed oil was lower than that of the control in terms of appearance, texture, odour, and taste. In terms of texture, fuet sausage made with melon seed oil had significant lower ($p < 0.05$) hardness value than control. Overall, from a nutritional point of view, the evidence in this study suggests the possibility of melon seed oil into sausage application, which could lead into nutritionally more balanced meat products.

The above studies highlight some promising progress with regards to melon seeds utilisation in food formulations. However, challenges in product quality and organoleptic aspects still exist and further work is required to optimise processing parameters and develop products acceptable by consumers. Additionally, food matrix can influence the bioactivity and bioavailability of nutrients during digestion process, and currently, there is still no sufficient information regarding the potentially nutritional properties of incorporated melon seed in food. Based on this point, further research is needed to identify and evaluate the bioavailability, absorption, and bioactivity of melon seed nutrients in *vivo*. Moreover, considering the safety and

quality of by-products is also an important part of valorisation strategies to ensure food safety for customer consumption. In general, allergens, microbiological safety, and contamination assessment (e.g. pesticides residues and toxins) are most common considerations in food safety evaluation (Ayuso et al., 2022; Socas-Rodríguez et al., 2021). Although there is no available information about melon seed safety and quality at present, it merits an important aspect of research in the future.

2.6. Biological activity of melon seed compounds

Melon seeds could be a potential source of natural bioactive compounds. Research studies have indicated the biological activity of melon seeds including their antioxidant and anti-proliferative activities, as well as their health benefits in preventing or reducing the risk of chronic diseases, such as diabetes, obesity, and cardiovascular conditions (Chen et al., 2014; Chen & Kang, 2013; Hao et al., 2020; Rasouli et al., 2017; Rolim et al., 2018). Studies by Chen & Kang (2013) demonstrated *in vitro* that hexane extracts of the melon (*Cucumis melo L. var. makuwa Makino*) seed have potential ability on anti-diabetic by inhibiting the activity of α-glucosidase and α-amylase by 35% and 62%, respectively; this is because the inhibition of these enzymes can delay carbohydrate digestion and glucose absorption thereby controlling the increase in post-prandial plasma glucose, which has potential benefit for type 2 diabetics. According to the study, this could be attributed to presence of unsaturated fatty acids in the hexane extracts of melon seeds. Additionally, Chen et al. (2014) further demonstrated that the three key fatty acids in the melon extracts, palmitic, linoleic

and oleic acid, had high enzyme inhibitory effects *in vitro*, especially linoleic acid which demonstrated the strongest inhibition towards α-glucosidase and α-amylase.

Rolim et al. (2018) studied the anti-proliferative effects of melon seed extracts on cancer cells (SiHa, Hela, HT-29, and 786-0) and attributed this to their phenolic content. The results showed that melon seed extracts, obtained using distilled water, aqueous ethanol (30:70 v/v), and aqueous methanol (30:70 v/v) as solvents, exhibited inhibition activity for all four types of cancer cells. Zhang et al. (2020) demonstrated that melon seed extracts obtained using methanol, distilled water, and chloroform showed highly effective anticancer activity in HeLa cell lines and were more cytotoxic to HCT116 cell lines.

Rasouli et al. (2017) purified protein from melon seed as trypsin inhibitor and explored its potential anti-angiogenesis and anti-proliferation activities. The results showed that the molecular mass of purified protein was 3 kDa and the trypsin inhibition activity was 765 CIU/mg (chymotrypsin inhibitory unit per mg of protein). When the concentration of purified protein reached 40 μg/ml, the angiogenesis in the Human Umbilical Vein Endothelial Cells (HUVEC) capillary tube formation model was completely inhibited. Moreover, even at very high concentrations of TICMS, i.e. 120 μg/mL, there was no cytotoxic effect. Thus, this study indicated that purified melon seed protein could be used as a botanical derivative promoting anti-angiogenesis and anti-proliferation effects.

Although melon seed extracts seem to exhibit promising bioactive properties *in vitro*

studies, there is lack of information deriving from *in vivo* studies that could further support these findings. Therefore, there is a need for *in vivo* studies to produce more comparable and reliable data, to validate the bioactive effects of melon seed extracts on health and assist in the design of melon seed valorisation strategies.

2.7. Conclusions

Melon seeds hold potential as a source of oil as well as other functional food ingredients (protein/peptides, polyphenols, minerals, and dietary fibre). Research on the valorisation of melon seeds should aim at maximising their utilisation, converting into value-added products, applicable in food or other sectors (e.g. pharmaceuticals and cosmetics), to reduce waste and promote sustainability. Moreover, efficient, environmentally friendly, and scalable extraction methods should be evaluated to contribute to the transition into a circular economy through producing diverse value-added products from melon seeds. Regarding melon seed applications in food, the main limitation relates to their quality and organoleptic aspects. Therefore, optimising intrinsic quality parameters influencing the incorporation of melon seeds or their components in foods is an important area of research which should be further pursued.

2.8. References

Alexandre, E. M. C., Moreira, S. A., Castro, L. M. G., Pintado, M., & Saraiva, J. A. (2018). Emerging technologies to extract high added value compounds from fruit residues: Sub/supercritical, ultrasound-, and enzyme-assisted extractions. In *Food Reviews International* (Vol. 34, Issue 6). https://doi.org/10.1080/87559129.2017.1359842

AlYammahi, J., Rambabu, K., Thanigaivelan, A., Bharath, G., Hasan, S. W., Show, P. L., & Banat, F. (2023). Advances of non-conventional green technologies for phyto-saccharides extraction: current status and future perspectives. Phytochemistry Reviews, 22(4), 1067–1088. https://doi.org/10.1007/s11101-022-09831-2

Ameer, K., Shahbaz, H. M., & Kwon, J. (2017). Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. Comprehensive Reviews in Food Science and Food Safety, 16(2), 295–315. https://doi.org/10.1111/1541-4337.12253

Apak, R. (2019). Current Issues in Antioxidant Measurement. *Journal of Agricultural and Food Chemistry*, *67*(33), 9187–9202. https://doi.org/10.1021/acs.jafc.9b03657

Ayuso, M., Carpena, M., Taofiq, O., Albuquerque, T. G., Simal-Gandara, J., Oliveira, M. B. P. P., Prieto, M. A., Ferreira, I. C. F. R., & Barros, L. (2022). Fig "Ficus carica L." and its by-products: A decade evidence of their health-promoting benefits towards the development of novel food formulations. *Trends in Food Science & Technology*, *127*, 1–13. https://doi.org/10.1016/j.tifs.2022.06.010

BBC. (2022). *Melon seeds recipes*. BBC News, FOOD, Melon Seed. https://www.bbc.co.uk/food/melon_seeds

Bora, P. S., Narain, N., & de MeIIo, M. L. S. (2000). Characterization of the seed oils of some commercial cultivars of melon. *European Journal of Lipid Science and Technology*, *102*(4). https://doi.org/10.1002/(SICI)1438-9312(200004)102:4<266::AID-EJLT266>3.0.CO;2-1

Bouazzaoui, N., & Mulengi, J. K. (2018). Fatty acids and mineral composition of melon (Cucumis Melo) and pumpkin (Cucurbita moschata) seeds. *Journal of Herbs, Spices and Medicinal Plants*, *24*(4). https://doi.org/10.1080/10496475.2018.1485125

Bruno Siewe, F., Kudre, T. G., & Narayan, B. (2021). Optimisation of ultrasound-assisted enzymatic extraction conditions of umami compounds from fish by-products using the combination of fractional factorial design and central composite design. *Food Chemistry*, *334*. https://doi.org/10.1016/j.foodchem.2020.127498

Burger, Y., Paris, H. S., Cohen, R., Katzir, N., Tadmor, Y., Lewinsohn, E., & Schaffer, A. A. (2010). Genetic Diversity of Cucumis Melo . In *Horticultural Reviews*. https://doi.org/10.1002/9780470527238.ch3

Cecily Dignan, Barbara Burlingame, Shailesh Kumar, & William Aalbersberg. (2004).

The Pacific Islands food composition tables (Second).

Cepanec, K., Vugrinec, S., Cvetković, T., & Ranilović, J. (2017). Potassium Chloride-Based Salt Substitutes: A Critical Review with a Focus on the Patent Literature. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5). https://doi.org/10.1111/1541-4337.12291

Chemat, F., Abert Vian, M., Fabiano-Tixier, A. S., Nutrizio, M., Režek Jambrak, A., Munekata, P. E. S., Lorenzo, J. M., Barba, F. J., Binello, A., & Cravotto, G. (2020). A review of sustainable and intensified techniques for extraction of food and natural products. In *Green Chemistry* (Vol. 22, Issue 8). https://doi.org/10.1039/c9gc03878g

Chen, L., Kang, Y. H., & Suh, J. K. (2014). Roasting processed oriental melon (Cucumis melo L. var. makuwa Makino) seed influenced the triglyceride profile and the inhibitory potential against key enzymes relevant for hyperglycemia. *Food Research International*, *56*. https://doi.org/10.1016/j.foodres.2013.11.040

Chen, L., & Kang, Y.-H. (2013). In vitro inhibitory effect of oriental melon (Cucumis melo L. var. makuwa Makino) seed on key enzyme linked to type 2 diabetes. *Journal of Functional Foods*, *5*(2). https://doi.org/10.1016/j.jff.2013.01.008

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 5, Issue 4). https://doi.org/10.1111/j.1541-4337.2006.00009.x

da Cunha, J. A., Rolim, P. M., da Silva Chaves Damasceno, K. S. F., de Sousa, F. C., Nabas, R. C., & Seabra, L. M. A. J. (2020). From seed to flour: Sowing sustainability in the use of cantaloupe melon residue (Cucumis melo L. Var. Reticulatus). *PLoS ONE*, *15*(1). https://doi.org/10.1371/journal.pone.0219229

da Silva, A. C., & Jorge, N. (2014). Bioactive compounds of the lipid fractions of agro-industrial waste. *Food Research International*, *66*. https://doi.org/10.1016/j.foodres.2014.10.025

da Silva, A. C., & Jorge, N. (2017). Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste. *European Journal of Lipid Science and Technology*, *119*(4). https://doi.org/10.1002/ejlt.201600024

De Mello, M. L. S., Bora, P. S., & Narain, N. (2001). Fatty and Amino Acids Composition of Melon (Cucumis melo Var. saccharinus) Seeds. *Journal of Food Composition and Analysis*, *14*(1). https://doi.org/10.1006/jfca.2000.0952

De Melo, M. L. S., Narain, N., & Bora, P. S. (2000). Characterisation of some nutritional constituents of melon (Cucumis melo hybrid AF-522) seeds. *Food Chemistry*, *68*(4). https://doi.org/10.1016/S0308-8146(99)00209-5

Dhar Dubey, K. K., Sharma, G., & Kumar, A. (2019). Conjugated Linolenic Acids: Implication in Cancer. *Journal of Agricultural and Food Chemistry*, *67*(22). https://doi.org/10.1021/acs.jafc.9b01379

Du, S., Wang, H., Zhang, B., & Popkin, B. M. (2020). Dietary potassium intake remains low and sodium intake remains high, and most sodium is derived from home food preparation for chinese adults, 1991–2015 trends. In *Journal of Nutrition* (Vol. 150, Issue 5). https://doi.org/10.1093/jn/nxz332

Ekinci, M. S., & Gürü, M. (2019). Extraction of phytosterols from melon (Cucumis melo) seeds by supercritical CO2 as a clean technology. *Green Processing and Synthesis*, *8*(1). https://doi.org/10.1515/gps-2019-0038

Ekman, A., Campos, M., Lindahl, S., Co, M., Börjesson, P., Karlsson, E. N., & Turner, C. (2013). Bioresource utilisation by sustainable technologies in new value-added biorefinery concepts - Two case studies from food and forest industry. *Journal of Cleaner Production*, *57*. https://doi.org/10.1016/j.jclepro.2013.06.003

Ellen MacArthur Foundation. (2019). *Cities and circular economy for food*.

Endl, J., Achigan-Dako, E. G., Pandey, A. K., Monforte, A. J., Pico, B., & Schaefer, H. (2018). Repeated domestication of melon (Cucumis melo) in Africa and Asia and a new close relative from India. *American Journal of Botany*, *105*(10). https://doi.org/10.1002/ajb2.1172

European Commission. (2015). Closing the Loop - An EU action plan for the Circular Economy - (ANNEX 1). *Communication from the Commission to the European Parliament, the Council, the Europena Economic and Social Committee and the Committee of the Regions*.

European Commission. (2020). *A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system*. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1590404602495&uri=CELEX:52020DC0381

FAO. (2015). Global initiative on food loss and food waste reduction. *United Nations*.

Fine, F., Brochet, C., Gaud, M., Carre, P., Simon, N., Ramli, F., & Joffre, F. (2016). Micronutrients in vegetable oils: The impact of crushing and refining processes on vitamins and antioxidants in sunflower, rapeseed, and soybean oils. In *European Journal of Lipid Science and Technology* (Vol. 118, Issue 5). https://doi.org/10.1002/ejlt.201400400

Fontes, A. L., Pimentel, L. L., Simões, C. D., Gomes, A. M. P., & Rodríguez-Alcalá, L. M. (2017). Evidences and perspectives in the utilization of CLNA isomers as bioactive compounds in foods. *Critical Reviews in Food Science and Nutrition*, *57*(12). https://doi.org/10.1080/10408398.2015.1063478

Frankowska, A., Jeswani, H. K., & Azapagic, A. (2019). Life cycle environmental impacts of fruits consumption in the UK. *Journal of Environmental Management*, *248*, 109111. https://doi.org/10.1016/j.jenvman.2019.06.012

Fundo, J. F., Miller, F. A., Garcia, E., Santos, J. R., Silva, C. L. M., & Brandão, T. R. S. (2018). Physicochemical characteristics, bioactive compounds and antioxidant activity in juice, pulp, peel and seeds of Cantaloupe melon. *Journal of Food*

Measurement and Characterization, *12*(1). https://doi.org/10.1007/s11694-017-9640-0

Gao, Y., Dong, Q., Zhao, S., Zhao, Y., Zhang, Y., Wang, H., Wang, Y., Wang, W., Wang, L., & Wang, H. (2022). Efficient ultrasound-assisted enzymatic method for extraction of immunostimulant QS-21 from Quillaja saponaria Molina. *Industrial Crops and Products*, *189*. https://doi.org/10.1016/j.indcrop.2022.115807

Garavand, F., Rahaee, S., Vahedikia, N., & Jafari, S. M. (2019). Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients. In *Trends in Food Science and Technology* (Vol. 89). https://doi.org/10.1016/j.tifs.2019.05.005

Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., Gonźalez, V. M., Heńaff, E., Caṁara, F., Cozzuto, L., Lowy, E., Alioto, T., Capella-Gutieŕrez, S., Blancae, J., Cañizares, J., Ziarsolo, P., Gonzalez-Ibeas, D., Rodriǵuez-Moreno, L., Droege, M., Du, L., … Puigdomeǹech, P. (2012). The genome of melon (Cucumis melo L.). *Proceedings of the National Academy of Sciences of the United States of America*, *109*(29). https://doi.org/10.1073/pnas.1205415109

Geow, C. H., Tan, M. C., Yeap, S. P., & Chin, N. L. (2021). A Review on Extraction Techniques and Its Future Applications in Industry. European Journal of Lipid Science and Technology, 123(4). https://doi.org/10.1002/ejlt.202000302

Gligor, O., Mocan, A., Moldovan, C., Locatelli, M., Crișan, G., & Ferreira, I. C. F. R. (2019). Enzyme-assisted extractions of polyphenols – A comprehensive review. Trends in Food Science & Technology, 88, 302–315. https://doi.org/10.1016/j.tifs.2019.03.029

Górnaś, P., Soliven, A., & Segliņa, D. (2015). Seed oils recovered from industrial fruit by-products are a rich source of tocopherols and tocotrienols: Rapid separation of α/β/γ/δ homologues by RP-HPLC/FLD. *European Journal of Lipid Science and Technology*, *117*(6). https://doi.org/10.1002/ejlt.201400566

Granato, D., Shahidi, F., Wrolstad, R., Kilmartin, P., Melton, L. D., Hidalgo, F. J., Miyashita, K., Camp, J. van, Alasalvar, C., Ismail, A. B., Elmore, S., Birch, G. G., Charalampopoulos, D., Astley, S. B., Pegg, R., Zhou, P., & Finglas, P. (2018). Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods? *Food Chemistry*, *264*, 471–475. https://doi.org/10.1016/j.foodchem.2018.04.012

Hao, W., Zhu, H., Chen, J., Kwek, E., He, Z., Liu, J., Ma, N., Ma, K. Y., & Chen, Z. Y. (2020). Wild Melon Seed Oil Reduces Plasma Cholesterol and Modulates Gut Microbiota in Hypercholesterolemic Hamsters. *Journal of Agricultural and Food Chemistry*, *68*(7). https://doi.org/10.1021/acs.jafc.9b07302

Hashemi, S. M. B., Mousavi Khaneghah, A., Koubaa, M., Lopez-Cervantes, J., Yousefabad, S. H. A., Hosseini, S. F., Karimi, M., Motazedian, A., & Asadifard, S. (2017). Novel edible oil sources: Microwave heating and chemical properties. *Food Research International*, *92*. https://doi.org/10.1016/j.foodres.2016.11.033

He, Z., Zhu, H., Li, W., Zeng, M., Wu, S., Chen, S., Qin, F., & Chen, J. (2016). Chemical components of cold pressed kernel oils from different Torreya grandis cultivars. *Food Chemistry*, *209*. https://doi.org/10.1016/j.foodchem.2016.04.053

Hua, M., Lu, J., Qu, D., Liu, C., Zhang, L., Li, S., Chen, J., & Sun, Y. (2019). Structure, physicochemical properties and adsorption function of insoluble dietary fiber from ginseng residue: A potential functional ingredient. *Food Chemistry*, *286*. https://doi.org/10.1016/j.foodchem.2019.01.114

Huang, Y., Liu, C., Ge, Z., Huang, F., Tang, H., Zhou, Q., Liu, R., Huang, J., & Zheng, C. (2023). Influence of different thermal treatment methods on the processing qualities of sesame seeds and cold-pressed oil. *Food Chemistry*, *404*, 134683. https://doi.org/10.1016/j.foodchem.2022.134683

Jew, S., Antoine, J. M., Bourlioux, P., Milner, J., Tapsell, L. C., Yang, Y., & Jones, P. J. H. (2015). Nutrient essentiality revisited. In *Journal of Functional Foods* (Vol. 14). https://doi.org/10.1016/j.jff.2015.01.024

Jha, A. K., & Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. In *Trends in Food Science and Technology* (Vol. 119). https://doi.org/10.1016/j.tifs.2021.11.019

Jin, Q., Yang, L., Poe, N., & Huang, H. (2018). Integrated processing of plant-derived waste to produce value-added products based on the biorefinery concept. In *Trends in Food Science and Technology* (Vol. 74). https://doi.org/10.1016/j.tifs.2018.02.014

Karakaya, S., Kavas, A., El, S. N., Gündüç, N., & Akdoğan, L. (1995). Nutritive value of a melon seed beverage. *Food Chemistry*, *52*(2). https://doi.org/10.1016/0308-8146(94)P4193-J

Kaur, G., Kaur, D., Kansal, S. K., Garg, M., & Krishania, M. (2022). Potential cocoa butter substitute derived from mango seed kernel. *Food Chemistry*, *372*. https://doi.org/10.1016/j.foodchem.2021.131244

Kotecka-Majchrzak, K., Sumara, A., Fornal, E., & Montowska, M. (2020). Oilseed proteins – Properties and application as a food ingredient. In *Trends in Food Science and Technology* (Vol. 106). https://doi.org/10.1016/j.tifs.2020.10.004

Koubaa, M., Roselló-Soto, E., Šic Žlabur, J., Režek Jambrak, A., Brnčić, M., Grimi, N., Boussetta, N., & Barba, F. J. (2015). Current and New Insights in the Sustainable and Green Recovery of Nutritionally Valuable Compounds from Stevia rebaudiana Bertoni. Journal of Agricultural and Food Chemistry, 63(31), 6835–6846. https://doi.org/10.1021/acs.jafc.5b01994

Kumar T., A., Pareek, S., Kaur, R., Sagar, N. A., Singh, L., Sami, R., Aljuraide, N. I., Elhakem, A., Alsharari, Z. D., Alruwais, R. S., Aljabri, M. D., & Rahman, M. M. (2022). Optimization of Ultrasonic-Assisted Enzymatic Extraction of Freeze-Dried Sea Buckthorn (Hippophae rhamnoides L.) Berry Oil Using Response Surface Methodology. *Sustainability*, *14*(17), 10849. https://doi.org/10.3390/su141710849

Laghetti, G., Accogli, R., & Hammer, K. (2008). Different cucumber melon (Cucumis melo L.) races cultivated in Salento (Italy). *Genetic Resources and Crop Evolution*, *55*(4). https://doi.org/10.1007/s10722-008-9341-y

Lin, C. S. K., Pfaltzgraff, L. A., Herrero-Davila, L., Mubofu, E. B., Abderrahim, S., Clark, J. H., Koutinas, A. A., Kopsahelis, N., Stamatelatou, K., Dickson, F., Thankappan, S., Mohamed, Z., Brocklesby, R., & Luque, R. (2013). Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. In *Energy and Environmental Science* (Vol. 6, Issue 2). https://doi.org/10.1039/c2ee23440h

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2018). Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. *Process Safety and Environmental Protection*, *113*. https://doi.org/10.1016/j.psep.2017.09.016

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2019). Phytochemical profile, nutraceutical potential and functional properties of Cucumis melo L. seeds. *Journal of the Science of Food and Agriculture*, *99*(3). https://doi.org/10.1002/jsfa.9304

Manohar, S. H., & Murthy, H. N. (2014). Fatty acid profile of Cucumis melo var. acidulus (culinary melon) seed oil. In *JAOCS, Journal of the American Oil Chemists' Society* (Vol. 91, Issue 5). https://doi.org/10.1007/s11746-014-2422-5

Maran, J. P., & Priya, B. (2015). Supercritical fluid extraction of oil from muskmelon (Cucumis melo) seeds. *Journal of the Taiwan Institute of Chemical Engineers*, *47*. https://doi.org/10.1016/j.jtice.2014.10.007

Marangoni, F., Agostoni, C., Borghi, C., Catapano, A. L., Cena, H., Ghiselli, A., La Vecchia, C., Lercker, G., Manzato, E., Pirillo, A., Riccardi, G., Risé, P., Visioli, F., & Poli, A. (2020). Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. In *Atherosclerosis* (Vol. 292). https://doi.org/10.1016/j.atherosclerosis.2019.11.018

Marić, M., Grassino, A. N., Zhu, Z., Barba, F. J., Brnčić, M., & Rimac Brnčić, S. (2018). An overview of the traditional and innovative approaches for pectin extraction from plant food wastes and by-products: Ultrasound-, microwaves-, and enzyme-assisted extraction. In *Trends in Food Science and Technology* (Vol. 76). https://doi.org/10.1016/j.tifs.2018.03.022

Martinelli, E., Granato, D., Azevedo, L., Gonçalves, J. E., Lorenzo, J. M., Munekata, P. E. S., Simal-Gandara, J., Barba, F. J., Carrillo, C., Riaz Rajoka, M. S., & Lucini, L. (2021). Current perspectives in cell-based approaches towards the definition of the antioxidant activity in food. *Trends in Food Science & Technology*, *116*, 232–243. https://doi.org/10.1016/j.tifs.2021.07.024

Medic, J., Atkinson, C., & Hurburgh, C. R. (2014). Current knowledge in soybean composition. In *JAOCS, Journal of the American Oil Chemists' Society* (Vol. 91, Issue 3). https://doi.org/10.1007/s11746-013-2407-9

Mian-Hao, H., & Yansong, A. (2007). Characteristics of some nutritional composition of melon (Cucumis melo hybrid 'ChunLi') seeds. *International Journal of Food Science and Technology*, *42*(12). https://doi.org/10.1111/j.1365-2621.2006.01352.x

Miller, F. A., Fundo, J. F., Garcia, E., Santos, J. R., Silva, C. L. M., & Brandão, T. R. S. (2020). Physicochemical and Bioactive Characterisation of Edible and Waste Parts of "Piel de Sapo" Melon. *Horticulturae*, *6*(4), 60. https://doi.org/10.3390/horticulturae6040060

Miller, F. A., Fundo, J. F., Silva, C. L. M., & Brandão, T. R. S. (2018). Physicochemical and Bioactive Compounds of 'Cantaloupe' Melon: Effect of Ozone Processing on Pulp and Seeds. *Ozone: Science and Engineering*, *40*(3). https://doi.org/10.1080/01919512.2017.1414582

Moing, A., William Allwood, J., Aharoni, A., Baker, J., Beale, M. H., Ben-Dor, S., Biais, B., Brigante, F., Burger, Y., Deborde, C., Erban, A., Faigenboim, A., Gur, A., Goodacre, R., Hansen, T. H., Jacob, D., Katzir, N., Kopka, J., Lewinsohn, E., … Schaffer, A. A. (2020). Comparative metabolomics and molecular phylogenetics of melon (Cucumis melo, cucurbitaceae) biodiversity. *Metabolites*, *10*(3). https://doi.org/10.3390/metabo10030121

Monforte, A. J., Diaz, A., Caño-Delgado, A., & Van Der Knaap, E. (2014). The genetic basis of fruit morphology in horticultural crops: Lessons from tomato and melon. In *Journal of Experimental Botany* (Vol. 65, Issue 16). https://doi.org/10.1093/jxb/eru017

More, P. R., Jambrak, A. R., & Arya, S. S. (2022). Green, environment-friendly and sustainable techniques for extraction of food bioactive compounds and waste valorization. *Trends in Food Science & Technology*, *128*, 296–315. https://doi.org/10.1016/j.tifs.2022.08.016

Mwaurah, P. W., Kumar, S., Kumar, N., Attkan, A. K., Panghal, A., Singh, V. K., & Garg, M. K. (2020). Novel oil extraction technologies: Process conditions, quality parameters, and optimization. *Comprehensive Reviews in Food Science and Food Safety*, *19*(1). https://doi.org/10.1111/1541-4337.12507

Nee, M., & Kirkbride, J. H. (1994). Biosystematic Monograph of the Genus Cucumis (Cucurbitaceae)-Botanical Identification of Cucumbers and Melons. *Bulletin of the Torrey Botanical Club*, *121*(3). https://doi.org/10.2307/2997187

Nehdi, I. A., Sbihi, H., Tan, C. P., & Al-Resayes, S. I. (2013). Evaluation and characterisation of Citrullus colocynthis (L.) Schrad seed oil: Comparison with Helianthus annuus (sunflower) seed oil. *Food Chemistry*, *136*(2). https://doi.org/10.1016/j.foodchem.2012.09.009

Nguyen, N. M. P., Le, T. T., Vissenaekens, H., Gonzales, G. B., Van Camp, J., Smagghe, G., & Raes, K. (2019). *In vitro* antioxidant activity and phenolic profiles of tropical fruit by-products. *International Journal of Food Science & Technology*, *54*(4), 1169–1178. https://doi.org/10.1111/ijfs.14093

Nyam, K. L., Tan, C. P., Lai, O. M., Long, K., & Che Man, Y. B. (2009). Physicochemical properties and bioactive compounds of selected seed oils. *LWT*, *42*(8). https://doi.org/10.1016/j.lwt.2009.03.006

Orsavova, J., Misurcova, L., Ambrozova, J., Vicha, R., & Mlcek, J. (2015). Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *International Journal of Molecular Sciences*, *16*(12), 12871–12890. https://doi.org/10.3390/ijms160612871

Osorio, L. L. D. R., Flórez-López, E., & Grande-Tovar, C. D. (2021). The Potential of Selected Agri-Food Loss and Waste to Contribute to a Circular Economy: Applications in the Food, Cosmetic and Pharmaceutical Industries. *Molecules*, *26*(2), 515. https://doi.org/10.3390/molecules26020515

Paris, H. S., Amar, Z., & Lev, E. (2012). Medieval emergence of sweet melons, Cucumis melo (Cucurbitaceae). *Annals of Botany*, *110*(1). https://doi.org/10.1093/aob/mcs098

Pasrija, D., & Sogi, D. S. (2022). Extraction optimization and functional properties of muskmelon seed protein concentrate. *Journal of Food Measurement and Characterization*, *16*(5), 4137–4150. https://doi.org/10.1007/s11694-022-01523-x

Pérez-Vich, B., Velasco, L., & Fernández-Martínez, J. M. (1998). Determination of seed oil content and fatty acid composition in sunflower through the analysis of intact seeds, husked seeds, meal and oil by near-infrared reflectance spectroscopy. *JAOCS, Journal of the American Oil Chemists' Society*, *75*(5). https://doi.org/10.1007/s11746-998-0064-1

Petkova, Z., & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (Cucumis melo L.) cultivated in Bulgaria. *Cogent Food and Agriculture*, *1*(1). https://doi.org/10.1080/23311932.2015.1018779

Pitrat, M. (2008). Melon (<i>Cucumis melo<i/> L.). In *Hand-book of crop breeding vegetables (Vol 1.)*.

Rabadán, A., Antónia Nunes, M., Bessada, S. M. F., Pardo, J. E., Beatriz Oliveira, M. P. P., & Álvarez-Ortí, M. (2020). From by-product to the food chain: Melon (cucumis melo l.) seeds as potential source for oils. *Foods*, *9*(10). https://doi.org/10.3390/foods9101341

Rai, A., Mohanty, B., & Bhargava, R. (2016). Fitting of broken and intact cell model to supercritical fluid extraction (SFE) of sunflower oil. *Innovative Food Science and Emerging Technologies*, *38*. https://doi.org/10.1016/j.ifset.2016.08.019

Rasouli, H., Parvaneh, S., Mahnam, A., Rastegari-Pouyani, M., Hoseinkhani, Z., & Mansouri, K. (2017). Anti-angiogenic potential of trypsin inhibitor purified from Cucumis melo seeds: Homology modeling and molecular docking perspective. *International Journal of Biological Macromolecules*, *96*.

https://doi.org/10.1016/j.ijbiomac.2016.12.027

Renard, C. M. G. C. (2018). Extraction of bioactives from fruit and vegetables: State of the art and perspectives. LWT, 93, 390–395. https://doi.org/10.1016/j.lwt.2018.03.063

Rezig, L., Chouaibi, M., Meddeb, W., Msaada, K., & Hamdi, S. (2019). Chemical composition and bioactive compounds of Cucurbitaceae seeds: Potential sources for new trends of plant oils. *Process Safety and Environmental Protection*, *127*. https://doi.org/10.1016/j.psep.2019.05.005

Rezig, L., Chouaibi, M., Msaada, K., & Hamdi, S. (2012). Chemical composition and profile characterisation of pumpkin (Cucurbita maxima) seed oil. *Industrial Crops and Products*, *37*(1). https://doi.org/10.1016/j.indcrop.2011.12.004

Ribeiro, S. A. O., Nicacio, A. E., Zanqui, A. B., Biondo, P. B. F., de Abreu-Filho, B. A., Visentainer, J. V., Gomes, S. T. M., & Matsushita, M. (2016). Improvements in the quality of sesame oil obtained by a green extraction method using enzymes. *LWT*, *65*. https://doi.org/10.1016/j.lwt.2015.08.053

Ritchie, Roser, Mispy, & Ortiz-Ospina. (2018). *Measuring progress towards the Sustainable Development Goals*. SDG-Tracker. https://sdg-tracker.org/sustainable-consumption-production

Rolim, P. M., Fidelis, G. P., Padilha, C. E. A., Santos, E. S., Rocha, H. A. O., & Macedo, G. R. (2018). Phenolic profile and antioxidant activity from peels and seeds of melon (Cucumis melo L. var. reticulatus) and their antiproliferative effect in cancer cells. *Brazilian Journal of Medical and Biological Research*, *51*(4). https://doi.org/10.1590/1414-431x20176069

Rolim, P. M., Seabra, L. M. J., & de Macedo, G. R. (2020). Melon By-Products: Biopotential in Human Health and Food Processing. In *Food Reviews International* (Vol. 36, Issue 1). https://doi.org/10.1080/87559129.2019.1613662

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., & Lobo, M. G. (2018). Fruit and Vegetable Waste: Bioactive Compounds, Their Extraction, and Possible Utilization. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3). https://doi.org/10.1111/1541-4337.12330

Sahin, E., Erem, E., Güzey, M., Kesen, M. S., Icyer, N. C., Ozmen, D., Toker, O. S., & Cakmak, H. (2022). High potential food wastes: Evaluation of melon seeds as spreadable butter. *Journal of Food Processing and Preservation*, *46*(10). https://doi.org/10.1111/jfpp.16841

Shahzad, N., Khan, W., MD, S., Ali, A., Saluja, S. S., Sharma, S., Al-Allaf, F. A., Abduljaleel, Z., Ibrahim, I. A. A., Abdel-Wahab, A. F., Afify, M. A., & Al-Ghamdi, S. S. (2017). Phytosterols as a natural anticancer agent: Current status and future perspective. In *Biomedicine and Pharmacotherapy* (Vol. 88). https://doi.org/10.1016/j.biopha.2017.01.068

Sharif, K. M., Rahman, M. M., Azmir, J., Mohamed, A., Jahurul, M. H. A., Sahena, F., & Zaidul, I. S. M. (2014). Experimental design of supercritical fluid extraction - A review. In *Journal of Food Engineering* (Vol. 124). https://doi.org/10.1016/j.jfoodeng.2013.10.003

Siddeeg, A., Xu, Y., Jiang, Q., & Xia, W. (2015). *In vitro* antioxidant activity of protein fractions extracted from seinat (*Cucumis melo* var. *tibish*) seeds. *CyTA - Journal of Food*, *13*(3), 472–481. https://doi.org/10.1080/19476337.2014.1003199

Silva, M. A., Albuquerque, T. G., Alves, R. C., Oliveira, M. B. P. P., & Costa, H. S. (2020). Melon (Cucumis melo L.) by-products: Potential food ingredients for novel functional foods? In *Trends in Food Science and Technology* (Vol. 98). https://doi.org/10.1016/j.tifs.2018.07.005

Socas-Rodríguez, B., Álvarez-Rivera, G., Valdés, A., Ibáñez, E., & Cifuentes, A. (2021). Food by-products and food wastes: are they safe enough for their valorization? In *Trends in Food Science and Technology* (Vol. 114). https://doi.org/10.1016/j.tifs.2021.05.002

Swamy, K. R. M. (2017). Origin, distribution and systematics of culinary cucumber (Cucumis melo subsp. agrestis var. conomon). *Journal of Horticultural Sciences*, *12*(1).

Tang, C., Wu, L., Zhang, F., Kan, J., & Zheng, J. (2022). Comparison of different extraction methods on the physicochemical, structural properties, and in vitro hypoglycemic activity of bamboo shoot dietary fibers. *Food Chemistry*, *386*. https://doi.org/10.1016/j.foodchem.2022.132642

Tarjuelo, L., Pardo, J. E., Álvarez-Ortí, M., Pardo-Giménez, A., Millán, C., & Rabadán, A. (2022). Development of Seed-Oil Based Dried Sausages, Considering Physicochemical and Nutritional Quality and the Role of Food Neophobia. *Nutrients*, *14*(15), 3106. https://doi.org/10.3390/nu14153106

Teigiserova, D. A., Hamelin, L., & Thomsen, M. (2019). Review of high-value food waste and food residues biorefineries with focus on unavoidable wastes from processing. *Resources, Conservation and Recycling*, *149*. https://doi.org/10.1016/j.resconrec.2019.05.003

Thakur, H., Sharma, S., & Thakur, M. (2019). Recent trends in muskmelon (Cucumis melo L.) research: an overview. In *Journal of Horticultural Science and Biotechnology* (Vol. 94, Issue 4). https://doi.org/10.1080/14620316.2018.1561214

Titchenal, C. A., & Dobbs, J. (2007). A system to assess the quality of food sources of calcium. *Journal of Food Composition and Analysis*, *20*(8). https://doi.org/10.1016/j.jfca.2006.04.013

Wang, D. H., Wang, Z., Le, K. P., Cortright, J. R., Park, H. G., Tobias, H. J., & Brenna, J. T. (2019). Potentially High Value Conjugated Linolenic Acids (CLnA) in Melon Seed Waste. *Journal of Agricultural and Food Chemistry*, *67*(37). https://doi.org/10.1021/acs.jafc.9b04744

Wang, M. L., Raymer, P., Chinnan, M., & Pittman, R. N. (2012). Screening of the USDA peanut germplasm for oil content and fatty acid composition. *Biomass and Bioenergy*, *39*. https://doi.org/10.1016/j.biombioe.2012.01.025

Wen, L., Zhang, Z., Sun, D. W., Sivagnanam, S. P., & Tiwari, B. K. (2020). Combination of emerging technologies for the extraction of bioactive compounds. In *Critical Reviews in Food Science and Nutrition* (Vol. 60, Issue 11). https://doi.org/10.1080/10408398.2019.1602823

Wen, L., Álvarez, C., Zhang, Z., Poojary, M. M., Lund, M. N., Sun, D.-W., & Tiwari, B. K. (2021). Optimisation and characterisation of protein extraction from coffee silverskin assisted by ultrasound or microwave techniques. Biomass Conversion and Biorefinery, 11(5), 1575–1585. https://doi.org/10.1007/s13399-020-00712-2

WHO. (2013). WHO issues new guidance on dietary salt and potassium. In *Central European journal of public health* (Vol. 21, Issue 1).

Wu, K., Ju, T., Deng, Y., & Xi, J. (2017). Mechanochemical assisted extraction: A novel, efficient, eco-friendly technology. In *Trends in Food Science and Technology* (Vol. 66). https://doi.org/10.1016/j.tifs.2017.06.011

Xu, X., Li, W., Lu, Z., Beta, T., & Hydamaka, A. W. (2009). Phenolic Content, Composition, Antioxidant Activity, and Their Changes during Domestic Cooking of Potatoes. *Journal of Agricultural and Food Chemistry*, *57*(21), 10231–10238. https://doi.org/10.1021/jf902532q

Yannone, S. M., Hartung, S., Menon, A. L., Adams, M. W. W., & Tainer, J. A. (2012). Current Opinion in Biotechnology: Analytical Biotech: Metals in Biology: Defining Metalloproteomes. *Current Opinion in Biotechnology*, *23*(1).

Yanty, N. A. M., Lai, O. M., Osman, A., Long, K., & Ghazali, H. M. (2008). Physicochemical properties of cucumis melo var. inodorus (honeydew melon) seed and seed oil. *Journal of Food Lipids*, *15*(1). https://doi.org/10.1111/j.1745-4522.2007.00101.x

Zanetti, F., Monti, A., & Berti, M. T. (2013). Challenges and opportunities for new industrial oilseed crops in EU-27: A review. In *Industrial Crops and Products* (Vol. 50). https://doi.org/10.1016/j.indcrop.2013.08.030

Zeb, A. (2016). Phenolic profile and antioxidant activity of melon (Cucumis melo l.) seeds from pakistan. *Foods*, *5*(4). https://doi.org/10.3390/foods5040067

Zhang, H., Yuan, Y., Zhu, X., Xu, R., Shen, H., Zhang, Q., & Ge, X. (2022). The Effect of Different Extraction Methods on Extraction Yield, Physicochemical Properties, and Volatile Compounds from Field Muskmelon Seed Oil. *Foods*, *11*(5). https://doi.org/10.3390/foods11050721

Zhang, T., Guan, E., Yang, Y., Liu, F., Zhang, L., Pang, J., & Bian, K. (2021). Fatty acid profiles of vegetable oils from four different plant sources and their effects on dough rheology and Chinese steamed bread quality. *International Journal of Food Science &*

Technology, *56*(5), 2407–2414. https://doi.org/10.1111/ijfs.14868

Zhang, X., Bai, Y., Wang, Y., Wang, C., Fu, J., Gao, L., Liu, Y., Feng, J., Swamy, M. K., Yogi, M., Rudramurthy, G. R., Purushotham, B., & Deng, Y. (2020). Anticancer Properties of Different Solvent Extracts of Cucumis melo L. Seeds and Whole Fruit and Their Metabolite Profiling Using HPLC and GC-MS. *BioMed Research International*, *2020*. https://doi.org/10.1155/2020/5282949

Zungur Bastıoğlu, A., Tomruk, D., Koç, M., & Ertekin, F. K. (2016). Spray dried melon seed milk powder: physical, rheological and sensory properties. *Journal of Food Science and Technology*, *53*(5). https://doi.org/10.1007/s13197-016-2214-z

Chapter 3. Impact of processing on the composition of melon seeds (*Cucumis melo L.*)

Abstract

This study aimed to investigate the composition of two varieties of melon seeds (Galia and Cantaloupe) (*Cucumis melo L.*) and evaluate the impact of processing. The seeds were processed by three methods including soaking, boiling, and roasting, and analysed in terms of their proximate composition, mineral content, anti-nutritional compounds, as well as fatty acid and amino acid contents. Soaking and boiling reduced the tannins content (by 13% - 20%, 10% - 26%, respectively). Boiling had a positive effect on the extractability of oil, while it resulted in a slight decrease in protein content (by approximately 6%) and a significant potassium loss (up to 36% decrease) ($p < 0.05$). Roasting enhanced mineral content (especially in zinc and iron), but increased tannin (by 40% - 114%) and phytic acid (3% - 5%) content. Of the three processing methods, roasting was not effective in reducing the levels of anti-nutritional compounds. Overall, this study demonstrated that the melon seeds have considerable potential to be valorised through their use as a nutritious food ingredient.

3.1. Introduction

Melon (*Cucumis melo L.*) is a member of the Cucurbitaceae family; it is one of the most important commercial horticultural crops in the world (Yanty et al., 2008). Melons are increasingly cultivated and consumed due to their sweet flesh and attractive aroma, with the global production being about 28 million tons in 2018 (FAOSTAT, 2018). Melon seeds (accounts for 10% of melon weight), is a major by-product in the melon supply chain, and are usually generated from household consumption and food industrial processing, such as the production of fruit salads and drinks (Gómez-García et al., 2020). However, melon seeds are scarcely utilised, mainly due to the lack of understanding of their nutritional value and suitable processing technologies. Recently, studies showed that melon seeds have high nutritional value, attributed to the high levels of proteins (15% - 45%), lipids (25% - 45%), dietary fibre (19% - 25%), and minerals (rich in potassium), indicating that they would be a potentially nutritious food ingredient for human consumption (De Melo et al., 2000; Mallek-Ayadi et al., 2018; Mian-Hao & Yansong, 2007; Petkova & Antova, 2015; Rabadán et al., 2020). The variation in the nutritional composition of melon seeds might be associated with the variety of melons, growing conditions, and seasonal variation of harvest (De Melo et al., 2000; Mallek-Ayadi et al., 2018; Mian-Hao & Yansong, 2007).

Despite their rich nutritional profile, melon seeds are not generally included in culinary or food formulations. However, in some countries, melon seeds can be consumed as a food following processing. Traditionally, in India and Nigeria, melon seeds can be added into sauces, soups, and desserts to provide flavour and a thick texture (Rabadán

et al., 2020). In addition, melon seeds after roasting are also regarded as a ready-to-eat snack in Arabian countries (Mallek-Ayadi et al., 2018). Overall, melon seeds need to be processed to improve their edibility before consumption as well as for safety (Tenyang et al., 2017).

In terms of processing technologies, soaking, boiling, and roasting are commonly used in both domestic cooking and in the food industry (Feizollahi et al., 2021; Zhao et al., 2019). During processing, the texture, sensory, and physicochemical properties of the food matrix can significantly change, which may have both beneficial or adverse effects. It has been shown that after processing, the sensory characteristics, food safety, and shelf life of food could be improved, whereas the bioavailability of nutrients could be enhanced by decreasing the levels of anti-nutritional compounds (Jain et al., 2016; Sharma et al., 2022; Xiong et al., 2019). On the other hand, processing could result in nutritional value loss, for example through leaching of minerals and degradation of unsaturated fatty acids (Yang et al., 2014; Zhao et al., 2019).

From a food sustainability and a nutrition point of view, investigating and developing processing technologies to add value to various agri-food by-products and residues is very important as it can reduce food waste and could result to products of good nutritional quality that are suitable for human consumption. To our knowledge, there is no available information in the literature on the effect of processing on the composition and nutritional quality of melon seeds. Therefore, this study aimed to investigate the effect of three processing methods, namely soaking, boiling, and roasting, on the proximate composition, as well as the mineral, anti-nutritional

compounds, fatty acid and amino acid contents of melon seeds. This will generate important knowledge on the impact of processing on the nutritional value of melon seeds, and thus contribute towards the development of appropriate valorisation processes for melon seeds and reduce food waste.

3.2. Materials and methods

3.2.1. Chemicals and standards

Mineral standards (potassium, zinc, magnesium, iron, calcium), hydrochloric acid (36%), and sulfuric acid (96%) were purchased from Fisher Scientific (UK). FAME mix standard (C4-C24) and isooctane (for gas chromatography ECD and FID) were purchased from Supelco (UK). Xylose (≥99%, GC grade), arabinose (≥99%), glucose (≥99.5%, GC grade), oxalate (≥99%), vanillin (99%), sodium methoxide solution (0.5 M, ACS reagent), and catechin (≥98%, HPLC grade) were purchased from Sigma Aldrich (UK).

3.2.2. Melon seed preparation

Galia and Cantaloupe melons (both from Spain) were purchased (50 melons for each variety) from Waitrose Supermarket (Reading, UK) in July 2021. The seeds were separated manually from the fresh fruits, and then washed to remove any flesh residuals from their surface. The melon seeds were processed by different cooking methods as described in **Section 3.2.3**. A portion of the melon seeds (control group) was dried at 50 °C for 16 h in a tray dryer (Wolverine proctor, USA), grounded in a food

grinder (Caterlite, CK686, Bristol, UK), passed through a 600 µm standard sieve, and then sealed in a plastic container and stored into freezer at -20 °C until further analysis.

3.2.3. Processing methods

3.2.3.1. Soaking

The soaking method described by Sahni & Sharma (2020) was followed, with some modifications. Briefly, 100 g of melon seeds were soaked in 1000 mL of tap water at room temperature for 12 h at 1:10 (w/v). In order to prevent rotting, the water was changed halfway. After treatment, the seeds were dried at 50 °C for 16 h in a tray dryer (Wolverine proctor, USA), grounded (Caterlite, CK686, Bristol, UK) for 30 s, and then passed through a 600 µm standard sieve. The seed powder was sealed in a plastic container and stored in a freezer at -20 °C until further analysis.

3.2.3.2. Boiling

100 g of melon seeds were cooked in 1000 mL of boiling tap water (100 °C) in a ratio of 1:10 (w/v) for 30 min. After boiling the seeds were washed with cold water. The samples were drained and then were dried at 50 °C for 16 h in a tray dryer (Wolverine proctor, USA), grounded (Caterlite, CK686, Bristol, UK) for 30 s, and then passed through a 600 µm standard sieve. The seed powder was sealed in a plastic container and stored in a freezer at -20 °C until further analysis.

3.2.3.3. Roasting

100 g of melon seeds were spread on an oven tray fitted tin foil at the base, and

roasted in an oven at 150 °C for 30 min. The roasted melon seeds were grounded (Caterlite, CK686, Bristol, UK) for 30 s and then passed through a 600 µm standard sieve. The seed powder was sealed in a plastic container and stored in a freezer at -20 °C until further analysis.

3.2.4. Proximate analysis

The proximate composition of the raw melon seeds (control) and processed melon seeds was determined by the AOAC (Association of Official Analytical Chemists) standard method (AOAC, 2005). The moisture content was determined using a moisture analyser (Mettler Toledo, UK). The protein content (conversion factor used was 6.25) was determined using the Kjeldahl method according to the AOAC method 979.09. The lipid content was determined by Soxhlet extraction according to the AOAC method 948.22. The ash content was determined according to the AOAC method 923.03.

The carbohydrate composition was determined using the protocol by The National Renewable Energy Laboratory, NREL/TP-510-42618 (Sluiter et al., 2008). Briefly, 300 mg of sample were hydrolysed with 3 mL of (72%, v/v) H_2SO_4 and incubated at 30 °C for 1 h. Afterwards, the mixture was diluted by adding 84 mL distilled water and autoclaved at 121°C for 30 min, and then was cooled to room temperature and filtered. The monosaccharides including glucose (derived from cellulose), xylose, and arabinose were quantified by using HPLC (Agilent, 1260 series) with an Aminex HPX-87H column (300 mm x 7.8 mm, Bio-Rad, California, USA); the operating conditions

were as follows: injection volume was 20 µL, mobile phase was 0.005 M sulphuric acid, flow rate was 0.6 mL/min, column temperature was at 65°C. Calibration standard curves were constructed using external standards [xylose (0 - 5 mg/mL, R^2 = 1), glucose (0 - 5 mg/mL, R^2 = 1), and arabinose (0 - 5 mg/mL, R^2 = 1)]. The acid-soluble lignin was measured using filtered acid-hydrolysed sample with a UV-Vis spectrometer (BioMate 3, Thermo, UK) at 320 nm. The acid-insoluble lignin was measured by gravimetric analysis and was calculated by the following the **Equation 4.1**. The calorific values (kcal/100 g) were calculated using Atwater general factor system (energy values of 4 kcal/g for carbohydrate, 4 kcal/for protein, g, and 9 kcal/g for lipid).

Equation 4.1:

The acid-insoluble lignin content (g) = the solid residue after hydrolysis (g) – [ash of solid residue after hydrolysis (g) + protein content of sample(g)].

3.2.5. Mineral content

The mineral content was analysed by digestion of the ash (Mbuma et al., 2022). Briefly, 1 g of melon seed was ashed. The ash was digested with 5 mL concentrated hydrochloric acid (36%) and evaporated to dryness on a hot plate (100 °C), followed by dilution to 50 mL using water (HPLC grade), and then determining the concentration of different minerals [zinc (0 - 2 mg/L, R^2 = 0.99), magnesium (0 - 2 mg/L, R^2 = 0.99), calcium (0 - 10 mg/L, R^2 = 0.99), iron (0 - 10 mg/L, R^2 = 0.99)] using an atomic absorption spectrophotometer (Nov AA 350, Analytik Jena GmbH, Germany). Potassium (0 - 200 mg/L, R^2 = 0.92) was determined using a flame photometer (PFP7,

Janway, UK).

3.2.6. Anti-nutritional compounds analysis

3.2.6.1 Phytic acid

The phytic acid content was determined using a phytic acid kit (Megazyme, Ireland) and following the manufacturer's assay procedure (Megazyme, 2017). Briefly, 1 g of sample was mixed with 20 mL of 0.66 M HCL for 3 h at room temperature. 1 mL of the extract was collected and centrifuged (Mini Spin, Eppendorf, Germany) at 13,000 rpm for 10 min. Then, 0.5 mL of the supernatant was mixed with 0.5 mL of 0.75 M NaOH solution for neutralisation. The neutralised sample was used to determine the phytic acid content using Phytic Acid Assay Kit (Megazyme, Ireland), which was calculated following the **Equation 4.2** (provided by Megazyme).

Equation 4.2:

Phytic acid content = Phosphorus (g/100)/0.282

3.2.6.2 Tannins

The tannins content was determined according to Shawrang et al. (2011), with slight modifications. 0.5 g of seed powder was extracted with 10 mL of methanol on a shake plate (Variomag Poly, Thermo) at 600 rpm for 12 h at room temperature. 1.5 mL of extract was collected and centrifuged (Mini Spin, Eppendorf, Germany) at 13,000 rpm for 10 min. After this step, 1 mL of supernatant was mixed with 5 mL of freshly prepared vanillin-HCL reagent (the reagent was prepared by mixing 4% vanillin in methanol and 8% HCL in methanol at a ratio of 1:1). The mixture was incubated at

room temperature for 20 min and then the absorbance was measured at 500 nm using a UV-Vis spectrometer (BioMate 3, Thermo, UK). Catechin (0 - 50 µg/mL) was used for constructing a calibration curve (R^2 = 0.99). The tannins content was expressed as mg of CE (Catechin equivalent)/100 g of dry weight.

3.2.6.3. Oxalate

The oxalate content was determined using the method described by Israr et al. (2013). Briefly, 1 g of seed powder was added into 50 mL of 1 M of H_2SO_4 and incubated in a water bath at 15 °C for 15 min. After incubation, the mixture was transferred into a 100 mL volumetric flask and the volume was made up to 100 mL with 1 M of H_2SO_4. 1 mL of the mixture was centrifuged (Mini Spin, Eppendorf, Germany) at 3,000 rpm for 15 min; the supernatant was filtered (0.20 um filter) and then analysed by HPLC. The HPLC analysis was performed in an Agilent 1260 series (Agilent technologies, UK) with a UV-Vis detector set at 210 nm and an Aminex ion exclusion HPX-87H (300 x 7.8 mm) analytical column fitted with an Aminex cation-H guard column. The mobile phase was 0.005 M H_2SO_4 whereas a flow rate of 0.6 mL/min was used; the column temperature was 65 °C. A calibration curve (0 - 0.2 mg/mL, R^2 = 1) with oxalate standards was constructed for quantification.

3.2.7. Amino acid analysis

The amino acid composition of the processed melon seeds and control was determined according to Eze et al. (2022). Briefly, 0.1 g of sample was mixed with 6 M HCL in a sealed container with nitrogen flushed into it to prevent oxidation reactions;

the suspension was hydrolysed at 110 C° for 24 h. The hydrolysate was analysed for its amino acid content using the EZ-Faast amino acid analysis derivatisation kit (Phenomenex, Torrance, CA). The derivatised samples were analysed in electron impact mode using an Agilent -5975GC-MS system (Agilent, Santa, Clara, CA) equipped with a zebron ZB-AAA column (100 x 0.25 x 0.25). The analytical conditions were as follows: the oven temperature was held initially at 110 °C for 1 min, then increased at a rate of 30 °C/min to 310 °C; the temperature of the transfer line and ion source were kept 320 °C and 230 °C, respectively; the flow rate of the carrier gas was 1.5 mL/min and the split rate was 1:40. Amino acids were quantified from calibration curves (0 - 200 µmol/L, R^2 = 0.99) constructed using amino acid standard solutions provided in the EZ-Faast kit. Cysteine and tryptophan were not detected as they were degraded during acid hydrolysis. Samples and standards were analysed in duplicate, and the retention time of the standards were used to identify the respective amino acids peak.

3.2.8. Fatty acid composition analysis

The fatty acid composition was determined according to Milinsk et al. (2008) with certain modifications. 50 mg of seed oil, obtained by Soxhlet extraction were added to 2 mL of 0.5 M sodium methoxide solution in methanol and mixed for 5 min for methylation reaction to take place. Subsequently, 1 mL of isooctane and 5 mL of saturated sodium chloride solution were added and stirred vigorously for 15 min; the mixture was allowed to settle for 10 min. The upper layer was then collected and transferred into a GC vial, and was analysed by GC (7690B, Agilent, USA) equipped with a flame ionization detector (FID) and a fused silica capillary column HP-88 (100 x

0.25 x 0.2). The oven temperature was held initially at 120 °C for 1 min, then increased to 175 °C at a rate of 10 °C/min and remained at 175 °C for 10 min; it then increased to 210 °C at a rate of 5 °C/min and remained for 5 min; it then increased to 230 °C at a rate of 5 °C/min and remained for 10 min. The temperature of the injection and detector were kept at 250 °C and 280 °C, respectively. The split ratio was at 1:50. The carrier gas was helium and the flow rate was 1.5 mL/min. FAME (Fatty acid methyl esters) were identified by comparing the retention times to a FAME standard mixture. Samples and standards were analysed in duplicate. The individual fatty acid composition was expressed as a percentage of total fatty acids (%).

3.2.9. Statistical analysis

All experiments were carried out in triplicate unless otherwise stated. The data were analysed using the Minitab statistical software (version 20, State College, USA). One-way analysis of variance (ANOVA) and Tukey's test were used to compare the mean values ($p < 0.05$) among samples.

3.3. Results and Discussion

3.3.1. Proximate composition

Data in **Table 3.1** show the proximate composition of the two varieties of melon seeds without being subjected to any processing (control), and after processing, namely soaking, boiling, and roasting. Both varieties of melon seeds showed considerable levels of lipid (43.5% - 46%, w/w) and protein (30.4% - 30.7%, w/w). This result is

similar to that of Petkova & Antova (2015) who reported a range of 41% - 45% w/w for lipid and 34% - 40% w/w for protein, but higher than others (de Melo et al., 2000; Mallek-Ayadi et al., 2018; Mian-Hao & Yansong, 2007; Yanty et al., 2008) who reported a range of 25% - 35% w/w for lipid and 15% - 29% w/w for protein. These differences regarding the lipid and protein contents of the melon seeds could be attributed to differences in variety, region, seasonal variation of harvest, and growth conditions (Mallek-Ayadi et al., 2018). Metabolic and physiological traits of crops are affected by the genotypes and environmental factors (e.g. soil, rainfall, temperature, frost, and salinity), therefore, these factors can affect plant's growth as well as nutrient absorption, resulting in various change in nutritional quality (Gonzalez et al., 2012). To the best our knowledge, there is limited information regarding the presence of carbohydrates in melon seeds. The glucose content was 4.8% - 5.4%, w/w, indicating the presence of cellulose and mixed linkage-glucans, whereas xylose plus arabinose were 3.3% - 3.7%, w/w, indicating the presence of hemicellulose such as arabinoxylan; meanwhile, the lignin content was 6.0% - 6.8%, w/w. Overall, it was indicated that cellulose and hemicellulose-based dietary fibre was more likely the major carbohydrate in melon seeds; this aligned to the reports of other researchers (de Melo et al., 2000; Mallek-Ayadi et al., 2018; Yanty et al., 2008). The ash content ranged from 5.2 to 5.7%, w/w. The calorific value of both varieties of melon seeds (549.5 - 569.2 kcal/100 g) was similar to peanut (557.6 kcal/100 g) and cashew nut (563.6 kcal/100 g), indicating that it could be considered as a good contributor of energy (Freitas et al., 2012). Taking into account this and the proximate analysis, it can be suggested that

that melon seeds have good nutritional value and can be used as a food ingredient; this concept was supported by Mallek-Ayadi et al. (2018) study.

As expected, melon seeds after roasting showed significantly lower ($p < 0.05$) moisture content than the control melon seeds. No significant change ($p > 0.05$) in moisture content was observed after boiling, whereas a significant increase ($p < 0.05$) was observed only in the Galia seed after soaking. It is possible that during the long soaking process, the seeds absorbed water, thus increasing their moisture content compared to the control. In terms of protein content, a significant decrease ($p < 0.05$) was observed for both of varieties of melon seeds after boiling. This result was in line with previous studies, which reported a decrease in the protein content of sunflower seeds after boiling; it was suggested that boiling most likely helped in loosening the seed structure (particularly the seed coat), which resulted in the diffusion of some of the soluble proteins into the boiling water (Tenyang et al., 2022). On the other hand, according to DeVries et al. (2017) and Gao et al. (2015), the principle of Kjeldahl method is to measure the total nitrogen content and calculate protein content using an appropriate nitrogen-to-protein conversion factor, thus, some non-protein nitrogen compounds (e.g. non-protein amino acids) are also accounted for conversion to protein content. Therefore, the protein loss phenomenon after boiling could also be associated with the non-protein nitrogen loss rather than true protein loss. In contrast, in terms of lipid content, a significant increase ($p < 0.05$) was observed for both of varieties of melon seeds after boiling. This result was in line with Mariod et al. (2012) study, who reported that the oil content of safflower seed increased from 34.1% to

36.1% after 40 min boiling processing. The increase in oil content after boiling could be attributed to the protein denaturation and alteration of the oil body lipoprotein membranes after boiling, thereby enhancing oil release from oil bodies and membranes, resulting in increased oil extractability (Cai et al., 2021). The above suggests that boiling could be potentially used as a pre-treatment method, especially before oil extraction, to improve the oil yield. In terms of the ash content, no significant changes ($p > 0.05$) were observed in both of varieties of melon seeds after the three processing methods. In terms of carbohydrates, a significant increase ($p < 0.05$) in glucose content was observed for both varieties of melon seeds after roasting. It is possible that roasting disrupted the crystalline structure of lignocellulosic component of the seeds, causing the release of the free cellulose from its lignin seal (McIntosh & Vancov, 2011).

Table 3.1. Chemical composition of Galia and Cantaloupe melon seeds after different processing methods.

Composition (%, w/w DW)	Galia				Cantaloupe			
	Control	Soaking	Boiling	Roasting	Control	Soaking	Boiling	Roasting
Moisture	5.8 ± 0.1 ^{b}	6.4 ± 0.2 ^{a}	5.8 ± 0.3 ^{b}	3.3 ± 0.0 ^{c}	5.7 ± 0.2 ^{a}	5.8 ± 0.3 ^{a}	5.6 ± 0.2 ^{a}	3.7 ± 0.1 ^{b}
Lipid	43.5 ± 0.3 ^{b}	45.0 ± 0.2 ^{a}	44.0 ± 0.4 ^{a}	44.5 ± 0.3 ^{a}	46.0 ± 0.4 ^{b}	46.0 ± 0.3 ^{b}	48.3 ± 0.2 ^{a}	44.6 ± 0.2 ^{c}
Protein	30.4 ± 0.1 ^{a}	29.4 ± 0.2 ^{b}	28.4 ± 0.1 ^{c}	29.2 ± 0.1 ^{b}	30.7 ± 0.3 ^{a}	30.9 ± 0.1 ^{a}	28.9 ± 0.2 ^{b}	30.8 ± 0.1 ^{a}
Ash	5.2 ± 0.1 ^{ab}	5.1 ± 0.0 ^{ab}	4.9 ± 0.2 ^{b}	5.3 ± 0.0 ^{a}	5.7 ± 0.1 ^{ab}	5.3 ± 0.2 ^{b}	5.6 ± 0.2 ^{ab}	5.9 ± 0.0 ^{a}
Total carbohydrate	9.1 ± 0.2	9.3 ± 0.2	9.9 ± 0.4	9.9 ± 0.4	8.1 ± 0.1	8.0 ± 0.2	8.1 ± 0.3	9.6 ± 0.2
Glucose	5.4 ± 0.1 ^{b}	5.7 ± 0.1 ^{ab}	6.0 ± 0.2 ^{a}	6.0 ± 0.2 ^{a}	4.8 ± 0.1 ^{bc}	4.7 ± 0.0 ^{c}	5.0 ± 0.2 ^{b}	5.8 ± 0.1 ^{a}
Xylose	3.1 ± 0.1 ^{a}	3.0 ± 0.0 ^{a}	3.2 ± 0.2 ^{a}	3.2 ± 0.1 ^{a}	2.8 ± 0.0 ^{b}	2.8 ± 0.0 ^{b}	2.6 ± 0.1 ^{c}	3.3 ± 0.0 ^{a}
Arabinose	0.6 ± 0.0 ^{a}	0.6 ± 0.0 ^{a}	0.7 ± 0.1 ^{a}	0.7 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}
Lignin	6.8 ± 0.3	6.6 ± 0.1	6.7 + 0.3	7.6 ± 0.6	6.0 ± 0.7	6.7 ± 0.2	6.1 ± 0.2	7.3 ± 0.2
Acid insoluble lignin	2.3 ± 0.2 ^{a}	2.1 ± 0.1 ^{a}	2.6 ± 0.4 ^{a}	2.9 ± 0.4 ^{a}	2.3 ± 0.3 ^{b}	2.2 ± 0.2 ^{b}	2.7 ± 0.1 ^{ab}	3.3 ± 0.3 ^{a}
Acid soluble lignin	4.5 ± 0.1 ^{a}	4.5 ± 0.1 ^{a}	4.1 ± 0.4 ^{a}	4.7 ± 0.4 ^{a}	3.7 ± 0.5 ^{ab}	4.5 ± 0.3 ^{a}	3.4 ± 0.3 ^{b}	4.0 ± 0.3 ^{ab}
Calorific value	549.5	559.8	549.2	556.9	569.2	569.6	582.7	563.0

Data represented as mean ± standard deviation (n = 3). Values with different lowercase letters in the same row within each variety are significantly different ($p < 0.05$).

3.3.2. Mineral composition

Data in **Table 3.2** show the mineral content of the two varieties of melon seeds without any processing (control) and after processing. In the two varieties of the control melon seeds, potassium (988.3 - 1076.6 mg/100 g) was the major mineral, followed by magnesium (514.3 - 541 mg/100 g), and calcium (189.1 - 196.9 mg/100 g); Iron and zinc were present in relative low content, 22.2 - 34.6 (mg/100 g) and 4.7 - 6.5 (mg/100 g), respectively. These results agree with previous reports indicating that potassium was the most abundant mineral (Mallek-Ayadi et al., 2018; Morais et al., 2017). Compared to the above studies, the potassium level of the melon seeds in the present study was similar to the Mallek-Ayadi et al. (2018) study (~1150 mg/100 g), but considerably lower than the Morais et al. (2017) study (~2080 mg/100 g). Petropoulos et al. (2019) indicated that the variations in the seed mineral contents were mostly due to the different growing conditions, such as soil, climate, and location of cultivation, since these factors play an important role in determining the solubility and availability of nutrients in the root zone of plants thereby influencing nutrient uptake of plants. According to the WHO guidelines for potassium intake, the recommended potassium intake in adults is at least 3510 mg (WHO, 2012), therefore, the high amount of potassium in the melon seeds, indicates that they could be potentially used as a functional food ingredient.

After processing, significant changes were observed in some cases. In terms of potassium, a significant reduction ($p < 0.05$) was observed in the case of boiled melon seeds compared to the control melon seeds. The reduction in potassium could be attributed to a leaching effect of potassium into the boiling water (Avanza et al., 2013).

In contrast, with the exception of soaked and boiled melon seeds, the zinc content was significantly increased ($p < 0.05$) for both varieties of melon seeds after roasting. Tenyang et al. (2022) reported a similar result for roasted sunflower seeds (at 120 °C for 20 min) compared to control (raw sunflower seed), as the zinc content increased from 7.37 to 10.02 (mg/100 g). According to Klepacka et al. (2020), during processing, minerals can be released from some complexes, indicating that the roasting process could have induce certain modifications in some complexes' structures causing the liberation of bound zinc. The iron content showed a significant increase ($p < 0.05$) in roasted Cantaloupe melon seeds as compared to control. On the other hand, the magnesium and calcium contents showed no significant difference ($p > 0.05$) for both varieties of melon seeds after processing.

Table 3.2. The mineral content of Galia and Cantaloupe seeds after different processing methods.

	Minerals (mg/100 g DW)				
Galia	**Potassium**	**Magnesium**	**Calcium**	**Iron**	**Zinc**
Control	1076.6 ± 29.2 ^{a}	541.0 ± 12.4 ^{a}	189.1 ± 19.7 ^{a}	34.6 ± 4.0 ^{a}	4.7 ± 0.9 ^{b}
Soaking	1050.1 ± 11.0 ^{a}	517.8 ± 9.1 ^{a}	175.3 ± 15.9 ^{a}	39.9 ± 0.1 ^{a}	4.0 ± 1.3 ^{b}
Boiling	833.0 ± 26.1 ^{b}	531.6 ± 10.5 ^{a}	205.7 ± 34.4 ^{a}	36.9 ± 3.0 ^{a}	4.2 ± 0.1 ^{b}
Roasting	1115.4 ± 37.1 ^{a}	511.2 ± 40.4 ^{a}	240.9 ± 30.9 ^{a}	38.5 ± 1.8 ^{a}	7.3 ± 1.0 ^{a}
Cantaloupe					
Control	988.3 ± 41.4 ^{a}	514.3 ± 17.6 ^{a}	196.9 ± 17.3 ^{ab}	22.2 ± 1.9 ^{b}	6.5 ± 0.1 ^{b}
Soaking	836.5 ± 18.6 ^{b}	502.2 ± 9.6 ^{a}	162.7 ± 10.5 ^{b}	29.9 ± 4.5 ^{ab}	6.0 ± 0.2 ^{bc}
Boiling	637.0 ± 41.1 ^{c}	519.7 ± 29.0 ^{a}	209.5 ± 5.6 ^{a}	31.6 ± 4.9 ^{ab}	5.8 ± 0.1 ^{c}
Roasting	1013.0 ± 41.1 ^{a}	474.6 ± 21.9 ^{a}	213.4 ± 20.3 ^{a}	32.0 ± 2.8 ^{a}	7.0 ± 0.3 ^{a}

Data represented as mean ± standard deviation (n = 3). Values with different lowercase letters in the same column within each variety are significantly different ($p < 0.05$).

3.3.3. Fatty acid composition

Data in **Table 3.3** show the fatty acid content of the two varieties of melon seeds without any processing (control), and after processing; the individual fatty acid content was expressed as a percentage of total fatty acids (%). Linoleic acid (74.9% - 75.7%) was the most abundant fatty acid in melon seeds, followed by palmitic acid (9.6% - 10.6%) and oleic acid (8.7% - 10.2%). These results were in agreement with previous studies (Mallek-Ayadi et al., 2018; Rabadán et al., 2020; Yanty et al., 2008). Comparing the linoleic acid content of melon seed oil with most conventional vegetable oils, it is considerably higher than many other oils, such as sesame (41% - 59%), corn (47% - 60%), and sunflower (31% - 60%), suggesting that melon seed oil could be considered as a good source of linoleic acid (Moreau et al., 2009; Nehdi et al., 2013; Tenyang et al., 2017). From a nutritional value point, several published works have shown that there is a link between increasing dietary intake of unsaturated fatty acids, such as linoleic acid and oleic acid, and reducing the risk of cardiovascular disease (Bowen et al., 2019; Marangoni et al., 2020). The presence of high amounts of unsaturated fatty acid (around 85%, consisting primarily of linoleic acid, oleic acid, and α-linolenic acic) in melon seeds oil demonstrates its potential to be used as a novel source of plant oil into the human diet.

During processing, the levels of unsaturated fatty acids were affected, primarily of linoleic acid. After roasting, the linoleic acid content was significantly decreased ($p < 0.05$) for both varieties of melon seeds (reduced from 74.9% to 69.5% in Galia, and from 75.7% reduced to 69.6% in Cantaloupe). This finding is supported by Jain et al.

(2016), who reported a similar result for the garden cress (*Lepidium sativum*) seeds after roasting (at 150 °C for 3 min), where the linoleic acid content decreased from 11.4% to 10.3%. This could be attributed to the oxidation of linoleic acid due to the high temperature; it has been reported that temperature is an important factor to cause unsaturated fatty acid oxidation, with a higher temperature resulting in a higher oxidation rate (Jain et al., 2016; Lin et al., 2016). Furthermore, the higher rate of fatty acid oxidation could be attributed to the increasing the number of double bonds, since the hydrogen attached to the carbon between two double bonds is removed more easily, therefore, polyunsaturated fatty acid (PUFA) (e.g. linoleic acid) is more susceptible to oxidation than monounsaturated fatty acid (MUFA) (Valdés et al., 2015). After boiling, the linoleic acid content significantly decreased ($p < 0.05$) for the Galia variety (74.9% reduced to 68.3%), while it did not significantly change ($p > 0.05$) for the Cantaloupe variety. The overall differences between roasting and boiling could be attributed to the differences in temperature; a higher temperature increases the rate of linoleic acid oxidation, and thus results in more linoleic acid becoming oxidised (Suri et al., 2019; Tenyang et al., 2017). In addition, soaking of the melon seeds resulted in similar changes to boiling, with a decrease in the linoleic acid content observed only for the Galia variety (from 74.9% to 71.9%). Overall, the results indicate that roasting has a more negative impact on the linoleic acid content compared to boiling and soaking.

Table 3.3. The fatty acid composition of Galia and Cantaloupe seeds after different processing methods.

Fatty acids (%)	Galia				Cantaloupe			
	Control	Soaking	Boiling	Roasting	Control	Soaking	Boiling	Roasting
Palmitic acid (C16:0)	9.6 ± 0.1 [a]	9.3 ± 0.0 [b]	9.7 ± 0.0 [a]	9.4 ± 0.0 [b]	10.6 ± 0.1 [a]	10.8 ± 0.0 [a]	10.8 ± 0.2 [a]	9.6 ± 0.0 [b]
Stearic acid (C18:0)	4.3 ± 0.1 [d]	4.8 ± 0.0 [c]	5.4 ± 0.0 [a]	5.1 ± 0.0 [b]	4.3 ± 0.0 [b]	4.3 ± 0.0 [b]	4.4 ± 0.0 [b]	5.4 ± 0.0 [a]
Arachidic acid (C 20:0)	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]
Tricosanoic acid (C23:0)	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	_	_	_	_
Oleic acid (C18:1)	10.2 ± 0.0 [d]	13.1 ± 0.0 [c]	15.6 ± 0.0 [a]	14.9 ± 0.0 [b]	8.7 ± 0.0 [b]	8.6 ± 0.0 [b]	8.7 ± 0.1 [b]	14.7 ± 0.1 [a]
Linoleic acid (C18:2)	74.9 ± 0.2 [a]	71.9 ± 0.1 [b]	68.3 ± 0.1 [d]	69.5 ± 0.0 [c]	75.7 ± 0.1 [a]	75.5 ± 0.1 [a]	75.4 ± 0.1 [a]	69.6 ± 0.1 [b]
α-Linolenic acid (C18:3)	0.4 ± 0.0 [a]	0.3 ± 0.0 [a]	0.3 ± 0.0 [a]	0.3 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.01 [a]	0.2 ± 0.0 [a]
SFA	14.3	14.5	15.5	14.9	15.1	15.3	15.4	15.2
MUFA	10.2	13.1	15.6	14.9	8.7	8.6	8.7	14.7
PUFA	75.3	72.2	68.6	69.8	75.9	75.7	75.6	69.8
Unknown	0.2	0.2	0.2	0.4	0.3	0.4	0.3	0.3

Data represented as mean ± standard deviation (n = 2). Values with different lowercase letters in the same row within each variety are significantly different ($p < 0.05$). SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid.

3.3.4. Amino acid composition

Data in **Table 3.4** show the amino acid composition of the two varieties of melon seeds without any processing (control), and after processing. The two varieties of raw melon seeds had similar amino acid profiles. In terms of essential amino acids, leucine (approximately 2.4% w/w), valine (approximately 1.8% w/w), and phenylalanine (1.4 - 1.5%, w/w) were the major essential amino acids present in melon seeds. However, melon seeds were relatively low concentration in methionine (approximately 0.2% w/w), followed by lysine (approximately 0.5% w/w). This result was in accordance with previous reports (Mallek-Ayadi et al., 2019; Mian-Hao & Yansong, 2007). In terms of the non-essential amino acids, glutamic acid (6.5 - 7.8%, w/w) and aspartic acid (2.9 - 3.0%, w/w) were the most predominant in melon seeds. Dos Santos et al. (2020) reported that ingredients that are naturally rich in glutamic acid can be used as flavour-enhancers for culinary applications and help to reduce salt without reducing the sensory properties. Results from the current study validate this and indicate that melon seeds are potential flavour ingredients that could be potentially used as a complementary strategy for achieving sodium content reduction in food.

The results indicated no significant changes ($p > 0.05$) in the amino acid profiles of the melon seeds as a result of the three processing methods. Gurumoorthi et al. (2008) reported a similar result for velet bean after soaking, boiling, and roasting. During processing, the side chains of some protein-bound amino acids can react chemically with each other or with other components (e.g. fat and polysaccharides) under appropriate conditions, resulting in a change in the composition of amino acids and/or

in individual amino acid content, therefore, changes in amino acid content may depend on processing methods and parameters (e.g. time, temperature, and pressure), and investigated species of melons (Cobas et al., 2022; Korus, 2012).

Table 3.4. Amino acid composition (%, in g/100 g seed DW) of Galia and Cantaloupe seeds after different cooking processing methods.

Essential amino acids	Galia				Cantaloupe			
	Control	Soaking	Boiling	Roasting	Control	Soaking	Boiling	Roasting
Leucine	2.3 ± 0.0 [b]	2.4 ± 0.0 [ab]	2.6 ± 0.1 [a]	2.4 ± 0.1 [ab]	2.4 ± 0.1 [a]	2.4 ± 0.0 [a]	2.4 ± 0.1 [a]	2.4 ± 0.0 [a]
Methionine	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	0.2 ± 0.0 [a]	0.2 ± 0.1 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]
Phenylalanine	1.4 ± 0.1 [a]	1.5 ± 0.0 [a]	1.5 ± 0.0 [a]	1.4 ± 0.1 [a]	1.5 ± 0.1 [a]	1.5 ± 0.0 [a]	1.5 ± 0.0 [a]	1.4 ± 0.1 [a]
Threonine	0.7 ± 0.1 [a]	0.8 ± 0.1 [a]	0.7 ± 0.2 [a]	0.7 ± 0.1 [a]	0.7 ± 0.1 [a]	0.7 ± 0.1 [a]	0.7 ± 0.1 [a]	0.7 ± 0.0 [a]
Lysine	0.5 ± 0.2 [a]	0.6 ± 0.2 [a]	0.6 ± 0.4 [a]	0.5 ± 0.1 [a]	0.5 ± 0.2 [a]	0.5 ± 0.3 [a]	0.4 ± 0.2 [a]	0.5 ± 0.2 [a]
Valine	1.8 ± 0.1 [b]	1.9 ± 0.1 [ab]	2.0 ± 0.0 [a]	1.9 ± 0.0 [ab]	1.8 ± 0.0 [a]	1.9 ± 0.1 [a]	1.9 ± 0.1 [a]	1.8 ± 0.1 [a]
Isoleucine	1.3 ± 0.1 [a]	1.3 ± 0.0 [a]	1.4 ± 0.0 [a]	1.4 ± 0.0 [a]	1.3 ± 0.0 [a]	1.4 ± 0.0 [a]	1.4 ± 0.1 [a]	1.3 ± 0.1 [a]
Non-essential amino acids								
Alanine	1.1 ± 0.1 [a]	1.1 ± 0.0 [a]	1.2 ± 0.0 [a]	1.2 ± 0.0 [a]	1.2 ± 0.1 [a]	1.1 ± 0.1 [a]	1.1 ± 0.0 [a]	1.1 ± 0.0 [a]
Glycine	1.7 ± 0.1 [a]	1.7 ± 0.1 [a]	1.8 ± 0.1 [a]	1.8 ± 0.1 [a]	1.8 ± 0.1 [a]	1.7 ± 0.0 [ab]	1.6 ± 0.1 [b]	1.8 ± 0.1 [a]
Tyrosine	0.4 ± 0.2 [a]	0.4 ± 0.1 [a]	0.4 ± 0.1 [a]	0.4 ± 0.1 [a]	0.4 ± 0.1 [a]	0.4 ± 0.1 [a]	0.4 ± 0.1 [a]	0.4 ± 0.1 [a]
Glutamine	1.9 ± 0.1 [a]	1.7 ± 0.2 [a]	1.8 ± 0.5 [a]	2.1 ± 0.2 [a]	1.7 ± 0.0 [a]	2.0 ± 0.3 [a]	2.0 ± 0.2 [a]	2.0 ± 0.2 [a]
Serine	0.9 ± 0.1 [a]	1.0 ± 0.1 [a]	1.0 ± 0.0 [a]	0.9 ± 0.2 [a]	0.9 ± 0.1 [a]	0.8 ± 0.2 [a]	0.8 ± 0.1 [a]	0.8 ± 0.1 [a]
Proline	1.1 ± 0.1 [a]	1.1 ± 0.1 [a]	1.1 ± 0.1 [a]	1.1 ± 0.0 [a]	1.1 ± 0.0 [a]	1.1 ± 0.0 [a]	1.1 ± 0.0 [a]	1.1 ± 0.1 [a]
Aspartic acid	3.0 ± 0.1 [a]	3.0 ± 0.2 [a]	2.9 ± 0.2 [a]	2.9 ± 0.2 [a]	2.9 ± 0.1 [a]	2.8 ± 0.2 [a]	2.7 ± 0.1 [a]	2.7 ± 0.2 [a]
Glutamic acid	7.8 ± 0.3 [a]	7.9 ± 0.3 [a]	7.1 ± 0.3 [a]	7.5 ± 0.1 [a]	6.5 ± 0.8 [a]	6.4 ± 0.6 [a]	5.7 ± 0.3 [a]	6.4 ± 0.7 [a]

Data represented as mean ± standard deviation (n = 2). Values with different lowercase letters in the same row within each variety are significantly different ($p < 0.05$).

3.3.5. Analysis of anti-nutritional compounds

Data in **Table 3.5** show the anti-nutritional compounds of the two varieties of melon seeds without any processing (control), and after processing. To the best our knowledge, there is limited information on the presence of anti-nutritional compounds in melon seeds. For this reason, we analysed phytic acid, tannins, and oxalate, because they are widely distributed in edible seeds and are regarded as a major limitation for seeds' nutritional quality and their applications as ingredient in food production (Nikmaram et al., 2017).

Phytic acid (**Figure 3.1**), with its six reactive phosphate groups, is a strong chelator naturally present in plants. It has the ability to form insoluble complexes with minerals and protein, and thus reduce their bioavailability, hence it is regarded as an anti-nutritional compound in food (Samtiya et al., 2020; Shawrang et al., 2011). Phytic acid content in melon seeds was approximately 4.1% w/w. Compared with the control, no statistically significant ($p > 0.05$) reduction in phytic acid was observed after soaking and boiling. However, for the Cantaloupe variety, roasting resulted in a significant increase ($p < 0.05$) in phytic acid. Sharma et al. (2022) observed a similar result for quinoa grains after roasting (at 180 °C for 6.5 min); it was suggested that the increase in phytic acid after roasting could be attributed to the complete inactivation of the endogenous phytase enzyme. The phytase enzyme is present endogenously in seeds and has been suggested as being responsible for the degradation of phytic acid during processing; at high-temperature roasting, the intrinsic phytase enzyme could be deactivated completely, thus, it cannot degrade phytic acid further down the production and supply chain process (Embaby, 2010; Kumar et al., 2021; Sharma et al.,

2022).

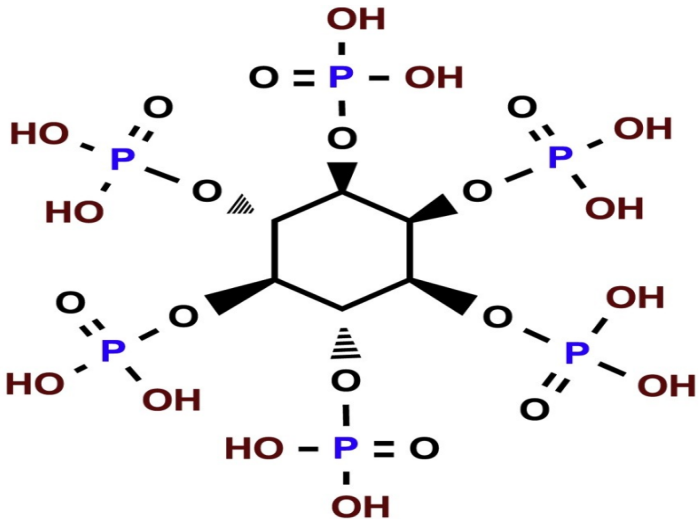

Figure 3.1. Chemical structure of phytic acid (Sykam et al., 2021).

Tannins (**Figure 3.2**) are water-soluble phenolic compounds (molecular weight of more than 500 Da), and their anti-nutritional effect is associated with protein digestibility. They can inhibit digestive enzymes or bind to proteins resulting in the formation of complexes, thus, interfering with protein digestibility (Nikmaram et al., 2017). The tannins content of the control melon seeds ranged from 7.8 to 9.8 (mg CE/100 g). Soaking and boiling resulted in significant tannins reduction ($p < 0.05$) for both varieties of melon seeds. This could be attributed to a leaching effect because tannins are water-soluble (Kataria et al., 2021; Yang et al., 2014). In contrast, the tannins content was significantly increased ($p < 0.05$) after roasting for both varieties. Godrich et al. (2023) observed a similar result as tannins increased after roasting chickpeas and red kidney beans at 180 °C for 20 min. There are several possible explanations for this increase: (1) roasting can modify the structure of cellular membranes and walls

thereby releasing more tannins; (2) at high temperature, high-molecular weight tannins are broken down into lower molecular weight forms that are more soluble which results to a higher content using the spectrophotometric method (Babiker et al., 2021; Kataria et al., 2021; Xiong et al., 2019). Overall, the results of this study suggest that soaking and boiling are the most effective cooking methods for reducing the tannins content.

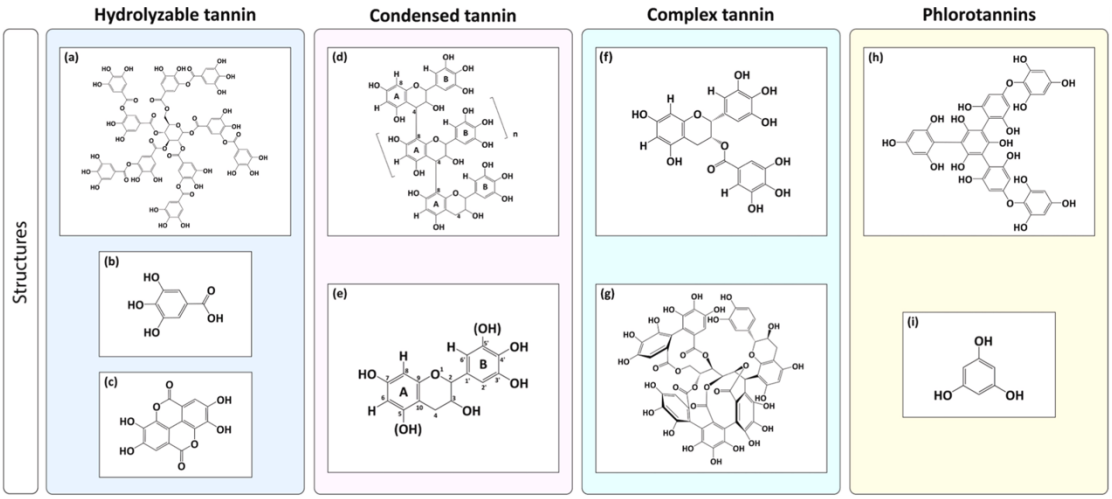

Figure 3.2. Chemical structure of tannins. Hydrolysable tannins: (a) tannic acid, (b) gallic acid, (c) ellagic acid. Condensed tannins: (d) prodelphinidin, (e) monomeric flavonoid unit. Complex tannins: (f) epigallocatechin-gallate and (g) acutissimin A. Phlorotannins: (h) phlorotannin hexamer, and (i) monomeric phloroglucinol (1,3,5-trihydroxybenzene) unit (Vera et al., 2023).

Oxalate (**Figure 3.3**) has a negative effect on mineral absorption due to its ability to bind divalent metallic cations, such as calcium. For example, a high intake of oxalate in the diet could lead to the formation of calcium oxalate stones in the kidney (Israr et al., 2013; Nikmaram et al., 2017). In this study, oxalate was not detected in melon seeds. Ruan et al. (2013) determined oxalate content in some foods; almond (296.1 mg/100 g), cashew nut (265.9 mg/100 g), hazel (194.4 mg/100 g) were considered as high oxalate foods, whereas sweet corn (6.1 mg/100 g), mung bean (14.3 mg/100 g),

and millet (12.7 mg/100 g) were considered as low oxalate food. Comparing melon seeds with the above results, it can be suggested that melon seeds should be considered as a low oxalate foods.

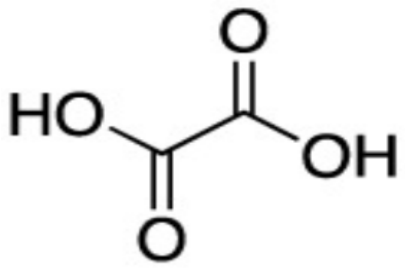

Figure 3.3. Chemical structure of oxalate (Salgado et al., 2023).

.

Table 3.5. Anti-nutritional compounds of Galia and Cantaloupe seeds after three different processing methods.

Anti-nutritional compounds	Galia				Cantaloupe			
	Control	Soaking	Boiling	Roasting	Control	Soaking	Boiling	Roasting
Phytic acid (%, in g/100 g DW)	4.1 ± 0.1 ^{ab}	4.0 ± 0.1 ^{ab}	3.8 ± 0.1 ^{b}	4.2 ± 0.1 ^{a}	4.1 ± 0.1 ^{b}	4.0 ± 0.1 ^{b}	3.9 ± 0.1 ^{b}	4.3 ± 0.0 ^{a}
Tannins (mg CE/100 g DW)	9.8 ± 0.0 ^{b}	7.8 ± 0.1 ^{d}	8.8 ± 0.0 ^{c}	13.8 ± 0.1 ^{a}	7.8 ± 0.1 ^{b}	6.8 ± 0.1 ^{c}	5.8 ± 0.0 ^{d}	18.8 ± 0.0 ^{a}

Data represented as mean ± standard deviation (n = 3). Values with different lowercase letters in the same row within each variety are significantly different ($p < 0.05$); ND: not detected. Oxalate was not detected in any of the samples.

3.4. Conclusions

This study demonstrated that melon seeds are a good food source of protein, oil, minerals and dietary fibre. Additionally, the high level of unsaturated fatty acids (especially linoleic acid) in the seed oil indicated its potential nutritional quality as a novel plant oil. In terms of processing of melon seeds, boiling increased the lipid content (by 2% - 5%) and reduced the tannin content (by 10% - 26%), while it led to considerable potassium loss (by 23% - 36%) and slightly reduced linoleic acid content (by 0.3% - 9%). Roasting slightly improved the zinc and iron contents (by 8% - 55% and 11% -44%, respectively), while it had a negative effect on the linoleic acid content (~8% reduction) and anti-nutritional compounds (phytic acid and tannins); the latter could be unfavourable for the bioavailability of nutrients (e.g. mineral and protein). Soaking reduced the tannin content (by 13 - 20%), although not as effectively as boiling. Future work will aim to investigate the sensory characteristics (e.g. colour and flavour) and acceptability of melon seeds treated under different food industrial processing and home cooking methods, as well as the bioavailability of melon seed nutrients (e.g. protein and minerals), in order to develop a novel and highly nutritious food ingredient from a low value feedstock.

3.5. References

AOAC. (2005). Official Methods of Analysis of AOAC International. In *Association of Official Analysis Chemists International*.

Avanza, M., Acevedo, B., Chaves, M., & Añón, M. (2013). Nutritional and anti-nutritional components of four cowpea varieties under thermal treatments: Principal component analysis. *LWT - Food Science and Technology*, *51*(1). https://doi.org/10.1016/j.lwt.2012.09.010

Babiker, E. E., Uslu, N., Al Juhaimi, F., Mohamed Ahmed, I. A., Ghafoor, K., Özcan, M. M., & Almusallam, I. A. (2021). Effect of roasting on antioxidative properties, polyphenol profile and fatty acids composition of hemp (Cannabis sativa L.) seeds. *LWT*, *139*. https://doi.org/10.1016/j.lwt.2020.110537

Bowen, K. J., Kris-Etherton, P. M., West, S. G., Fleming, J. A., Connelly, P. W., Lamarche, B., Couture, P., Jenkins, D. J. A., Taylor, C. G., Zahradka, P., Hammad, S. S., Sihag, J., Chen, X., Guay, V., Maltais-Giguère, J., Perera, D., Wilson, A., Juan, S. C. S., Rempel, J., & Jones, P. J. H. (2019). Diets enriched with conventional or high-oleic acid canola oils lower atherogenic lipids and lipoproteins compared to a diet with a western fatty acid profile in adults with central adiposity. *Journal of Nutrition*, *149*(3). https://doi.org/10.1093/jn/nxy307

Cai, Z., Li, K., Lee, W. J., Reaney, M. T. J., Zhang, N., & Wang, Y. (2021). Recent progress in the thermal treatment of oilseeds and oil oxidative stability: A review. Fundamental Research, 1(6), 767–784. https://doi.org/10.1016/j.fmre.2021.06.022

Cobas, N., Gómez-Limia, L., Franco, I., & Martínez, S. (2022). Amino acid profile and protein quality related to canning and storage of swordfish packed in different filling media. Journal of Food Composition and Analysis, 107, 104328. https://doi.org/10.1016/j.jfca.2021.104328

De Melo, M. L. S., Narain, N., & Bora, P. S. (2000). Characterisation of some nutritional constituents of melon (Cucumis melo hybrid AF-522) seeds. *Food Chemistry*, *68*(4). https://doi.org/10.1016/S0308-8146(99)00209-5

DeVries, J. W., Greene, G. W., Payne, A., Zbylut, S., Scholl, P. F., Wehling, P., Evers, J. M., & Moore, J. C. (2017). Non-protein nitrogen determination: A screening tool for nitrogenous compound adulteration of milk powder. International Dairy Journal, 68, 46–51. https://doi.org/10.1016/j.idairyj.2016.12.003

Dos Santos, F. F., Dantas, N. M., Simoni, N. K., Pontes, L. S., & Pinto-E-silva, M. E. M. (2020). Are foods naturally rich in glutamic acid an alternative to sodium reduction? *Food Science and Technology (Brazil)*, *40*. https://doi.org/10.1590/fst.08819

Embaby, H. E. S. (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. *Food Science and Biotechnology*, *19*(4). https://doi.org/10.1007/s10068-010-0148-1

Eze, O. F., Chatzifragkou, A., & Charalampopoulos, D. (2022). Properties of protein isolates extracted by ultrasonication from soybean residue (okara). *Food Chemistry*, *368*. https://doi.org/10.1016/j.foodchem.2021.130837

FAOSTAT. (2018). *Food and agriculture organization of the United Nations*.

https://www.fao.org/faostat/en/#data/QCL

Feizollahi, E., Mirmahdi, R. S., Zoghi, A., Zijlstra, R. T., Roopesh, M. S., & Vasanthan, T. (2021). Review of the beneficial and anti-nutritional qualities of phytic acid, and procedures for removing it from food products. *Food Research International*, *143*. https://doi.org/10.1016/j.foodres.2021.110284

Freitas, J. B., Fernandes, D. C., Czeder, L. P., Lima, J. C. R., Sousa, A. G. O., & Naves, M. M. V. (2012). Edible Seeds and Nuts Grown in Brazil as Sources of Protein for Human Nutrition. *Food and Nutrition Sciences*, *03*(06). https://doi.org/10.4236/fns.2012.36114

Gao, P., Li, Z., Zan, L., Yue, T., & Shi, B. (2015). A non-protein nitrogen index for discriminating raw milk protein adulteration via the Kjeldahl method. Analytical Methods, 7(21), 9166–9170. https://doi.org/10.1039/C5AY01422K

Godrich, J., Rose, P., Muleya, M., & Gould, J. (2023). The effect of popping, soaking, boiling and roasting processes on antinutritional factors in chickpeas and red kidney beans. *International Journal of Food Science and Technology*, *58*(1). https://doi.org/10.1111/ijfs.16190

Gómez-García, R., Campos, D. A., Aguilar, C. N., Madureira, A. R., & Pintado, M. (2020). Valorization of melon fruit (Cucumis melo L.) by-products: Phytochemical and Biofunctional properties with Emphasis on Recent Trends and Advances. In *Trends in Food Science and Technology* (Vol. 99). https://doi.org/10.1016/j.tifs.2020.03.033

Gonzalez, J. A., Konishi, Y., Bruno, M., Valoy, M., & Prado, F. E. (2012). Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (Chenopodium quinoa) cultivars from two different agroecological regions. Journal of the Science of Food and Agriculture, 92(6), 1222–1229. https://doi.org/10.1002/jsfa.4686

Gurumoorthi, P., Janardhanan, K., & Myhrman, R. V. (2008). Effect of differential processing methods on L-Dopa and protein quality in velvet bean, an underutilized pulse. *LWT*, *41*(4). https://doi.org/10.1016/j.lwt.2007.04.016

Israr, B., Frazier, R. A., & Gordon, M. H. (2013). Effects of phytate and minerals on the bioavailability of oxalate from food. *Food Chemistry*, *141*(3). https://doi.org/10.1016/j.foodchem.2013.04.130

Jain, T., Grover, K., & Kaur, G. (2016). Effect of processing on nutrients and fatty acid composition of garden cress (Lepidium sativum) seeds. *Food Chemistry*, *213*. https://doi.org/10.1016/j.foodchem.2016.07.034

Kataria, A., Sharma, S., & Dar, B. N. (2021). Changes in phenolic compounds, antioxidant potential and antinutritional factors of Teff (Eragrostis tef) during different thermal processing methods. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.15210

Klepacka, J., Najda, A., & Klimek, K. (2020). Effect of buckwheat groats processing on the content and bioaccessibility of selected minerals. *Foods*, *9*(6). https://doi.org/10.3390/foods9060832

Korus, A. (2012). Effect of technological processing and preservation method on

amino acid content and protein quality in kale (Brassica oleracea L. var. acephala) leaves. Journal of the Science of Food and Agriculture, 92(3), 618–625. https://doi.org/10.1002/jsfa.4619

Kumar, A., Singh, B., Raigond, P., Sahu, C., Mishra, U. N., Sharma, S., & Lal, M. K. (2021). Phytic acid: Blessing in disguise, a prime compound required for both plant and human nutrition. In *Food Research International* (Vol. 142). https://doi.org/10.1016/j.foodres.2021.110193

Lin, J. T., Liu, S. C., Hu, C. C., Shyu, Y. S., Hsu, C. Y., & Yang, D. J. (2016). Effects of roasting temperature and duration on fatty acid composition, phenolic composition, Maillard reaction degree and antioxidant attribute of almond (Prunus dulcis) kernel. *Food Chemistry*, *190*. https://doi.org/10.1016/j.foodchem.2015.06.004

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2018). Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. *Process Safety and Environmental Protection*, *113*. https://doi.org/10.1016/j.psep.2017.09.016

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2019). Phytochemical profile, nutraceutical potential and functional properties of Cucumis melo L. seeds. *Journal of the Science of Food and Agriculture*, *99*(3). https://doi.org/10.1002/jsfa.9304

Marangoni, F., Agostoni, C., Borghi, C., Catapano, A. L., Cena, H., Ghiselli, A., La Vecchia, C., Lercker, G., Manzato, E., Pirillo, A., Riccardi, G., Risé, P., Visioli, F., & Poli, A. (2020). Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. In *Atherosclerosis* (Vol. 292). https://doi.org/10.1016/j.atherosclerosis.2019.11.018

Mariod, A. A., Ahmed, S. Y., Abdelwahab, S. I., Cheng, S. F., Eltom, A. M., Yagoub, S. O., & Gouk, S. W. (2012). Effects of roasting and boiling on the chemical composition, amino acids and oil stability of safflower seeds. International Journal of Food Science & Technology, 47(8), 1737–1743. https://doi.org/10.1111/j.1365-2621.2012.03028.x

Mbuma, N. W., Labuschagne, M., Siwale, J., & Hugo, A. (2022). Diversity in seed protein content, selected minerals, oil content and fatty acid composition of the Southern African Bambara groundnut germplasm collection. *Journal of Food Composition and Analysis*, *109*. https://doi.org/10.1016/j.jfca.2022.104477

McIntosh, S., & Vancov, T. (2011). Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw. *Biomass and Bioenergy*, *35*(7). https://doi.org/10.1016/j.biombioe.2011.04.018

Megazyme. (2017). *Phytic Acid (phytate)/Total Phosphorus*. Megazyme. https://www.megazyme.com/documents/Booklet/K-PHYT_DATA.pdf

Mian-Hao, H., & Yansong, A. (2007). Characteristics of some nutritional composition of melon (Cucumis melo hybrid 'ChunLi') seeds. *International Journal of Food Science and Technology*, *42*(12). https://doi.org/10.1111/j.1365-2621.2006.01352.x

Milinsk, M. C., Matsushita, M., Visentainer, J. v., de Oliveira, C. C., & de Souza, N. E. (2008). Comparative analysis of eight esterification methods in the quantitative determination of vegetable oil fatty acid methyl esters (FAME). *Journal of the Brazilian Chemical Society*, *19*(8). https://doi.org/10.1590/S0103-

50532008000800006

Morais, D. R., Rotta, E. M., Sargi, S. C., Bonafe, E. G., Suzuki, R. M., Souza, N. E., Matsushita, M., & Visentainer, J. V. (2017). Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. *Journal of the Brazilian Chemical Society*, *28*(2). https://doi.org/10.5935/0103-5053.20160178

Moreau, R. A., Lampi, A. M., & Hicks, K. B. (2009). Fatty acid, phytosterol, and polyamine conjugate profiles of edible oils extracted from corn germ, corn fiber, and corn kernels. *JAOCS, Journal of the American Oil Chemists' Society*, *86*(12). https://doi.org/10.1007/s11746-009-1456-6

Nehdi, I. A., Sbihi, H., Tan, C. P., & Al-Resayes, S. I. (2013). Evaluation and characterisation of Citrullus colocynthis (L.) Schrad seed oil: Comparison with Helianthus annuus (sunflower) seed oil. *Food Chemistry*, *136*(2). https://doi.org/10.1016/j.foodchem.2012.09.009

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., Oey, I., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. In *Food Control* (Vol. 79). https://doi.org/10.1016/j.foodcont.2017.03.027

Petkova, Z., & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (Cucumis melo L.) cultivated in Bulgaria. *Cogent Food and Agriculture*, *1*(1). https://doi.org/10.1080/23311932.2015.1018779

Petropoulos, S., Fernandes, Â., Pereira, C., Tzortzakis, N., Vaz, J., Soković, M., Barros, L., & Ferreira, I. C. F. R. (2019). Bioactivities, chemical composition and nutritional value of Cynara cardunculus L. seeds. *Food Chemistry*, *289*. https://doi.org/10.1016/j.foodchem.2019.03.066

Rabadán, A., Antónia Nunes, M., Bessada, S. M. F., Pardo, J. E., Beatriz Oliveira, M. P. P., & Álvarez-Ortí, M. (2020). From by-product to the food chain: Melon (cucumis melo l.) seeds as potential source for oils. *Foods*, *9*(10). https://doi.org/10.3390/foods9101341

Ruan, Q. Y., Zheng, X. Q., Chen, B. L., Xiao, Y., Peng, X. X., Leung, D. W. M., & Liu, E. E. (2013). Determination of total oxalate contents of a great variety of foods commonly available in Southern China using an oxalate oxidase prepared from wheat bran. *Journal of Food Composition and Analysis*, *32*(1). https://doi.org/10.1016/j.jfca.2013.08.002

Sahni, P., & Sharma, S. (2020). Influence of processing treatments on cooking quality, functional properties, antinutrients, bioactive potential and mineral profile of alfalfa. *LWT*, *132*. https://doi.org/10.1016/j.lwt.2020.109890

Salgado, N., Silva, M. A., Figueira, M. E., Costa, H. S., & Albuquerque, T. G. (2023). Oxalate in Foods: Extraction Conditions, Analytical Methods, Occurrence, and Health Implications. Foods, 12(17), 3201. https://doi.org/10.3390/foods12173201

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. In *Food Production, Processing and Nutrition* (Vol. 2, Issue 1). https://doi.org/10.1186/s43014-020-0020-5

Sharma, S., Kataria, A., & Singh, B. (2022). Effect of thermal processing on the bioactive compounds, antioxidative, antinutritional and functional characteristics of quinoa (Chenopodium quinoa). *LWT*, *160*. https://doi.org/10.1016/j.lwt.2022.113256

Shawrang, P., Sadeghi, A. A., Behgar, M., Zareshahi, H., & Shahhoseini, G. (2011). Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chemistry*, *125*(2). https://doi.org/10.1016/j.foodchem.2010.09.010

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of structural carbohydrates and lignin in Biomass - NREL/TP-510-42618. In *National Renewable Energy Laboratory*.

Suri, K., Singh, B., Kaur, A., Yadav, M. P., & Singh, N. (2019). Impact of infrared and dry air roasting on the oxidative stability, fatty acid composition, Maillard reaction products and other chemical properties of black cumin (Nigella sativa L.) seed oil. *Food Chemistry*, *295*. https://doi.org/10.1016/j.foodchem.2019.05.140

Sykam, K., Försth, M., Sas, G., Restás, Á., & Das, O. (2021). Phytic acid: A bio-based flame retardant for cotton and wool fabrics. Industrial Crops and Products, 164, 113349. https://doi.org/10.1016/j.indcrop.2021.113349

Tenyang, N., Ponka, R., Tiencheu, B., Djikeng, F. T., Azmeera, T., Karuna, M. S. L., Prasad, R. B. N., & Womeni, H. M. (2017). Effects of boiling and roasting on proximate composition, lipid oxidation, fatty acid profile and mineral content of two sesame varieties commercialized and consumed in Far-North Region of Cameroon. *Food Chemistry*, *221*. https://doi.org/10.1016/j.foodchem.2016.11.025

Tenyang, N., Ponka, R., Tiencheu, B., Tonfack Djikeng, F., & Womeni, H. M. (2022). Effect of boiling and oven roasting on some physicochemical properties of sunflower seeds produced in Far North, Cameroon. *Food Science and Nutrition*, *10*(2). https://doi.org/10.1002/fsn3.2637

Valdés, A., Beltrán, A., Karabagias, I., Badeka, A., Kontominas, M. G., & Garrigós, M. C. (2015). Monitoring the oxidative stability and volatiles in blanched, roasted and fried almonds under normal and accelerated storage conditions by DSC, thermogravimetric analysis and ATR-FTIR. *European Journal of Lipid Science and Technology*, *117*(8). https://doi.org/10.1002/ejlt.201400384

Vera, M., Mella, C., García, Y., Jiménez, V. A., & Urbano, B. F. (2023). Recent advances in tannin-containing food biopackaging. Trends in Food Science & Technology, 133, 28–36. https://doi.org/10.1016/j.tifs.2023.01.014

WHO. (2012). World Health Organization - Guideline: Potassium intake for adults and children Potassium intake for adults and children. *Geneva*.

Xiong, Y., Zhang, P., Luo, J., Johnson, S., & Fang, Z. (2019). Effect of processing on the phenolic contents, antioxidant activity and volatile compounds of sorghum grain tea. *Journal of Cereal Science*, *85*. https://doi.org/10.1016/j.jcs.2018.10.012

Yang, H. W., Hsu, C. K., & Yang, Y. F. (2014). Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. *Journal of the Science of Food and Agriculture*, *94*(9). https://doi.org/10.1002/jsfa.6494

Yanty, N. A. M., Lai, O. M., Osman, A., Long, K., & Ghazali, H. M. (2008). Physicochemical properties of cucumis melo var. inodorus (honeydew melon) seed and seed oil. *Journal of Food Lipids*, *15*(1). https://doi.org/10.1111/j.1745-4522.2007.00101.x

Zhao, C., Liu, Y., Lai, S., Cao, H., Guan, Y., San Cheang, W., Liu, B., Zhao, K., Miao, S., Riviere, C., Capanoglu, E., & Xiao, J. (2019). Effects of domestic cooking process on the chemical and biological properties of dietary phytochemicals. In *Trends in Food Science and Technology* (Vol. 85). https://doi.org/10.1016/j.tifs.2019.01.004

Chapter 4. Effect of extraction methods on the physicochemical properties, and oxidative stability of melon seed oil

Abstract

Melon seed oil was extracted with three different methods (Soxhlet, cold-pressed, and aqueous enzymatic extraction), aiming to evaluate the physicochemical properties, and oxidative stability of melon seed oil. Melon seed oil contained high levels of linoleic acid (53.6% - 70.8%, w/w), squalene (101.1 - 164.7 mg/100 g), and β-sitosterol (119.5 - 291.9 mg/100 g). In terms of oil yield, Soxhlet extraction was the most efficient (37% - 40.9%, w/w), followed by cold-pressed (16.2% - 17.5%, w/w) and aqueous enzymatic extraction (10.7% - 11.3%, w/w). Results shown that the choice of the extraction method did not alter fatty acid composition, but impacted on the physicochemical properties, content of bioactive compounds and oxidative stability of the oil. Specifically, melon seed oil obtained by aqueous enzymatic extraction (AEE) exhibited higher tocopherol content and exhibited better oxidative stability compared to oil samples obtained by other two extraction methods. Overall, melon seed oil has high potential as an alternative source of edible oil.

4.1. Introduction

Edible oil is an important ingredient in daily diet, providing nutritional and phytochemical compounds associated with energy and health benefits (Cicero et al., 2018). With the increasing world population, the identification of sustainable alternative oil sources with high nutritional quality is necessary to satisfy the growing food demand. On the basis of developing resilient food systems, oil recovery from agricultural residues or by-products has recently attracted research interest (Grajzer, Wiatrak, et al., 2020; Haller et al., 2022).

Melon (*Cucumis melo L.*) belongs to the *Curcurbitaceae* family and is one of the most important commercial tropical fruits cultivated in the world. Melon seeds represent 10% of the total melon weight; currently, they are underutilised and are usually discarded as waste (Silva et al., 2020). Recent studies have shown that melon seeds contain considerable amounts of oil (30.7% - 44.5%, w/w) (Petkova & Antova, 2015; Rabadán et al., 2020), rich in unsaturated fatty acids and bioactive compounds, such as linoleic acid and tocopherols (Mallek-Ayadi et al., 2018; Zhang et al., 2022).

Conventional solvent and cold-pressed extraction methods are widely used in the oil industry. Conventional solvent extraction uses large amounts of organic or nonpolar solvents, and has limitations with regards to oil quality (Nie et al., 2020). In contrast, cold-pressed extraction takes place in the absence of organic solvents and can maintain oil quality, but may cause lipid oxidation issues (Tura et al., 2022). The limitations of the above mentioned traditional extraction methods have prompted the development of novel, environmentally friendly, and sustainable extraction processes that yield edible oil of high quality. Aqueous enzymatic extraction (AEE) has received

much attention in this regard (Nonviho et al., 2015). Compared with traditional extraction methods, the use of water as extraction solvent and the potential of enzyme reusability are major advantages in AEE (Nguyen et al., 2020). Nevertheless, low oil recovery is one of the major challenges for AEE; recent studies have shown that its combination with other extraction technologies such as ultrasonication and microwave, could improve oil yield (Latif & Anwar, 2011; Mwaurah et al., 2020). Studies have also reported that oil quality and nutritional components can vary depending on extraction methods (Nie et al., 2020; Zhang et al., 2022). However, to date, there is scarce information regarding the quality and nutritional value of melon seed oil obtained by different extraction methods.

Therefore, the aim of this study was to evaluate the effect of three extraction methods (Soxhlet, cold-pressed and aqueous enzymatic extraction) on the physicochemical properties, content of bioactive compounds, and oxidative stability of melon seed oil.

4.2. Materials and methods

4.2.1. Chemicals and standards

Methanol (HPLC grade), 2-propanol (Laboratory reagent grade), n-hexane (HPLC grade), petroleum ether (laboratory reagent grade), and acetonitrile (HPLC grade) were purchased from Fisher Scientific (UK). Tri-sil HTP reagent was purchased from Thermo Scientific (UK). FAME standard mixture (C4-C24) and isooctane (for gas chromatography ECD and FID) were purchased from Supelco (UK). Protease (from *Bacillus amyloliquefaciens*), cellulase (from *Trichoderma reesei*), 5α-cholestan-3β-ol (≥95%), sodium methoxide solution (0.5 M, ACS grade), β-sitosterol (≥95%), cholesterol (≥99%, sigma grade), squalene (≥98%), campesterol (~65%), stigmasterol

(~95%), α-tocopherol (≥96%, HPLC grade), γ-tocopherol (≥96%, HPLC grade), and δ-tocopherol (≥90%) standards were purchased from Sigma Aldrich (UK).

4.2.2. The preparation of sample

Galia melon (from Honduras), Honeydew melon (from Brazil) and Cantaloupe melon (from Brazil) were obtained (200 melons for each variety) from Sainsbury Supermarket (Reading, UK) in March 2021. Seeds were manually separated from the fresh, and were washed with water to remove any flesh residual on the seeds' surface. Seeds were dried in vacuum dryer (Townson & Mercer Ltd, Croydon, UK) at 75 ^{o}C and 25 kPa for 24 h. Afterwards, melon seeds were stored at - 18 ^{o}C for further analysis.

4.2.3. Soxhlet extraction (SE)

30 g of melon seed powder (grounded and passed through 600 μm sieve) were weighted and extracted in a Soxhlet apparatus with petroleum ether for 6 h at 40 ^{o}C. After the extraction process, a rotary evaporator (R-144, BUCHI, UK) was used to remove the residual solvent from the extracted oil. The melon seed oils were stored in the freezer at -18 ^{o}C to avoid oil oxidation until further analysis. Oil yield was calculated according to **Equation 4.1**. The oil yield was 37% - 40.9%, w/w.

Equation 4.1:

Oil yield (g/g, %) = Mass oil extracted (g)/Mass of the sample initially processed (g) x 100

4.2.4. Cold-pressed extraction (CPE)

200 g of melon seeds were pressed at room temperature using a cold-press machine (KK 20F SPEZ, oil press GmbH & Co, KG, Germany). After pressing, the oils were

centrifuged at 1107 x g for 15 min (ST 8 Centrifuge, Thermo) at room temperature to separate the oil from the residue; then the cold-pressed oil was collected and stored in the freezer (-18 °C) to avoid oil oxidation for future analysis. Oil yield was calculated following the **Equation 4.1** (**Section 4.2.3**). The oil yield was 16.2% - 17.5%, w/w.

4.2.5. Aqueous enzymatic extraction (AEE)

Aqueous enzymatic extraction of melon seed oil was based on the method reported by Mat Yusoff et al. (2016). Briefly, 5 g of melon seed powder (grounded and passed through 600 µm of sieve) were mixed with 25 mL of distilled water, at a liquid/solid ratio of 5:1. The pH was adjusted at 6 with using 1.0 M HCL/NaOH, and then 3% (v/w) of protease and cellulase, in a ratio of 3:1, were added to the mixture. Then, the mixture was incubated in a water bath at 50 °C and 150 rpm for 6 h. After extraction, the suspension was centrifuged at 1107 x g for 20 min (ST 8 Centrifuge, Thermo). The oil (upper layer) was collected using micropipette and stored at -18 °C to avoid oil oxidation for further analysis. Oil yield was calculated following the **Equation 4.1** (**Section 4.2.2**). The oil yield was 10.7% - 11.3%, w/w.

4.2.6. Oil physicochemical properties

The following parameters were evaluated based on their respective AOAC method (AOAC, 2005): acid value (AOAC method, 969.17), iodine value (AOAC method, 993.20), saponification value (AOAC method, 920.160), and peroxide value (AOAC method, 965.33).

4.2.7. Fatty acid composition analysis

The fatty acid composition was determined according to Milinsk et al. (2008) with some modifications. Briefly, 50 mg of melon seed oil samples were added into 2 mL of

0.5 M sodium methoxide solution in methanol and mixed for 5 min for methyl esterification. After this step, 1 mL of isooctane and 5 mL of saturated sodium chloride solution were added and stirred vigorously for 15 min. The upper layer was collected and transferred into a GC vial, and was analysed by GC (7690B, Agilent, USA) equipped a flame ionization detector (FID). The analysis of fatty acid methyl esters (FAME) was conducted using a fused silica capillary column HP-88 (100 x 0.25 x 0.2). The oven temperature was held initially at 120 °C for 1 min, then increased to 175 °C at a rate of 10 °C/min and remained at 175 °C for 10 min; then increased to 210 °C at a rate of 5 °C/min and remained at 210 °C for 5 min; then increased to 230 °C at a rate of 5 °C/min and remained for 10 min. The temperature of the injection and detector were kept at 250 °C and 280 °C, respectively. The split ratio was 1:50. The carrier gas was helium and flow rate at 1.5 mL/min. FAME were identified by comparison of retention time of FAME standard mixture. The individual fatty acid composition was expressed as a relative percentage of total fatty acids identified (%).

4.2.8. Determination of sterols and squalene content

Sterols and squalene content were determined according to the method described by Liu et al. (2019) with some modifications. Briefly, 0.2 g of oil sample was mixed with 20 mL of 1.0 M KOH in ethanol and 1 mL of 1.0 M internal standard (5α-cholestan-3β-ol), and heated at 90 °C for 1 h in a reflux condenser. After that, 10 mL of distilled water and 5 mL of n-hexane were added to the mixture, followed by vigorous mixing for 30 s. Then, samples were left to rest for 5 min and the n-hexane layer was collected with a micropipette. The extraction with n-hexane was repeated three times and all extracts were combined and evaporated under N_2 flow at 40 °C until dryness. The residue was

derivatized by using 0.5 mL of Tri-sil HTP reagent for 30 min at 60 °C in a water bath. Afterwards, the derivatized sample was transferred into GC vials, and was analysed by GC (7690B, Agilent, USA) equipped a flame ionization detector (FID) using a HP-5ms column (30 m x 0.25 mm x 0.25 µm; J&W Scientific, Folsom, CA, USA). The column temperature was initially held at 200 °C for 0.5 min, then increased to 270 °C at a rate of 10 °C/min, and then held at 270 °C for 25 min. FID detector temperature was set at 290 °C and the temperature of injection port was set at 280 °C. Helium was used as carrier gas and kept the flow rate was 1.0 mL/min. The split ratio was 20:1. Sterols and squalene were quantified according to calibration curves made with known concentrations of external standards, including β-sitosterol (0 - 0.4 mg/mL, R^2 = 1), campesterol (0 - 0.2 mg/mL, R^2 = 0.99), stigmasterol (0 - 1 mg/mL, R^2 = 0.99), cholesterol (0 - 1 mg/mL, R2 = 0.99), and squalene (0 - 1 mg/mL, R^2 = 0.99).

4.2.9. Analysis of tocopherol content

The tocopherol content of melon seeds oil was determined according to Martakos et al. (2020) with slight modifications. Briefly, 100 µL of oil sample was dissolved into 900 µL of 2-propanol, mixed, filtered (0.20 µm filter), and analysed by HPLC coupled with a diode array detector (DAD) (Agilent 1260, Agilent Technologies, Stockport, UK) in a Zorbax SB-C18 column (150 x 4.6 mm, Agilent, UK). An isocratic method was applied using (A) methanol (50%) and (B) acetonitrile (50%) as mobile phase. The flow rate was 1.0 mL/min, and the DAD was set at 295 nm. Each tocopherol compound was quantified according to calibration curves made with known concentrations of external standard, including α-tocopherol (0 - 0.5 mg/mL, R^2 = 0.99), γ-tocopherol (0 - 0.5 mg/mL, R^2 = 0.99), and δ-tocopherol (0 - 0.5 mg/mL, R^2 = 0.99).

4.2.10. Determination of oil oxidation stability

The determination of oxidation stability of melon seeds oil was carried out as described by Kiralan et al. (2014) with some modifications. Briefly, 10 g of oil sample were placed in an air oven at 60 °C for 30 days. Peroxide value was measured according to AOAC method (965.33), in three-day-intervals for a total of 30 days storage duration.

4.2.11. Statistical analysis

All samples were analysed in triplicate. The data was analysed by Minitab (version 20) statistical analysis software. One-way analysis of variance (ANOVA) with Tukey's HSD test were used to evaluate significant difference ($p < 0.05$) between samples.

4.3. Results and Discussion

4.3.1. Oil yield and physicochemical properties of melon seed oil

The oil yield differed depending on the extraction method, with the highest achieved via Soxhlet extraction (SE, 37% - 40.9%, w/w), followed by cold-pressed extraction (CPE, 16.2% - 17.5%, w/w) and aqueous enzymatic extraction (AEE, 10.7% - 11.3%, w/w). The differences in the obtained oil yield are primarily due to the mechanism of extraction for each technique (Zhang et al., 2022; Zhang et al., 2023). The principle of SE is based on the contact and interaction between organic solvents and solute compounds to extract oil by dissolving, thus there are usually high extraction yields associated with the method, which is also AOAC approved (Zhang et al., 2022). CPE is based on the mechanical pressing technique to force oil to be squeezed out of plant cells; due to inherent limitations during pressing, not all the oil can be removed from

pressed oilseeds, and this method is often accompanied by a second solvent extraction step, in order to remove most of the oil from the matrix (Zhang et al., 2023). AEE is using water as extraction solvent with selected enzymes for oil extraction, thus, numerous variables can affect oil yield, such as type of enzyme, temperature, and chemical structure and composition of oilseeds. In addition, AEE can lead to the formation of an emulsion during extraction, which will further negatively affect oil yield and often requires subsequent steps to retrieve the extracted oil (Mat Yusoff et al., 2016).

The physicochemical parameters of melon seed oils from three varieties, obtained by different extraction methods, are shown in **Table 4.1**. The parameters assessed, namely acid value, peroxide value, iodine value, and saponification value, are all related to oil quality.

Acid value represents the free fatty acid content in oils, which reflects the degree of lipid rancidity and is associated with oil quality, since free fatty acids could denote triglyceride hydrolysis during storage (He et al., 2016). The acid value of melon seed oils ranged from 0.7 to 1.8 mg KOH/g, indicating low lipid rancidity. Comparing the acid values of oils extracted by different methods, there was no significant difference between cold-pressed and aqueous enzymatic extraction ($p > 0.05$), but both were lower than Soxhlet extracted oil, indicating that the latter had a relative higher free fatty acid content. This could be attributed to prolonged extraction processing and other non-triglyceride content (e.g. monoacylglycerols and diacylglycerols) that can be extracted by polar solvents (Mat Yusoff et al., 2020).

Peroxide value is an indicator of the degree of oil oxidation (Mat Yusoff et al., 2020).

There was no significant differences between extraction methods ($p > 0.05$) (**Table 4.1**). The peroxide value of all melon seed oils ranged from 6.7 to 8.9 meq O_2/kg, consistently lower than the Codex standard for edible oils (up to 15 meq O_2/kg), indicating that melon seed oil could be considered as edible (Codex Alimentarius Commission, 2001).

Iodine value reflects the unsaturation degree of fatty acids. Based on data in **Table 4.1**, no consistent changes were observed in iodine values with different extraction method. Values between 117.0 - 147.3 g I_2/100 g were obtained, which were higher than those reported for sunflower, olive, and rapeseed oil (80.0 - 107.5 g I_2/100 g), indicating the unsaturated nature of melon seed oil (Konuskan et al., 2019).

Saponification value reflects the average chain length of fatty acids, a higher saponification value indicates a shorter chain length of fatty acids (He et al., 2016). The saponification value of melon seed oil ranged from 108.9 to 205.4 mg KOH/g, whereas the saponification values of melon seed oil obtained by cold-pressed and aqueous enzymatic extraction were higher than that of Soxhlet extracted oil. This indicated that the latter contained longer fatty acids (Wu et al., 2020). According to Górnaś et al. (2013), high saponification value in oils indicates their suitability for soap production. As such, another avenue that could be explored based on this quality parameter is the utilisation of melon seed oil obtained by cold-pressed and aqueous enzymatic for soap making.

Table 4.1. The physicochemical parameters of Galia, Cantaloupe, and Honeydew seed oil from different extraction methods.

Variety	Extraction method	Acid value (mg KOH/g)	Peroxide value (meq O_2/kg)	Iodine value (g I_2/100 g)	Saponification value (mg KOH/g)
Galia	SE	1.8 ± 0.1 ^{a}	8.1 ± 0.4 ^{a}	147.3 ± 2.0 ^{a}	129.0 ± 1.4 ^{c}
	CPE	1.3 ± 0.0 ^{b}	7.8 ± 0.3 ^{a}	137.7 ± 2.1 ^{b}	190.2 ± 1.6 ^{b}
	AEE	1.2 ± 0.2 ^{b}	8.4 ± 0.5 ^{a}	132.6 ± 3.3 ^{b}	205.4 ± 2.0 ^{a}
Cantaloupe	SE	1.8 ± 0.3 ^{a}	8.9 ± 0.4 ^{a}	141.1 ± 2.6 ^{a}	108.9 ± 5.0 ^{b}
	CPE	1.1 ± 0.0 ^{b}	8.3 ± 0.4 ^{a}	133.6 ± 3.1 ^{b}	190.2 ± 0.9 ^{a}
	AEE	1.0 ± 0.2 ^{b}	6.7 ± 0.1 ^{b}	126.9 ± 1.4 ^{c}	183.9 ± 1.4 ^{a}
Honeydew	SE	1.4 ± 0.2 ^{a}	8.1 ± 0.4 ^{a}	124.2 ± 3.5 ^{ab}	123.0 ± 2.3 ^{c}
	CPE	0.7 ± 0.1 ^{b}	7.8 ± 0.4 ^{a}	129.7 ± 7.4 ^{a}	185.5 ± 0.9 ^{a}
	AEE	1.0 ± 0.2 ^{ab}	8.2 ± 0.5 ^{a}	117.0 ± 4.3 ^{b}	152.2 ± 1.2 ^{b}

Data represented as mean ± standard deviation (n = 3). Different lower letters in the same column within each variety indicates significant difference associated with extraction method ($p < 0.05$). SE - Soxhlet extraction; CPE - cold-pressed extraction; AEE - Aqueous enzymatic extraction.

4.3.2. Fatty acid profile of melon seed oil

Data in **Table 4.2** show the fatty acid profile of extracted melon seed oil. In relation to the extraction methods, no significant differences ($p > 0.05$) were observed in the fatty acid compositions of each variety. In terms of fatty acid profile, all samples were rich in unsaturated fatty acids (over 80%, w/w), with linoleic acid representing the predominant one (53.6% - 70.8%, w/w), followed by oleic acid (14.5% - 29.9%, w/w). In terms of saturated fatty acids, palmitic acid (8.8% - 10.2%, w/w) was found to be the main one, followed by stearic acid (4.5% - 6.1%, w/w). The results of this study are in agreement with previous reports, highlighting the abundance of linoleic acid in melon seed oil (Mallek-Ayadi et al., 2018; Rabadán et al., 2020). With regards to fatty acid profile differences among the varieties, large differences were found in linoleic and oleic acid content, and less in palmitic and stearic acid content. Among the three varieties, Galia and Cantaloupe exhibited much higher linoleic acid content than Honeydew, whereas Honeydew exhibited higher oleic acid content compared to the other two varieties (**Table 4.2**). Similar results was reported by Coetzee et al. (2008), who investigated the fatty acid composition in eight varieties of kenaf seed oil from South Africa; these differences could be due to genotypic variations and the impact of varying growth parameters (e.g. climate, soil, and year of harvest) (Boschin et al., 2008; Coetzee et al., 2008; Wang et al., 2013).

Overall, the considerable levels of unsaturated fatty acids (mainly due to linoleic and oleic acid) render melon seed oil a nutritionally valuable oil as compared to other commercial vegetable oils, such as olive (80%) and sunflower oil (85%) (Cerchiara et al., 2010).

Table 4.2. Fatty acid profile (%) of Galia, Cantaloupe, and Honeydew melon seed oil from different extraction methods.

Fatty acid	Galia			Cantaloupe			Honeydew		
(%)	SE	CPE	AEE	SE	CPE	AEE	SE	CPE	AEE
Palmitic acid (C16:0)	9.6 ± 0.1 [a]	9.6 ± 0.0 [a]	8.8 ± 0.0 [b]	9.9 ± 0.0 [b]	9.9 ± 0.0 [b]	10.2 ± 0.1 [a]	9.4 ± 0.1 [a]	9.4 ± 0.0 [a]	9.4 ± 0.0 [a]
Stearic acid (C18:0)	4.5 ± 0.0 [c]	4.7 ± 0.0 [b]	4.8 ± 0.0 [a]	6.0 ± 0.0 [a]	6.1 ± 0.0 [a]	5.5 ± 0.1 [b]	5.9 ± 0.1 [b]	6.1 ± 0.0 [a]	5.7 ± 0.0 [c]
Oleic acid (C18:1)	14.9 ± 0.1 [b]	15.5 ± 0.1 [a]	14.5 ± 0.1 [c]	15.4 ± 0.0 [b]	15.8 ± 0.0 [a]	14.5 ± 0.1 [c]	29.1 ± 0.1 [b]	29.9 ± 0.1 [a]	28.2 ± 0.1 [c]
Linoleic acid (C18:2)	69.9 ± 0.1 [b]	69.3 ± 0.1 [c]	70.8 ± 0.1 [a]	67.4 ± 0.0 [b]	67.3 ± 0.0 [b]	68.8 ± 0.2 [a]	54.3 ± 0.1 [b]	53.6 ± 0.1 [c]	55.3 ± 0.1 [a]
α-Linolenic acid (C18:3)	0.2 ± 0.0 [b]	0.2 ± 0.0 [b]	0.3 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.1 ± 0.0 [b]	0.2 ± 0.0 [a]
Arachidic acid (C20:0)	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.3 ± 0.0 [a]	0.2 ± 0.0 [b]	0.2 ± 0.0 [b]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]
Gondoic acid (C20:1)	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	–	–	–	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]
Tricosanoic acid (C23:0)	0.1 ± 0.0 [b]	0.1 ± 0.0 [b]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.2 ± 0.0 [a]	0.1 ± 0.0 [b]
SFA	14.5	14.7	14.0	16.4	16.4	16.1	15.8	16.0	15.5
MUFA	15.0	15.6	14.6	15.4	15.8	14.5	29.3	30.0	28.3
PUFA	70.1	69.5	71.1	67.6	67.5	69.0	54.5	53.7	55.5
Unknown	0.4	0.2	0.3	0.6	0.3	0.4	0.4	0.3	0.7

Data represented as mean ± standard deviation (n = 3). Different lower letters in the same row within each variety indicate significant difference associated with extraction methods ($p < 0.05$). SE - Soxhlet extraction; CPE - cold-pressed extraction; AEE - Aqueous enzymatic extraction; SFA - total saturated fatty acid; MUFA - total monounsaturated fatty acid; PUFA - total polyunsaturated fatty acid.

4.3.3. Tocopherol content in melon seed oil

Tocopherols (Vitamin E) are oil-soluble natural antioxidants, which play an important role in oil oxidative stability and human health (Górnaś, 2015; Nie et al., 2020). The tocopherol content of three melon seed oil varieties extracted with different methods are presented in **Table 4.3**. γ-Tocopherol was the major tocopherol in melon seed oils, ranging from 6.8 to 67.3 mg/100 g. In addition, α-tocopherol was not detected in any of the oils, whereas δ-tocopherol was only detected in low amounts in the Cantaloupe seed oil (0.9 - 1.2 mg/100 g). This indicated that γ-tocopherol was the dominant tocopherol in melon seed oil, in agreement with previous studies (Rabadán et al., 2020; Zhang et al., 2022). Notably, the highest γ-tocopherol content was found in Galia melon seed oil (**Table 4.3**). Compared with previous studies, Rabadán et al. (2020) and Zhang et al. (2022) detected low concentrations of α-tocopherol (1.2 - 7.4 mg/100 g) and δ-tocopherol (0.9 - 2.7 mg/100 g) in melon seed oil. In addition, tocopherol content varied significantly among the three melon varieties in this study; previous studies reported that tocopherol content in plant materials is mostly influenced by agronomical factors including variety, region, soil, and climate (Górnaś, 2015; Zhang et al., 2019). Considering the total tocopherol content of melon seed oils, significant differences on tocopherol content were observed with different extraction methods ($p < 0.05$); the sequence was listed as AEE > CPE > SE, except Galia variety. It can be seen that extraction methods affect tocopherol content. High tocopherol content in AEE extracted oil could be attributed to enzymatic hydrolysis that degrades substrate molecules (proteins and polysaccharides) and reduces the formation of tocopherol complexes with protein or polysaccharides, thereby enhancing their final concentration in the extracted oil (Gai et al., 2013; Latif & Anwar, 2011).

Table 4.3. Tocopherol content (mg/100 g) of Galia, Cantaloupe, and Honeydew melon seed oil from different extraction methods.

Variety	Extraction	α-tocopherol	γ-tocopherol	δ-tocopherol	Total
Galia	SE	ND	52.4 ± 0.4 [b]	ND	52.4
	CPE	ND	47.4 ± 0.3 [c]	ND	47.4
	AEE	ND	67.3 ± 0.2 [a]	ND	67.3
Cantaloupe	SE	ND	28.6 ± 0.4 [c]	1.2 ± 0.0 [a]	29.8
	CPE	ND	32.5 ± 0.0 [b]	0.9 ± 0.0 [b]	33.4
	AEE	ND	46.9 ± 0.3 [a]	ND	46.9
Honeydew	SE	ND	6.8 ± 0.2 [c]	ND	6.8
	CPE	ND	14.6 ± 0.0 [b]	ND	14.6
	AEE	ND	15.0 ± 0.1 [a]	ND	15.0

Data represented as mean ± standard deviation (n = 3). Different lower letters in the same column within each variety indicate significant difference associated with extraction method ($p < 0.05$). SE - Soxhlet extraction; CPE - cold-pressed extraction; AEE - Aqueous enzymatic extraction; ND - not detected.

4.3.4. Sterol and squalene content in melon seed oil

Sterols are commonly present as components of the unsaponifiable part in vegetable oils. Numerous studies have reported that sterols have anti-inflammatory activity, inhibit cholesterol absorption, and may also exhibit antioxidant and anti-bacterial effects (Wu et al., 2020; Zhang et al., 2019).

Sterol content in melon seed oils obtained with different extraction methods is presented in **Table 4.4**. Results showed that β-sitosterol was the major sterol (119.5 - 291.9 mg/100 g), followed by campesterol (83.6 - 133.2 mg/100 g); cholesterol and stigmasterol were not detected. The abundance of β-sitosterol in melon seed oil has led to the suggestion of utilising this sterol as an identification maker for melon seed oil (da Silva & Jorge, 2014; Górnaś & Rudzińska, 2016; Mallek-Ayadi et al., 2018). As shown in **Table 4.4**, the amount of individual sterols and total sterols seemed to be

influenced by the oil extraction method. Li et al. (2007) and Zhang et al. (2020) reported similar results in sea buckthorn and milk thistle seed oils obtained by different extraction methods. Sterols are located in monolayers and bilayers of various cell organelles, such as oil bodies, chloroplast, and chromoplasts, form protective layers on seed surfaces or are components of cell membranes (Dąbrowski et al., 2019; Liao et al., 2021); during oil extraction, the extraction of these compounds requires disruption of the membrane matrix or contact and interaction with extraction solvent, followed by release into the oil (Dąbrowski et al., 2019). Therefore, the final concentration of these compounds in oil can be affected by their physicochemical properties (e.g. polarity, structure, and partition coefficient) and extraction methods as well as the relevant extraction conditions (e.g. temperature, pressure, polarity of solvent) (Li et al., 2007; Liao et al., 2021; Tir et al., 2012).

Squalene is a compound of high nutritional value with potential health benefits, including anti-photooxidative and anti-atherosclerotic effects (Lou-Bonafonte et al., 2018; Lyashenko et al., 2021). The squalene content of melon seed oils ranged from 101.1 to 164.7 mg/100 g (**Table 4.4**). Compared with other commercial vegetable oils, the squalene content of melon seed oil was higher than that of rapeseed sunflower, corn, and palm oil, all of which have been reported to contain squalene in the range of 11.9 to 43.7 mg/100 g (Cicero et al., 2018; Tuberoso et al., 2007).

Table 4.4. Sterols and squalene content (mg/100 g) of Galia, Cantaloupe, and Honeydew melon seed oil from different extraction methods.

Variety	Extraction	Cholesterol	Campesterol	Stigmasterol	β-sitosterol	Total sterols	Squalene
Galia	SE	ND	98.1 ± 1.3 [a]	ND	177.6 ± 1.9 [b]	275.7	118.7 ± 1.5 [b]
	CPE	ND	83.6 ± 1.6 [b]	ND	157.9 ± 3.7 [c]	241.5	101.1 ± 1.9 [c]
	AEE	ND	96.9 ± 1.5 [a]	ND	238.7 ± 2.1 [a]	335.6	156.2 ± 0.4 [a]
Cantaloupe	SE	ND	133.2 ± 1.6 [a]	ND	291.9 ± 3.9 [a]	425.1	164.7 ± 1.1 [a]
	CPE	ND	121.6 ± 1.6 [b]	ND	182.6 ± 2.7 [c]	304.2	146.5 ± 2.3 [b]
	AEE	ND	99.9 ± 1.5 [c]	ND	204.7 ± 5.7 [b]	304.6	107.8 ± 1.5 [c]
Honeydew	SE	ND	97.7 ± 4.1 [a]	ND	157.3 ± 8.5 [a]	255.0	135.0 ± 7.9 [a]
	CPE	ND	103.6 ± 2.3 [a]	ND	119.5 ± 4.9 [b]	223.1	141.1 ± 2.3 [a]
	AEE	ND	102.5 ± 2.5 [a]	ND	130.5 ± 0.6 [b]	233.0	112.8 ± 2.6 [b]

Data represented as mean ± standard deviation (n = 3). Different lower letter in the same column within each variety indicates significant difference associated with extraction method ($p < 0.05$). SE - Soxhlet extraction; CPE - cold-pressed extraction; AEE - Aqueous enzymatic extraction; ND - not detected.

4.3.5. Oil oxidative stability

Oil oxidative stability is an important index to evaluate oil quality and shelf life (Bettaieb Rebey et al., 2019). Data in **Figure 4.1** show the oxidative stability of melon seed oils, based on peroxide value changes under storage at 60 °C (accelerated storage conditions).

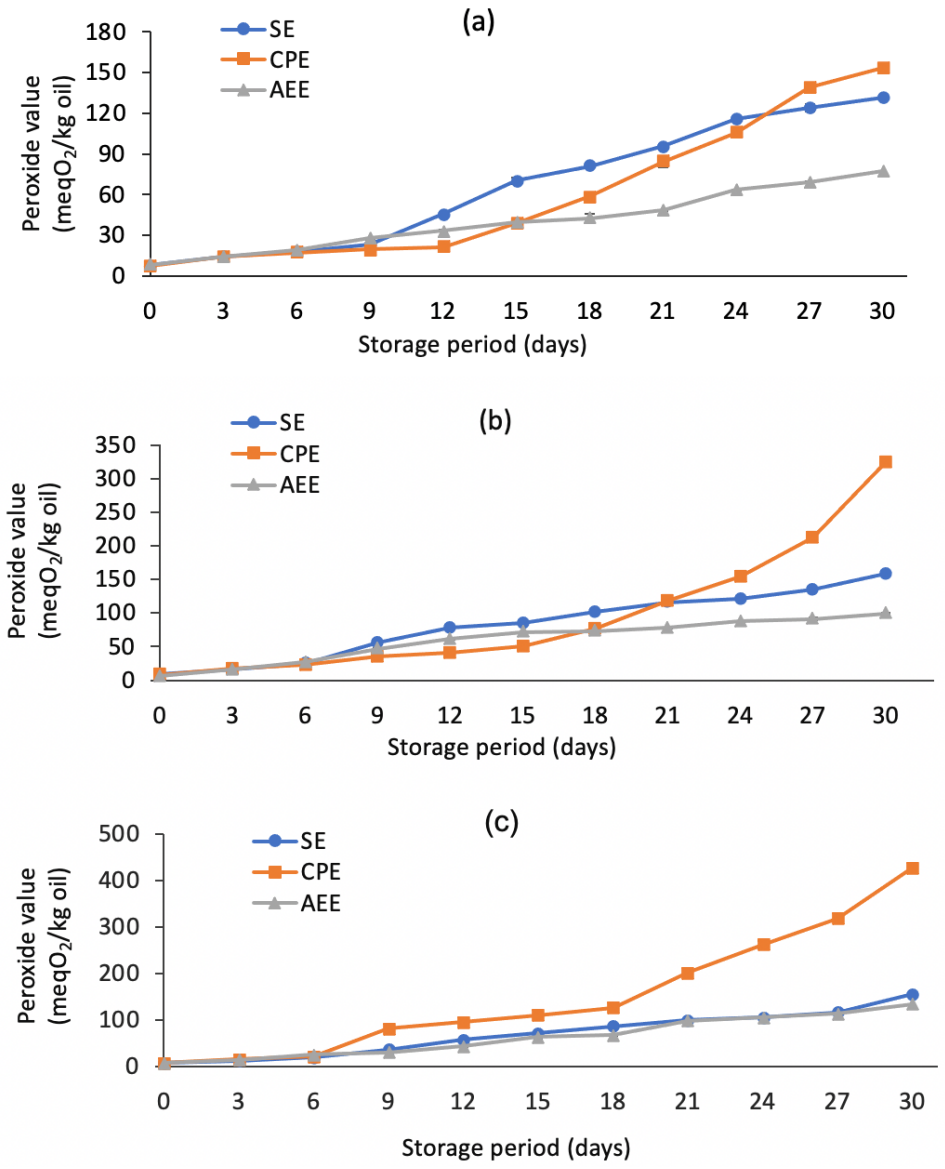

Figure 4.1. Peroxide value change in melon seed oils from different extraction methods during storage at 60 °C. (a) Galia variety; (b) Cantaloupe variety; (c) Honeydew variety; SE- Soxhlet extraction; CPE - cold-pressed extraction; AEE - aqueous enzymatic extraction. Error bars are shown in the symbol.

At the end of storage period (30th day), AEE-derived melon seed oils (Galia 77.7 meq O_2/kg, Cantaloupe 91.2 meq O_2/kg, and Honeydew 134.5 meq O_2/kg) had the lowest peroxide value compared to SE (Galia 131.6 meq O_2/kg, Cantaloupe 157.9 meq O_2/kg, and Honeydew 171.6 meq O_2/kg) and CPE (Galia 153.8 meq O_2/kg, Cantaloupe 325.1 meq O_2/kg, and Honeydew 428.7 meq O_2/kg), indicating that AEE-derived melon seed oil had better oxidative stability. Furthermore, under the same extraction method, Honeydew seed oil showed the lowest oxidative stability compared to other two varieties. These could be attributed to the tocopherol content in the extracted oil (**Table 4.3**), since the presence of tocopherol had positive effect on oil oxidative stability. In contrast, although CPE extracted oil contained higher tocopherol content than SE extracted oil (**Table 4.3**), their oxidative stability were lower than SE extracted oil, indicating that other compounds (e.g. sterols and squalene) also play important role in oil oxidative stability. As shown in **Figure 4.3**, the peroxide value at the end of storage (30th day) depends on many factors, but none of them showed a high correlation. It has been reported that synergistic effects between tocopherol and other antioxidants (phenolic acids, carotenoids, sterols, squalene as well as some Maillard reaction products) could play an important role for enhancing the oxidative stability in oils (Choe & Min, 2006; Lutterodt et al., 2011; Naziri et al., 2016). On the other hand, the oxidative stability of oil depends on the level of unsaturated fatty acid as well as the content of pro/antioxidant compositions in oil (Grosshagauer et al., 2019); according to Grajzer et al. (2020), cold-pressed oil could contain more pro-oxidant factors (e.g. metal ions), which are more susceptible to causing oil oxidation.

Data shown in **Figure 4.2** show the difference in linoleic acid (C18:2) content of melon seed oils between day 0 and day 30. The melon seed oil obtained by CPE showed higher loss rate of linoleic acid content compared to AEE and SE. Especially in Cantaloupe and Honeydew varieties, the linoleic acid content was decreased up to 9%. High loss rate of unsaturated fatty acids indicates high degree of oxidation and a subsequent reduction of the oil nutritional quality (Huang et al., 2021). In contrast, the loss rate of linoleic acid from melon seed oils obtained by SE and AEE were lower than CPE, further indicating that SE and AEE extracted oil had better oxidative stability than CPE extracted oil. Therefore, taken together, these results suggest that melon seed oils obtained by SE and AEE had better oxidative stability and relative higher linoleic acid retention, with samples obtained by AEE performing best.

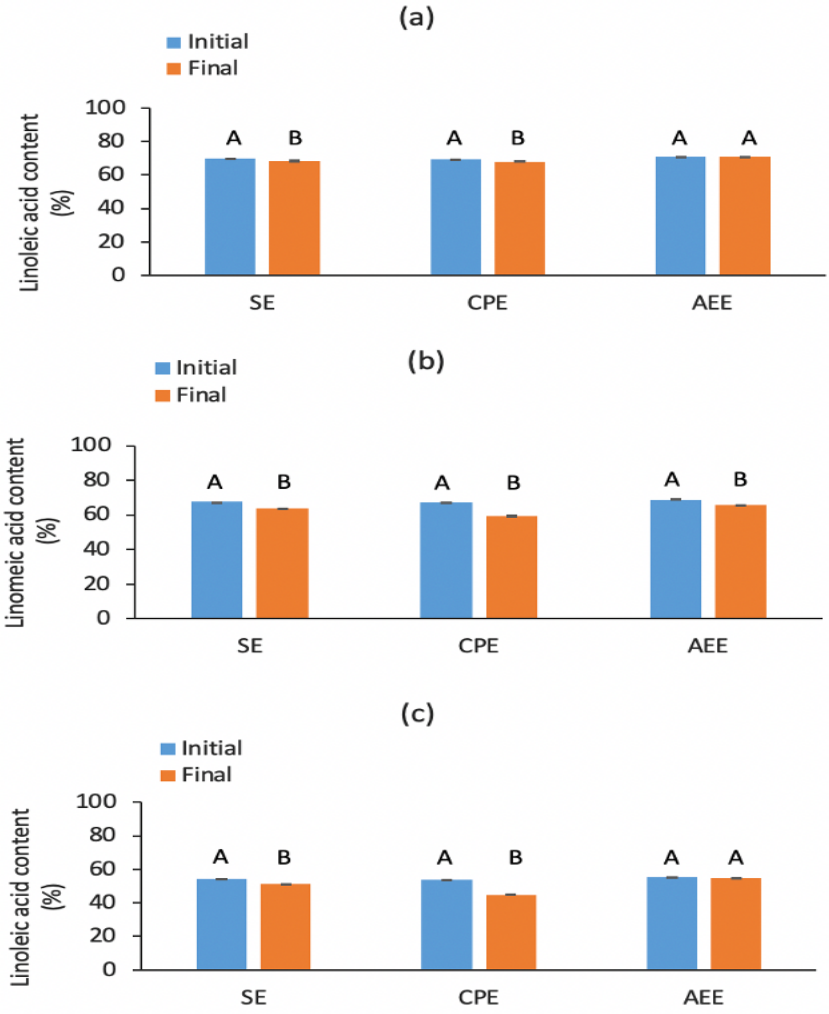

Figure 4.2. The linoleic acid (C18:2) content of melon seed oils obtained by different extraction methods before and after accelerated storage conditions. (a) Galia variety; (b) Cantaloupe variety; (c) Honeydew variety; SE - Soxhlet extraction; CPE - cold-pressed extraction; AEE - aqueous enzymatic extraction.

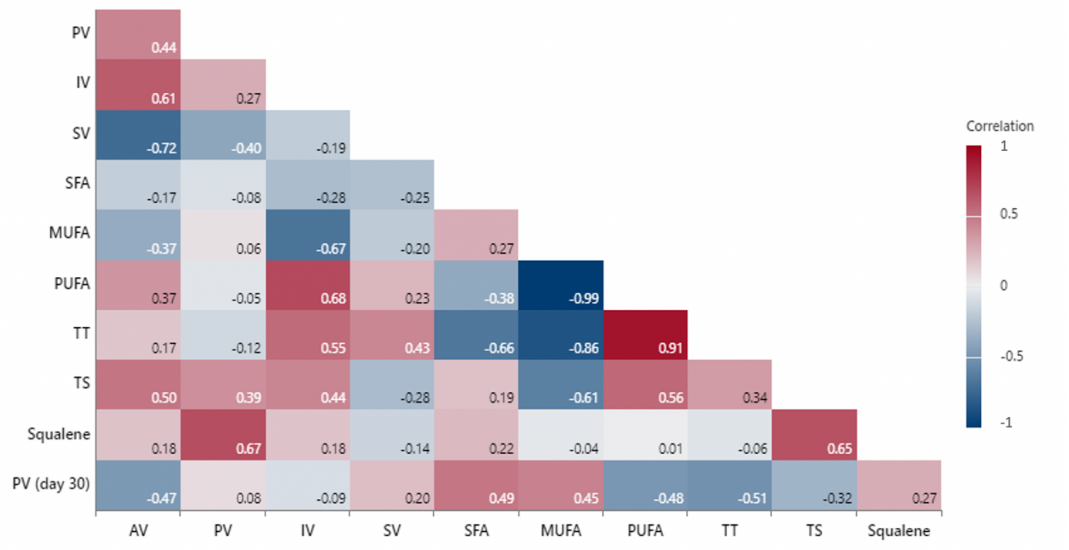

Figure 4.3. Analysis of Pearson correlation. Correlation values that are close to 1, −1, and 0 indicate a strong positive correlation, a strong negative correlation, and a weak or no linear relationship, respectively. PV - peroxide value; AV - acid value; IV - iodine value; SV - saponification value; SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; TT - total tocopherol content; TS - total sterol content; PV (day 30) - peroxide value at end of storage (30th day).

4.4. Conclusions

Melon seed oil was found rich in linoleic acid, phytosterols, and squalene; its composition could be translated into a wide range of applications in food, cosmetics, and pharmaceutical industries. In relation to the extraction method, the choice of extraction method did not affect fatty acid composition but impacted on physicochemical properties, content of bioactivity compounds, and oxidative stability of the oils. Melon seed oil obtained by AEE exhibited high tocopherol content and oxidative stability. Considering the oil quality, aqueous enzymatic extraction method (AEE) seemed to be more advantageous, but was accompanied by low oil yields. Further work on other assisted extraction methods (e.g. ultrasonic and microwave) combined with AEE could improve oil yield.

4.5. References

AOAC. (2005). Official Methods of Analysis of AOAC International. In *Association of Official Analysis Chemists International*.

Bettaieb Rebey, I., Bourgou, S., Detry, P., Wannes, W. A., Kenny, T., Ksouri, R., Sellami, I. H., & Fauconnier, M. L. (2019). Green Extraction of Fennel and Anise Edible Oils Using Bio-Based Solvent and Supercritical Fluid: Assessment of Chemical Composition, Antioxidant Property, and Oxidative Stability. *Food and Bioprocess Technology*, *12*(10). https://doi.org/10.1007/s11947-019-02341-8

Boschin, G., D'Agostina, A., Annicchiarico, P., & Arnoldi, A. (2008). Effect of genotype and environment on fatty acid composition of Lupinus albus L. seed. *Food Chemistry*, *108*(2). https://doi.org/10.1016/j.foodchem.2007.11.016

Cerchiara, T., Chidichimo, G., Ragusa, M. I., Belsito, E. L., Liguori, A., & Arioli, A. (2010). Characterization and utilization of Spanish Broom (Spartium junceum L.) seed oil. *Industrial Crops and Products*, *31*(2). https://doi.org/10.1016/j.indcrop.2009.11.003

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 5, Issue 4). https://doi.org/10.1111/j.1541-4337.2006.00009.x

Cicero, N., Albergamo, A., Salvo, A., Bua, G. D., Bartolomeo, G., Mangano, V., Rotondo, A., Di Stefano, V., Di Bella, G., & Dugo, G. (2018). Chemical characterization of a variety of cold-pressed gourmet oils available on the Brazilian market. *Food Research International*, *109*. https://doi.org/10.1016/j.foodres.2018.04.064

Codex Alimentarius Commission. (2001). Codex alimentarius, Volume 8: fats, oils and related products. In *Food and Agriculture Organization of the United Nations (FAO)*.

Coetzee, R., Labuschagne, M. T., & Hugo, A. (2008). Fatty acid and oil variation in seed from kenaf (Hibiscus cannabinus L.). *Industrial Crops and Products*, *27*(1). https://doi.org/10.1016/j.indcrop.2007.08.005

da Silva, A. C., & Jorge, N. (2014). Bioactive compounds of the lipid fractions of agro-industrial waste. *Food Research International*, *66*. https://doi.org/10.1016/j.foodres.2014.10.025

Dąbrowski, G., Czaplicki, S., & Konopka, I. (2019). Fractionation of sterols, tocols and squalene in flaxseed oils under the impact of variable conditions of supercritical CO2 extraction. *Journal of Food Composition and Analysis*, *83*, 103261. https://doi.org/10.1016/j.jfca.2019.103261

Gai, Q. Y., Jiao, J., Mu, P. S., Wang, W., Luo, M., Li, C. Y., Zu, Y. G., Wei, F. Y., & Fu, Y. J. (2013). Microwave-assisted aqueous enzymatic extraction of oil from Isatis indigotica seeds and its evaluation of physicochemical properties, fatty acid compositions and antioxidant activities. *Industrial Crops and Products*, *45*.

https://doi.org/10.1016/j.indcrop.2012.12.050

Górnaś, P. (2015). Unique variability of tocopherol composition in various seed oils recovered from by-products of apple industry: Rapid and simple determination of all four homologues (α, β, γ and δ) by RP-HPLC/FLD. *Food Chemistry*, *172*. https://doi.org/10.1016/j.foodchem.2014.09.051

Górnaś, P., & Rudzińska, M. (2016). Seeds recovered from industry by-products of nine fruit species with a high potential utility as a source of unconventional oil for biodiesel and cosmetic and pharmaceutical sectors. *Industrial Crops and Products*, *83*. https://doi.org/10.1016/j.indcrop.2016.01.021

Górnaś, P., Siger, A., & Segliņa, D. (2013). Physicochemical characteristics of the cold-pressed Japanese quince seed oil: New promising unconventional bio-oil from by-products for the pharmaceutical and cosmetic industry. *Industrial Crops and Products*, *48*. https://doi.org/10.1016/j.indcrop.2013.04.018

Grajzer, M., Szmalcel, K., Kuźmiński, Ł., Witkowski, M., Kulma, A., & Prescha, A. (2020). Characteristics and Antioxidant Potential of Cold-Pressed Oils—Possible Strategies to Improve Oil Stability. *Foods*, *9*(11). https://doi.org/10.3390/foods9111630

Grajzer, M., Wiatrak, B., Gębarowski, T., Matkowski, A., Grajeta, H., Rój, E., Kulma, A., & Prescha, A. (2020). Chemistry, oxidative stability and bioactivity of oil extracted from Rosa rugosa (Thunb.) seeds by supercritical carbon dioxide. *Food Chemistry*, *335*. https://doi.org/10.1016/j.foodchem.2020.127649

Grosshagauer, S., Steinschaden, R., & Pignitter, M. (2019). Strategies to increase the oxidative stability of cold pressed oils. In *LWT* (Vol. 106). https://doi.org/10.1016/j.lwt.2019.02.046

Haller, H., Fagerholm, A. S., Carlsson, P., Skoglund, W., van den Brink, P., Danielski, I., Brink, K., Mirata, M., & Englund, O. (2022). Towards a Resilient and Resource-Efficient Local Food System Based on Industrial Symbiosis in Härnösand: A Swedish Case Study. *Sustainability (Switzerland)*, *14*(4). https://doi.org/10.3390/su14042197

He, Z., Zhu, H., Li, W., Zeng, M., Wu, S., Chen, S., Qin, F., & Chen, J. (2016). Chemical components of cold pressed kernel oils from different Torreya grandis cultivars. *Food Chemistry*, *209*. https://doi.org/10.1016/j.foodchem.2016.04.053

Huang, Y., Xiang, X., Luo, X., Li, X., Yu, X., & Li, S. (2021). Study on the emulsification and oxidative stability of ovalbumin-pectin-pumpkin seed oil emulsions using ovalbumin solution prepared by ultrasound. *Ultrasonics Sonochemistry*, *78*. https://doi.org/10.1016/j.ultsonch.2021.105717

Kiralan, M., Özkan, G., Bayrak, A., & Ramadan, M. F. (2014). Physicochemical properties and stability of black cumin (Nigella sativa) seed oil as affected by different extraction methods. *Industrial Crops and Products*, *57*. https://doi.org/10.1016/j.indcrop.2014.03.026

Konuskan, D. B., Arslan, M., & Oksuz, A. (2019). Physicochemical properties of cold pressed sunflower, peanut, rapeseed, mustard and olive oils grown in the Eastern Mediterranean region. *Saudi Journal of Biological Sciences*, *26*(2). https://doi.org/10.1016/j.sjbs.2018.04.005

Latif, S., & Anwar, F. (2011). Aqueous enzymatic sesame oil and protein extraction. *Food Chemistry*, *125*(2). https://doi.org/10.1016/j.foodchem.2010.09.064

Li, T. S. C., Beveridge, T. H. J., & Drover, J. C. G. (2007). Phytosterol content of sea buckthorn (Hippophae rhamnoides L.) seed oil: Extraction and identification. *Food Chemistry*, *101*(4). https://doi.org/10.1016/j.foodchem.2006.04.033

Liao, Y., Fan, F., Zhu, A., Zhao, J., & Peng, Y. (2021). Kinetics characteristics and thermodynamics analysis of soybean pods sterols extraction process. *Environmental Challenges*, *5*, 100357. https://doi.org/10.1016/j.envc.2021.100357

Liu, R., Guo, X., Cheng, M., Zheng, L., Gong, M., Chang, M., Jin, Q., & Wang, X. (2019). Effects of chemical refinement on the quality of coconut oil. *Journal of Food Science and Technology*, *56*(6). https://doi.org/10.1007/s13197-019-03810-w

Lou-Bonafonte, J. M., Martínez-Beamonte, R., Sanclemente, T., Surra, J. C., Herrera-Marcos, L. V., Sanchez-Marco, J., Arnal, C., & Osada, J. (2018). Current Insights into the Biological Action of Squalene. In *Molecular Nutrition and Food Research* (Vol. 62, Issue 15). https://doi.org/10.1002/mnfr.201800136

Lutterodt, H., Slavin, M., Whent, M., Turner, E., & Yu, L. (2011). Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chemistry*, *128*(2). https://doi.org/10.1016/j.foodchem.2011.03.040

Lyashenko, S., González-Fernández, M. J., Borisova, S., Belarbi, E. H., & Guil-Guerrero, J. L. (2021). Mertensia (Boraginaceae) seeds are new sources of γ-linolenic acid and minor functional compounds. *Food Chemistry*, *350*. https://doi.org/10.1016/j.foodchem.2020.128635

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2018). Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. *Process Safety and Environmental Protection*, *113*. https://doi.org/10.1016/j.psep.2017.09.016

Martakos, I., Kostakis, M., Dasenaki, M., Pentogennis, M., & Thomaidis, N. (2020). Simultaneous determination of pigments, tocopherols, and squalene in Greek olive oils: A study of the influence of cultivation and oil-production parameters. *Foods*, *9*(1). https://doi.org/10.3390/foods9010031

Mat Yusoff, M., Gordon, M. H., Ezeh, O., & Niranjan, K. (2016). Aqueous enzymatic extraction of Moringa oleifera oil. *Food Chemistry*, *211*. https://doi.org/10.1016/j.foodchem.2016.05.050

Mat Yusoff, M., Niranjan, K., Mason, O. A., & Gordon, M. H. (2020). Oxidative

properties of Moringa oleifera kernel oil from different extraction methods during storage. *Journal of the Science of Food and Agriculture*, *100*(4). https://doi.org/10.1002/jsfa.10167

Milinsk, M. C., Matsushita, M., Visentainer, J. v., de Oliveira, C. C., & de Souza, N. E. (2008). Comparative analysis of eight esterification methods in the quantitative determination of vegetable oil fatty acid methyl esters (FAME). *Journal of the Brazilian Chemical Society*, *19*(8). https://doi.org/10.1590/S0103-50532008000800006

Mwaurah, P. W., Kumar, S., Kumar, N., Attkan, A. K., Panghal, A., Singh, V. K., & Garg, M. K. (2020). Novel oil extraction technologies: Process conditions, quality parameters, and optimization. *Comprehensive Reviews in Food Science and Food Safety*, *19*(1), 3–20. https://doi.org/10.1111/1541-4337.12507

Naziri, E., Mitić, M. N., & Tsimidou, M. Z. (2016). Contribution of tocopherols and squalene to the oxidative stability of cold-pressed pumkin seed oil (Cucurbita pepo L.). *European Journal of Lipid Science and Technology*, *118*(6). https://doi.org/10.1002/ejlt.201500261

Nguyen, H. C., Vuong, D. P., Nguyen, N. T. T., Nguyen, N. P., Su, C. H., Wang, F. M., & Juan, H. Y. (2020). Aqueous enzymatic extraction of polyunsaturated fatty acid–rich sacha inchi (Plukenetia volubilis L.) seed oil: An eco-friendly approach. *LWT*, *133*. https://doi.org/10.1016/j.lwt.2020.109992

Nie, R., Zhang, Y., Zhang, H., Jin, Q., Wu, G., & Wang, X. (2020). Effect of different processing methods on physicochemical properties, chemical compositions and in vitro antioxidant activities of Paeonia lactiflora Pall seed oils. *Food Chemistry*, *332*. https://doi.org/10.1016/j.foodchem.2020.127408

Nonviho, G., Paris, C., Muniglia, L., Sohounhloué, D., & Brosse, N. (2015). Lophira lanceolata seed oil extraction method (ancestral or modern) modifies the properties of the oil. *Industrial Crops and Products*, *67*. https://doi.org/10.1016/j.indcrop.2015.01.006

Petkova, Z., & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (Cucumis melo L.) cultivated in Bulgaria. *Cogent Food and Agriculture*, *1*(1). https://doi.org/10.1080/23311932.2015.1018779

Rabadán, A., Antónia Nunes, M., Bessada, S. M. F., Pardo, J. E., Beatriz Oliveira, M. P. P., & Álvarez-Ortí, M. (2020). From by-product to the food chain: Melon (cucumis melo l.) seeds as potential source for oils. *Foods*, *9*(10). https://doi.org/10.3390/foods9101341

Silva, M. A., Albuquerque, T. G., Alves, R. C., Oliveira, M. B. P. P., & Costa, H. S. (2020). Melon (Cucumis melo L.) by-products: Potential food ingredients for novel functional foods? In *Trends in Food Science and Technology* (Vol. 98). https://doi.org/10.1016/j.tifs.2018.07.005

Tir, R., Dutta, P. C., & Badjah-Hadj-Ahmed, A. Y. (2012). Effect of the extraction solvent polarity on the sesame seeds oil composition. *European Journal of Lipid Science and Technology*, *114*(12), 1427–1438. https://doi.org/10.1002/ejlt.201200129

Tuberoso, C. I. G., Kowalczyk, A., Sarritzu, E., & Cabras, P. (2007). Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. *Food Chemistry*, *103*(4). https://doi.org/10.1016/j.foodchem.2006.08.014

Tura, M., Mandrioli, M., Valli, E., Rubino, R. C., Parentela, D., & Gallina Toschi, T. (2022). Changes in the composition of a cold-pressed hemp seed oil during three months of storage. *Journal of Food Composition and Analysis*, *106*. https://doi.org/10.1016/j.jfca.2021.104270

Wang, M. L., Chen, C. Y., Tonnis, B., Barkley, N. A., Pinnow, D. L., Pittman, R. N., Davis, J., Holbrook, C. C., Stalker, H. T., & Pederson, G. A. (2013). Oil, fatty acid, flavonoid, and resveratrol content variability and FAD2A functional SNP genotypes in the U.S. peanut mini-core collection. *Journal of Agricultural and Food Chemistry*, *61*(11). https://doi.org/10.1021/jf305208e

Wu, Y., Yuan, W. Q., Han, X., Hu, J. Z., Yin, L. Q., & Lv, Z. L. (2020). Integrated analysis of fatty acid, sterol and tocopherol components of seed oils obtained from four varieties of industrial and environmental protection crops. *Industrial Crops and Products*, *154*. https://doi.org/10.1016/j.indcrop.2020.112655

Zhang, H., Yuan, Y., Zhu, X., Xu, R., Shen, H., Zhang, Q., & Ge, X. (2022). The Effect of Different Extraction Methods on Extraction Yield, Physicochemical Properties, and Volatile Compounds from Field Muskmelon Seed Oil. *Foods*, *11*(5). https://doi.org/10.3390/foods11050721

Zhang, R. Y., Liu, H. M., Ma, Y. X., & Wang, X. De. (2019). Characterization of fragrant oil extracted from pepper seed during subcritical propane extraction. *LWT*, *110*. https://doi.org/10.1016/j.lwt.2019.04.072

Zhang, R.-Y., Liu, A.-B., Liu, C., Zhu, W.-X., Chen, P.-X., Wu, J.-Z., Liu, H.-M., & Wang, X.-D. (2023). Effects of different extraction methods on the physicochemical properties and storage stability of tiger nut (Cyperus esculentus L.) oil. *LWT*, *173*, 114259. https://doi.org/10.1016/j.lwt.2022.114259

Zhang, Z. S., Wang, S., Liu, H., Li, B. Z., & Che, L. (2020). Constituents and thermal properties of milk thistle seed oils extracted with three methods. *LWT*, *126*. https://doi.org/10.1016/j.lwt.2020.109282

Zhang, Z. shan, Kang, Y. jie, & Che, L. (2019). Composition and thermal characteristics of seed oil obtained from Chinese amaranth. *LWT*, *111*. https://doi.org/10.1016/j.lwt.2019.05.007

Chapter 5. Defatted melon (*Cucumis melo L.*) seeds as a novel functional food ingredient: physicochemical and functional properties, anti-nutritional and bioactive compounds

Abstract

This study aimed to determine the chemical composition including phenolic acid profile and the antioxidant capacity, as well as the functional properties, of three varieties of defatted melon seeds (Galia, Cantaloupe, Honeydew), and compare these with defatted pumpkin seeds (as control group). The defatted melon seeds contained high level of protein (51.1% - 54.2%, w/w), dietary fibre (29.4% - 33.2%, w/w), potassium (1181.0 - 2373.1 mg/100 g), and GABA (γ-aminobutyric acid, 1.4 - 4.3 mmol/kg), whereas in terms of anti-nutritional compounds, they contained a relatively high amount of phytic acid (5.0% - 5.8%, w/w). They also exhibited good water and oil absorption capacity and emulsifying capacity. In terms of phenolics, the free phenolics (FP) fraction was the major component (75% - 77%), followed by the conjugated phenolics (CP) fraction (15% - 16%), and the bound phenolics (BP) fraction (about 8%); the antioxidant capacity of each fraction also followed the same sequence (FP > CP > BP). Considering the nutritional composition, functional properties, and the presence of potentially bioactive compounds, defatted melon seeds have considerable potential to be used as a functional food ingredient for the reformulation of foods.

5.1. Introduction

1.3 Billion tons of food by-products are estimated to be produced globally; these include, among others, plant-derived materials such as husks, seeds, stems, pulp, roots and peels (Rodríguez-García & Raghavan, 2022). Despite of their potential nutritional value (e.g. protein, polysaccharide, oil), generally, they are scarcely utilised for higher value applications, and are disposed to landfill due to the lack of sustainable management strategies (Sahin et al., 2022; Teigiserova et al., 2019). Currently the strategies for food waste management include landfilling, incineration and composting; these represent a low efficiency utilisation approach and are not considered as eco-friendly and sustainable, as they could lead to the generation of greenhouse gases (GHG), which have a negative effect for the environment (Gómez-García et al., 2021). Therefore, in order to achieve circularity across the food system, strengthen sustainability and efficient resource utilisation, food by-products and residues produced across agricultural production and food manufacture should be recovered and converted through a range of valorisation strategies to medium and high value products with applications in the food and other sectors (e.g. personal care, packaging, chemical and energy) (Comino et al., 2021; Teigiserova et al., 2019).

Melon seed (*Cucumis melo L.*) is a major by-product generated during consumption and melon processing, and constitutes 5% to 10% of the total melon weight (Silva et al., 2020). Previous studies have shown that melon seeds are rich in oil (25% - 38%, w/w) and are also high in protein (15% - 45%, w/w) and fibre (19% - 25%, w/w) (Petkova & Antova, 2015; Sahin et al., 2022; Yanty et al., 2008). The variation in the

nutritional composition of melon seeds might be associated with the variety of melons, growing conditions, and seasonal variation of harvest (Kolayli et al., 2010; Yanty et al., 2008). Due to their high oil content, particularly in unsaturated fatty acids, the focus in terms of valorisation of melon seeds has been on oil extraction (Hao et al., 2020; Mallek-Ayadi et al., 2018; Yanty et al., 2008). Defatted melon seeds is the main by-product generated after oil extraction, and which could also have potential value considering its high content in proteins, dietary fibre, and bioactive compounds. However, to date, there are no studies on the exploitation of defatted melon seeds.

It is necessary to evaluate the composition and nutritional value as well as the functional properties of defatted melon seeds in order to create value from this abundant resource. Therefore, the aim of this study was to investigate the physicochemical properties, functional properties, phenolic acid composition, and antioxidant capacity of three defatted melon seed varieties, in order to generate key knowledge for the valorisation of defatted melon seeds and its utilisation for food applications. To this end, defatted pumpkin seeds were selected as a control group for comparative purposes, since they belong to the same family (Cucurbitaceae).

5.2. Materials and Methods

5.2.1. Chemicals and standards

Methanol (HPLC grade), petroleum ether (laboratory reagent grade), sulfuric acid (96%), acetic acid (ACS reagent), ethyl acetate (GC grade), acetonitrile (HPLC grade),

and chlorogenic acid (98%) were purchased from Fisher Scientific (UK). Folin-Ciocalteu reagent, 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2- diphenyl-1-picryhydrazyl (DPPH), sodium hydroxide, sodium carbonate (≥99%), ascorbic acid (analytical reagent grade), ferric chloride (ACS reagent), oxalate (99%), xylose (≥99%, GC grade), arabinose (≥99%), glucose (≥99.5%, GC grade), vanillin (99%), catechin (≥98%, HPLC grade), caffeic acid (≥98%, HPLC grade), epicatechin (≥90%, HPLC grade), p-coumaric acid (≥98.0%, HPLC grade), protocatechuic acid (99.7%), cinnamic acid (≥9%, HPLC grade), ferulic acid, gallic acid, syringic acid (98%), sinapic (≥98%), and gentisic acid (98%) were purchased from Sigma Aldrich (UK).

5.2.2. The preparation of sample

Three commercial types of melons were used in this study, including Galia, Cantaloupe, and Honeydew. The Galia melon (from Honduras), Honeydew melon (from Brazil), Cantaloupe melon (from Brazil) were obtained (50 melons for each variety) from Sainsbury Supermarket (Reading, UK) in March 2021. Pumpkin (from UK) was obtained from Marks & Spencer (Reading, UK). All sample seeds were separated manually from the fresh fruits, and then washed to remove any flesh residual on the seeds' surface. The seeds were dried in a vacuum drier (Townson & Mercer Ltd, Croydon, UK) at 75 °C and 25 kPa for 24 h. They were then grounded in a food grinder (Caterlite CK686, Bristol, UK) and the powder passed through a 600 µm sieve. The seed powder was defatted by Soxhlet extraction with petroleum ether at 40 °C for 6 h. The defatted sample was air dried at room temperature for 18 h to remove the residual solvent. The

defatted sample was milled through a 600 µm sieve, sealed in a plastic container, and stored into the freezer at -20 °C until further analysis.

5.2.3. Proximate analysis

The proximal composition of grounded seed powder was determined by the AOAC standard methods (AOAC, 2005). More specifically, ash was determined by the AOAC 923.03 method, lipid by the AOAC 930.90 method, and protein (N x 6.25 used as the conversion factor) by the AOAC 979.09 method. The moisture content was determined using a moisture analyser (MA35 mode, Sartorius, Germany). The mineral content was determined using an atomic absorption spectrophotometer (Nov AA 350, Analytik Jena GmbH, Germany), the detailed procedure of analysis was the same as previously reported in **Chapter 3** in **Section 3.2.5**.

The carbohydrate composition was determined using the protocol by the National Renewable Energy Laboratory, NREL/TP-510-42618 (Sluiter et al., 2008). Briefly, 300 mg of sample were hydrolysed with 3 mL of (72%, v/v) H_2SO_4 and incubated at 30 °C for 1 h. Afterwards, the mixture was diluted by adding 84 mL distilled water and autoclaved at 121°C for 30 min, and then was cooled to room temperature and filtered (0.20 µm filter). The monosaccharides including glucose (derived from cellulose), xylose, and arabinose were quantified by using HPLC (Agilent, 1260 series) with an Aminex HPX-87H column (300 x 7.8 mm, Bio-Rad, California, USA); the operating conditions were as follows: injection volume 20 µL, mobile phase 0.005 M sulphuric acid, flow rate 0.6 mL/min, column temperature 65 °C. Calibration standard curves

were made using external standards [xylose (0 - 5 mg/mL, R^2 = 0.99), arabinose (0 - 5 mg/mL, R^2 = 0.99), and glucose (0 - 5 mg/mL, R^2 = 0.99)]. The acid-soluble lignin was measured using filtered acid hydrolysed sample with a UV-Vis spectrometer (BioMate 3, Thermo, UK) at 320 nm. The acid-insoluble lignin was measured by gravimetric analysis and was calculated using **Equation 5.1**.

Equation 5.1:

Acid-insoluble lignin content (g) = the solid residue after hydrolysis (g) – [ash of solid residue after hydrolysis (g) + protein content of sample (g)].

5.2.4. Anti-nutritional compounds

5.2.4.1. Phytic acid

The phytic acid content was determined using a phytic acid kit (Megazyme, Ireland) and following the manufacturer's assay procedure (Megazyme, 2017). Briefly, 1 g of sample was mixed with 20 mL of 0.66 M HCL for 3 h at room temperature. 1 mL of the extract was collected and centrifuged (Mini Spin, Eppendorf, Germany) at 13,000 rpm for 10 min. Then, 0.5 mL of the extract supernatant was mixed with 0.5 mL of 0.75 M NaOH solution for neutralisation. The neutralised sample was used to determine the phytic acid content using Phytic Acid Assay Kit (Megazyme, Ireland). The phytic acid content was calculated by using the following **Equation 5.2** (provided by Megazyme).

Equation 5.2:

$$\text{Phytic acid content} = \frac{\text{Phosphorus (g/100 g)}}{0.282}$$

5.2.4.2. Tannins

The tannins content of sample was determined according to Shawrang et al. (2011) described with slight modifications. 0.5 g of sample was extracted with 10 mL of methanol on a shake plate (Variomag Poly, Thermo) at 600 rpm for 12 h at room temperature. Then, 1.5 mL of extract was collected and centrifuged (Mini Spin, Eppendorf, Germany) at 13,000 rpm for 10 min. After this step, 1 mL of supernatant was mixed with 5 mL of freshly vanillin-HCL regent (the reagent was mixed with 4% vanillin in methanol and 8% HCL in methanol at the ratio of 1:1). The mixture was incubated at room temperature for 20 min and then the absorbance was measured at 500 nm using a UV-Vis spectrometer (BioMate 3, Thermo, UK). Catechin was used for constructing a calibration curve (0 - 100 µg/mL, R^2 = 0.99). The tannins content was expressed as mg of catechin equivalent (CE)/100 g of dry weight (DW).

5.2.5. Free amino acid analysis

The amino acid composition was determined according to Curtis et al. (2010). Briefly, 0.5 g of sample was mixed with 10 mL of 0.01 M of HCL for 15 min at room temperature. After that, 1.5 mL of mixture was collected and centrifuged (Mini Spin, Eppendorf, Germany) at 7,200 rpm for 15 min. 100 µL of supernatant was derivatised using the EZ-Faast amino acid kit (Phenomenex, UK) for Gas Chromatography and mass spectrometry analysis. The derivatised samples were analysed in electron impact mode using an Agilent -5975GC-MS system (Agilent, Santa, Clara, CA) equipped with a zebron ZB-AAA column (100 x 0.25 x 0.25). The analytical conditions were as follows: the oven temperature was held initially at 110 °C for 1 min, then increased at a rate of

30 °C/min to 310 °C; the temperature of the transfer line and ion source were kept 320 °C and 230 °C, respectively; the flow rate of the carrier gas was 1.5 mL/min and the split rate was 1:40. Amino acids were quantified from calibration curves (0 - 200 µmol/L, R^2 = 0.99) constructed using amino acid standard solutions provided with the EZ-Faast kit and the retention time of the standards were used to identify the respective amino acids peak.

5.2.6. Functional properties

5.2.6.1. Water absorption capacity and oil absorption capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) were determined as described by Teixeira et al. (2018) with slight modifications. Briefly, 0.5 g seed powder was added into a centrifuge tube (sample m1) and then 5 mL of distilled water were added for WAC and 3 mL of olive oil for OAC, respectively. The mixture was vortexed for 1 min and left standing for 30 min; it was then centrifuged (Centrifuge 5804 R, Eppendorf) for 20 min at 3,000 rpm at room temperature. The upper layer was removed and was weighted (sample m2). The WAC or OAC were expressed as the amount of water or oil per gram of sample (g of water or oil/g of sample), respectively, following the **Equation 5.3**.

Equation 5.3:

$$\text{WAC or OAC} = \frac{\text{m2} - \text{m1} - \text{sample weight}}{\text{sample weight}}$$

Where, m2 = weight of sample with absorbed water/oil plus centrifuge tube; m1 = weight of the centrifuge tube

5.2.6.2. Foaming capacity and stability

The foaming capacity (FC) and foaming stability (FS) were measured according to Embaby & Rayan (2016) with some modifications. 25 mL distilled water were added to 0.5 g seed powder and the volume recorded using a graduated cylinder (V1). The mixture was homogenised for 5 min at 6,400 rpm using an Ultra Turrax T18 digital (IKA, Germany). After homogenisation the mixture was transferred into a 50 mL graduated cylinder and the volume measured (V2). The FC (%, v/v) was calculated by the following **Equation 5.4**.

Equation 5.4:

FC (%, v/v) = (V2 – V1)/V1 X 100%

V1: volume of mixture before homogenisation; V2: volume of mixture plus the foam

The foaming stability was calculated as the ratio of the foam volume change after 30 min

Equation 5.5:

$$\text{FS (\%, v/v)} = \frac{\text{Final volume of foam}}{\text{Initial volume of foam}} \text{ x } 100\%$$

5.2.6.3. Emulsifying capacity

The emulsifying capacity (EC) was determined according to Shi et al. (2019) with some modifications. 5 mL distilled water were added to 0.5 g seed powder in a 50 mL centrifuge tube and the mixture homogenised for 30 s at 13,200 rpm using an Ultra Turrax T18 digital (IKA, Germany). Then, 5 mL olive oil were added and homogenized

again for 120 s, and the volume of the formed emulsion measured (V1). The emulsion was centrifuged for 5 min at 1100 x g and the volume of the emulsion was measured (V2). The emulsifying capacity (%, v/v) was calculated by the following **Equation 5.6**:

Equation 5.6:

EC (%, v/v) = V2/V1 x 100%

V1 = volume of formed emulsion before centrifugation; V2 = volume of emulsion after centrifugation

5.2.7. Extraction of free, conjugated, bound phenolics fraction from defatted melon seeds

5.2.7.1. Extraction of free phenolics

The extraction of the free phenolics fraction was conducted as described by Shewry & Ward (2010), with slight modifications. Briefly, 0.025 g sample powder was treated three times with 1mL of 80% (v/v) aqueous methanol in an ultrasonic bath for 10 min. Each time, the mixture was centrifuged (Mini Spin, Eppendorf, Germany) for 15 min at 5,000 rpm and then the supernatant collected and pooled together; the residue was also collected and stored at -20 ^{o}C for the analysis of bound phenolics. Subsequently, a speed vacuum concentrator (Life Technologies Ltd., Paisley, UK) was used to evaporate the sample until dryness. The dried sample was reconstituted in 100 µL of 2% (v/v) aqueous acetic acid and stored at -20 ^{o}C until further analysis.

5.2.7.2. Extraction of conjugated phenolics

The extraction of the conjugated phenolics fraction was conducted as described by Shewry & Ward (2010). Briefly, the dried sample from the free phenolics extraction process was treated with 400 µL of 2 M NaOH solution for 4 h at room temperature, for hydrolysis to occur. The hydrolysed solution was acidified to pH 2 with 12 M hydrochloric acid (80 µL). The solution was treated three times with 500 µL of ethyl acetate. Each time, the mixture was centrifuged (Mini Spin, Eppendorf, Germany) for 5 min at 13,200 rpm, and then the supernatant collected and pooled together. After that, a speed vacuum concentrator (Life Technologies Ltd., Paisley, UK) was used to evaporate the sample until dryness. The dried samples were reconstituted in 100 µL of 2% (v/v) aqueous acetic acid and then stored at -20 ^{o}C for further analysis.

5.2.7.3. Extraction of bound phenolics

The residue after the extraction of free phenolics was added to 400 µL of 2 M NaOH solution and mixed; the mixture was left for 4 h at room temperature for hydrolysis to occur. The hydrolysed solution was centrifuged (Mini Spin, Eppendorf, Germany) for 15 min at 5,000 rpm and the supernatant collected and acidified to pH 2 with 12 M HCL (80 µL). The solution was treated with 500 µL of ethyl acetate and then centrifuged (Mini Spin, Eppendorf, Germany) for 5 min at 13,200 rpm; the process was repeated three times. The upper layer was collected and evaporated to dryness using a speed vacuum concentrator (Life Technologies Ltd., Paisley, UK); the dried samples were reconstituted in 100 µL of 2% (v/v) aqueous acetic acid and then stored into -20 ^{o}C for further analysis (Shewry & Ward, 2010).

5.2.8. Determination of total phenolic content

The total phenolic content (TPC) of each of the phenolics fractions generated from the defatted melon seeds was determined according to Dudonné et al. (2009), with slightly modifications. Briefly, 200 µL of each phenolic fraction were mixed with 1 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) and then 800 µL of 7.5% sodium carbonate solution were added. The mixture was incubated for 1 h at room temperature in the dark; then the absorbance was measured at 765 nm using a UV-Vis spectrometer (BioMate 3, Thermo, UK). Gallic acid standards (0 - 10 mg/L) were used to construct a standard curve (R^2 = 0.99). The total phenolic acid content was expressed as g of gallic acid equivalent (GAE)/kg of dry weight (DW).

5.2.9. Antioxidant activity

5.2.9.1. DPPH radical scavenging assay

The DPPH (2,2- diphenyl-1-picryhydrazyl) radical scavenging assay was conducted as described by Yasir et al. (2016), with slight modifications. Briefly, 100 µL of each phenolics fraction were mixed with 1.5 mL of 0.1 mM DPPH (Sigma) solution in methanol and the mixture was left for 30 min at room temperature in the dark. The absorbance was then measured at 517 nm using a UV-Vis spectrometer (BioMate 3, Thermo, UK). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standards (0 - 600 µmol) were used to construct a standard curve (R^2 = 0.99) and the DPPH radical scavenging activity values were expressed as mmol of TE (Trolox equivalent) per kg of dry weight (DW) sample.

5.2.9.2. Ferric reducing antioxidant potential (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was conducted as described by Dudonné et al. (2009), with slight modifications. The FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6), with 1 volume of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCL, and 1 volume of 20 mM ferric chloride. Briefly, 100 µL of each phenolic fraction were mixed with 300 µL of deionized water and 3 mL of freshly made FRAP reagent. The mixture was incubated at 37°C for 30 min in a water bath, and then the absorbance was measured at 593 nm using a UV-Vis spectrometer (BioMate 3, Thermo, UK). Ascorbic acid standards (0 - 500 µmol) were used to construct a standard curve (R^2 = 0.99). The result was expressed as mmol of AA (ascorbic acid)/kg of dry weight (DW) sample.

5.2.10. Identification of phenolic compounds

Each phenolics fraction derived from the defatted melon seeds was analysed by HPLC (1260 series, Agilent Technologies, Stockport, UK) with a DAD (Diode Array Detector) and a Zorbax C18 reversed-phase column (100 x 4.6 mm), according to Lima et al. (2021). The mobile phase included two solvents: (A) 1% (v/v) aqueous acetic acid, and (B) acetonitrile. A linear gradient elution system was used: from 0 to 19 min, 95% A, 5% B; from 20 to 32 min, 85% A, 15% B; from 33 to 36 min, 50% A, 50% B; from 37 to 39 min, 30% A, 70% B, from 40 to 41 min, 0% A, 100% B; from 41 to 45 min, 95% A, 5% B. The injection volume was 10 µL, the flow rate was 1.0 mL/min, and the detector was set at 280 and 320 nm. Several phenolics [gallic acid (0 - 0.5 mg/mL, R2= 0.99),

protocatechuic acid (0 - 0.5 mg/mL, R^2 = 0.99), caffeic acid (0 - 0.5 mg/mL, R^2 = 0.99), catechin (0 - 0.5 mg/mL, R^2 = 0.99), syringic acid (0 - 0.5 mg/mL, R^2 = 0.99), epicatechin (0 - 0.5 mg/mL, R^2 = 0.99), sinapic acid (0 - 0.5 mg/mL R^2 = 0.97), chlorogenic acid (0 - 0.5 mg/mL, R^2 = 0.99), cinnamic acid (0 - 0.5 mg/mL, R^2 = 0.99), p-coumaric acid (0 - 0.5 mg/mL, R^2 = 0.99), ferulic acid (0 - 0.5 mg/mL, R^2 = 0.99), gentisic acid (0 - 0.5 mg/mL, R^2 = 0.99)]. were identified and quantified by comparing to the retention times and areas of individual standards.

5.2.11. Statistical analysis

All the experiments were carried out in triplicate unless otherwise stated. Results are expressed as mean ± standard deviation. The data were analysed using the Minitab statistical software (version 20, State College, USA). One-way analysis of variance (ANOVA) and Turkey's HSD test were used to determine differences among samples, where $p < 0.05$ was considered significantly different.

5.3. Results and Discussion

5.3.1. Proximal analysis

The proximal analysis of the three defatted melon seed varieties, Galia, Cantaloupe, and Honeydew, as well as the defatted pumpkin seeds (control), are presented in **Table 5.1**. The moisture contents of three defatted melon seeds ranged from 4.8% to 5.4% w/w, which were significantly lower than the moisture content of defatted pumpkin seed (5.8%, w/w) ($p < 0.05$). A flour moisture content lower than 14% can

prevent or minimise microbial growth and chemical deterioration during storage, potentially indicating a longer shelf life for the defatted melon seeds (Mokhtar et al., 2018). The protein content of the three defatted melon seeds ranged from 51.1% to 54.2% w/w, with only the Cantaloupe being slightly lower (51.1%, w/w) than the pumpkin seed protein content (52.3%, w/w). However, the protein content of defatted melon seeds was higher than other defatted oilseed powders such as sunflower (38%), sesame (35%), rapeseed (39%) and soybean (44%) (Grasso et al., 2019; Jia et al., 2021; Sá et al., 2022; Villalobos et al., 2016). The ash content ranged from 8.1% to 9.7% w/w, with all three melon seeds varieties having a higher ash content than defatted pumpkin seed (7.3%, w/w), indicating the presence of higher amounts of minerals. These results suggest that defatted melon seed could be considered as a potential source of protein and minerals and could be used as an ingredient for protein or mineral fortified foods. To the best of our knowledge, there is limited information on the presence of carbohydrate composition of defatted melon seeds. Three monosaccharides were present after analysis following acid hydrolysis, namely, glucose, arabinose and xylose. The glucose content of the three defatted melon seeds varieties ranged from 12.1% to 14.6% w/w (indicating the presence of cellulose and mixed linkage β-glucans), whereas the xylose plus arabinose contents ranged from 8.1% to 9.5% w/w, indicating the presence of hemicellulose polymers such as arabinoxylans. The likely hemicellulose content (xylose + arabinose) of all three defatted melon seeds was higher than that of defatted pumpkin seed (6.9%, w/w). In terms of the likely cellulose content (glucose), only Honeydew (12.1%, w/w)

was lower than defatted pumpkin seeds (12.2%, w/w) but no significant different ($p > 0.05$). In terms of the lignin content, the total lignin content (acid soluble + acid insoluble) in the three defatted melon seeds ranged from 8.4% to 9.2% w/w, and were all lower than defatted pumpkin seeds (10.1%, w/w). It has been reported that lignin is one of the most abundant natural polymers in plants, as lignin binds with cellulose and hemicellulose and provides rigidity to the plant cell wall (Agrawal et al., 2022).

In terms of minerals, potassium was the most abundant mineral in all three defatted melon seeds (1181.0 - 2273.1 mg/100 g), followed by magnesium (706.4 - 1014. mg/100 g) and calcium (149.1 - 267.8 mg/100 g). These results agree with a previous report on the mineral composition of melon seeds, where potassium and magnesium were found to be the most abundant minerals (Mallek-Ayadi et al., 2018). Considering the potassium content, it is worth highlighting that the defatted Galia melon seed had a very high content (2273.1 mg/100 g) which was almost twice that of defatted pumpkin seeds (1326.5 mg/100 g). Increasing potassium intake in the diet has many benefits for human health such as reducing the risk of cardiovascular disease and potentially preventing the development of vascular, glomerular, and tubular damage (He & MacGregor, 2008; Weaver, 2013). Moreover, the potassium content of defatted melon seeds was higher than some common potassium-rich food sources such as potato (610 mg/100 g), banana (358 mg/100 g), cod (516 mg/100 g), and dark chocolate (830 mg/100 g) (Lanham-New et al., 2012; Weaver, 2013). Therefore, it can be suggested that defatted melon seeds could be a potentially good dietary source of potassium. Overall, the compositional analysis showed that the valorisation of

defatted melon seeds can generate fractions of different compositions, functionalities and biological activities, which can find applications in the food and non-food sectors.

Table 5.1. Chemical composition of defatted Galia, Cantaloupe, Honeydew melon seeds and pumpkin seeds.

Composition (%, w/w of DW)	**Galia**	**Cantaloupe**	**Honeydew**	**Pumpkin**
Moisture	4.8 ± 0.1 ^{c}	5.4 ± 0.2 ^{b}	4.9 ± 0.1 ^{c}	5.8 ± 0.1 ^{a}
Protein	53.4 ± 0.5 ^{b}	51.1 ± 0.3 ^{d}	54.2 ± 0.0 ^{a}	52.3 ± 0.1 ^{c}
Ash	9.7 ± 0.1 ^{a}	8.3 ± 0.1 ^{b}	8.1 ± 0.2 ^{b}	7.3 ± 0.1 ^{c}
Total carbohydrate	22.3	24.1	20.2	19.1
Glucose	13.2 ± 0.2 ^{b}	14.6 ± 0.3 ^{a}	12.1 ± 0.1 ^{c}	12.2 ± 0.1 ^{c}
Xylose	8.1 ± 0.1 ^{a}	8.3 ± 0.2 ^{a}	7.1 ± 0.1 ^{b}	5.5 ± 0.1 ^{c}
Arabinose	1.0 ± 0.0 ^{c}	1.2 ± 0.0 ^{b}	1.0 ± 0.0 ^{c}	1.4 ± 0.0 ^{a}
Lignin	8.4	9.1	9.2	10.1
Acid insoluble lignin	2.9 ± 0.3 ^{b}	4.2 ± 0.2 ^{a}	3.6 ± 0.40 ^{ab}	3.3 ± 0.3 ^{b}
Acid soluble lignin	5.5 ± 0.1 ^{b}	4.9 ± 0.3 ^{c}	5.6 ± 0.1 ^{b}	6.8 ± 0.1 ^{a}
Minerals (mg/100 g of DW)				
Potassium	2273.1 ± 65.2 ^{a}	1415.7 ± 165.3 ^{b}	1181.0 ± 97.4 ^{b}	1326.5 ± 38.0 ^{b}
Magnesium	948.2 ± 12.0 ^{b}	706.4 ± 15.3 ^{c}	1014.1 ± 16.0 ^{a}	733.6 ± 4.3 ^{c}
Calcium	214.4 ± 21.0 ^{ab}	267.8 ± 27.4 ^{a}	149.1 ± 16.7 ^{c}	206.1 ± 23.6 ^{bc}
Anti-nutritional compounds				
Phytic acid (%, g/100 g of DW)	5.8 ± 0.1 ^{a}	5.0 ± 0.1 ^{b}	5.1 ± 0.1 ^{b}	4.0 ± 0.0 ^{c}
Tannins (mg CE/100 g of DW)	15.5 ± 0.0 ^{d}	18.0 ± 0.0 ^{c}	30.7 ± 0.4 ^{b}	32.9 ± 0.1 ^{a}

Data represent means ± standard deviation (n = 3). Values with the different lowercase letters in the same line are significantly different ($p < 0.05$).

5.3.2. Anti-nutritional compounds

The results from the analysis of anti-nutritional compounds, namely phytic acid and tannins, of the defatted melon seeds and defatted pumpkin seeds (control) are presented in **Table 5.1**. From a nutritional point of view, phytic acid is one of the most important anti-nutritional compounds, as it is a strong cation chelator, and can therefore reduce their bioavailability, for example for calcium, iron, and zinc (Cominelli et al., 2020). The phytic acid content of defatted melon seeds ranged from 5.0% to 5.8 % w/w, whereas in defatted pumpkin seed it was lower (4.0%, w/w). The phytic acid content of the defatted melon seeds was higher than some common cereals and legumes, such as wheat germ (3.91% w/w), oat (1.16%, w/w), and kidney bean (2.38%, w/w), but lower than some common nuts such as, walnuts (6.69%, w/w), and almond (9.42%, w/w) (Gupta et al., 2015). Overall, it could be considered that the contents are relatively high, but could be effectively reduced through processing such as thermal treatment, fermentation, and soaking (Raes et al., 2014; Rehman & Shah, 2005).

Tannins are polyphenolic compounds, which are usually considered as important anti-nutritional compounds because they can bind to proteins to form insoluble complexes and thus decrease protein digestibility (Bessada et al., 2019; Nikmaram et al., 2017). From **Table 5.1**, it can be seen that the tannins content of defatted melon seeds ranged from 15.5 to 30.7 (mg CE/100 g) - all lower than that of defatted pumpkin seeds (32.9 mg CE/100 g) - with Honeydew demonstrating the highest level. These values are significantly lower than some legumes, such as lentils (915 mg/100 g), chickpeas (770 mg/100 g), and red kidney beans (1100 mg/100 g) (Rehman & Shah, 2005), indicating

that the defatted melon seeds contain relatively low amount of tannins.

5.3.3. Free amino acids

The free amino acids of the three defatted melon seed varieties and the defatted pumpkin seeds (control) are presented in **Table 5.2**. In this study, 18 type of free amino acids were detected in the three defatted melon seeds and defatted pumpkin seeds. In terms of the total amino acid content, among the three defatted melon seeds (13.3 - 24.2 mmol/kg), the Cantaloupe seeds had the highest amount (24.2 mmol/kg) whereas the Galia melon seeds had the lowest amount (13.3 mmol/kg). Compared to the total amino acid content of defatted pumpkin seeds (46.4 mmol/kg), all three varieties of defatted melon seeds were lower than defatted pumpkin seeds. In terms of the essential amino acids, all three defatted melon seeds contain all 9 essential amino acids, with valine (0.4 - 1.0 mmol/kg), isoleucine (0.3 - 0.7 mmol/kg) and threonine (0.3 - 0.7 mmol/kg) the most abundant, whereas tryptophan (0.1 - 0.2 mmol/kg) and methionine (0.1 - 0.2 mmol/kg) the least prominent. Glutamic acid (4.7 - 8.8 mmol/kg), alanine (2.6 - 4.7 mmol/kg), glycine (1.1 - 3.0 mmol/kg), and serine (0.7 - 1.9 mmol/kg) were the most abundant amino acids in defatted melon seeds sample. These amino acids can contribute towards flavour (Wyllie et al., 1995). Glycine, serine, and alanine can contribute sweet taste, whereas glutamic acid can provide umami taste to the defatted melon seeds (Chen & Zhang, 2007).

GABA (γ-aminobutyric acid) is a non-protein amino acid neurotransmitter which has received increased attention due to its physiological functions such as maintaining

mental health, reducing stress and blood pressure, and enhancing brain protein synthesis (Diana et al., 2014; Nikmaram, Dar, et al., 2017; Poojary et al., 2017). According to data in **Table 5.2**, the GABA content of defatted melon seeds ranged from 1.4 mmol/kg to 4.3 mmol/kg, with Cantaloupe having the highest amount and Galia the lowest. Compared with the GABA content of defatted pumpkin seeds (3.1 mmol/kg), the Cantaloupe seeds had a higher content. Oh et al. (2003) reported the GABA contents in commercial cereals including corn (0.20 mmol/kg), barley (0.19 mmol/kg), and brown rice (0.12 mmol/kg). The GABA contents of all defatted melon seeds were higher than Oh et al. (2003) report, indicating that the defatted melon seeds could be used as a good source of GABA in everyday diet.

Table 5.2. Amino acid profile (mmol/kg of DW) of defatted Galia, Cantaloupe, Honeydew, and pumpkin seeds.

Amino acids	Galia	Cantaloupe	Honeydew	Pumpkin
Essential				
Methionine	0.2 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	0.3 ± 0.1 ^{a}
Phenylalanine	0.4 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	0.5 ± 0.1 ^{b}	1.8 ± 0.4 ^{a}
Valine	0.4 ± 0.0 ^{c}	1.0 ± 0.1 ^{b}	0.9 ± 0.0 ^{bc}	2.6 ± 0.4 ^{a}
Leucine	0.2 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	0.5 ± 0.1 ^{b}	2.5 ± 0.4 ^{a}
Isoleucine	0.3 ± 0.0 ^{b}	0.7 ± 0.0 ^{b}	0.7 ± 0.1 ^{b}	3.2 ± 0.5 ^{a}
Threonine	0.3 ± 0.0 ^{c}	0.6 ± 0.1 ^{bc}	0.7 ± 0.1 ^{b}	1.4 ± 0.2 ^{a}
Lysine	0.2 ± 0.0 ^{b}	0.3 ± 0.1 ^{b}	0.3 ± 0.0 ^{b}	1.3 ± 0.3 ^{a}
Histidine	0.3 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	0.4 ± 0.0 ^{b}	2.0 ± 0.1 ^{a}
Tryptophan	0.1 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	1.1 ± 0.1 ^{a}
Non-essential				
Aspartic acid	0.4 ± 0.0 ^{b}	0.4 ± 0.1 ^{b}	0.7 ± 0.1 ^{b}	3.8 ± 0.4 ^{a}
Alanine	2.6 ± 0.0 ^{c}	4.7 ± 0.5 ^{b}	3.2 ± 0.1 ^{bc}	7.6 ± 1.1 ^{a}
Glutamic acid	4.7 ± 0.1 ^{b}	8.8 ± 1.3 ^{a}	8.7 ± 0.5 ^{a}	7.5 ± 0.7 ^{a}
Glutamine	0.4 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.3 ± 0.1 ^{b}	1.4 ± 0.5 ^{a}
Glycine	1.1 ± 0.1 ^{c}	2.1 ± 0.2 ^{b}	3.0 ± 0.1 ^{a}	2.6 ± 0.5 ^{ab}
Serine	0.7 ± 0.1 ^{c}	1.9 ± 0.4 ^{b}	1.2 ± 0.2 ^{bc}	2.8 ± 0.5 ^{a}
Proline	0.1 ± 0.0 ^{c}	0.4 ± 0.0 ^{b}	0.6 ± 0.0 ^{b}	0.9 ± 0.1 ^{a}
Asparagine	0.6 ± 0.1 ^{b}	0.8 ± 0.2 ^{b}	1.0 ± 0.1 ^{b}	2.0 ± 0.3 ^{a}
Tyrosine	0.3 ± 0.0 ^{b}	0.5 ± 0.1 ^{b}	0.4 ± 0.0 ^{b}	1.6 ± 0.2 ^{a}
Total	13.3	24.2	23.3	46.4
γ-aminobutyric acid	1.4 ± 0.1 ^{c}	4.3 ± 0.9 ^{a}	3.0 ± 0.2 ^{b}	3.1 ± 0.4 ^{b}

Data represent means ± standard deviation (n = 3). Values with the different lowercase letters in the same row are significantly different ($p < 0.05$).

5.3.4. Functional properties

The results from the analysis of the functional properties of defatted melon seeds are presented in **Table 5.3**.

5.3.4.1. Water absorption capacity (WAC)

Water absorption capacity is an important property for assessing novel ingredients, particularly fibrous materials, in terms of their functionalities within food matrices. The water absorption capacity (WAC) of the three varieties of defatted melon seed powders varied from 1.6 to 1.9 g/g, with Galia having the lowest and Cantaloupe the highest. The WAC of all three varieties were lower than defatted pumpkin seed powder (2.5 g/g), but higher than some legume flours, such as chickpea flour (1.2 g/g) and lentil flour (1.3 g/g) (Du et al., 2014). The relatively high WAC could be due to the protein (51.1% - 54.2%, w/w) and carbohydrate (20.2% - 24.1%, w/w) contents (particularly polysaccharides), which being hydrophilic have high affinity for water molecules (Mokhtar et al., 2018; Ofori et al., 2020). Joshi et al. (2015) and Rodríguez-Miranda et al. (2012) reported that defatting could improve the water absorption capacity, especially for powders from seeds with high lipid content; it was suggested that some hydrophilic groups within proteins or carbohydrates could be blocked in a lipophilic environment, thus, defatting can expose more hydrophilic groups and bind with water. Considering potential food applications of melon seed powders (or its fractions), the levels of water absorption capacity could influence the product texture, mouth feel and viscosity, which play an important role in bakery and meat products (Ghanghas et al., 2020).

5.3.4.2. Oil absorption capacity (OAC)

The oil absorption capacity (OAC) of the three varieties of defatted melon seed powders ranged from 1.9 to 2.1 g/g; the OAC of the three melon seeds were similar to that defatted pumpkin seed powder (2.0 g/g), but higher than some legume or cereal flours, such as rice flour (0.8 g/g) and chickpea flour (0.9 g/g) (Joshi et al., 2015). The higher OAC is most likely associated with the number of nonpolar sites in protein (Rodríguez-Miranda et al., 2012); in addition, the secondary structure of protein could also affect the OAC (Nwokocha et al., 2023). The OAC can be an important parameter because it can influence flavour and texture (Sridaran et al., 2012). Therefore, it is likely that the defatted melon seed powder could perform well if used in meat product formulations (e.g. sausage) and bakery products (e.g. cookies).

5.3.4.3. Foaming capacity (FC) and Foaming stability (FS)

The foaming capacity (FC) of the three varieties of defatted melon seed powders ranged from 4.1% to 11.5%. Compared with the FC of the defatted pumpkin seed powder (9.0%), the FC of the defatted Cantaloupe seed powder and defatted Honeydew melon seed powder were lower than that of the defatted pumpkin seed powder, but the FC of the defatted Galia seed powder was higher than that of the pumpkin seed powder. The foaming stability (FS) is an important parameter to assess the potential of foaming agent (Cheng & Bhat, 2016). In terms of FS, all defatted melon seed powders had a high FS (76.3% - 80.8%); only Galia (76.3%) was lower than defatted pumpkin seeds (78.3%). The above results suggest that all defatted melon

seed powders have a good ability to maintain a strong air-water film for a long time, which is most likely associated with their very high protein content. The FS usually depends on the interfacial film formed by proteins; protein can absorb to air-water interfaces and form strong and cohesive viscoelastic films, which can maintain air bubble suspension and slow down the rate of coalescence (Rodríguez-Miranda et al., 2012). The high FS of defatted melon seeds are expected to contribute key functionalities when the seeds are incorporated in bakery and confectionery products.

5.3.4.4. Emulsifying capacity (EC)

The emulsifying capacity (EC) of food ingredients is an important property for food applications such as ice cream and bakery (Sridaran et al., 2012). The EC of three varieties of defatted melon seed powder ranged from 47.5% to 50.7%; only the EC of defatted honeydew seed powder (47.5%) was lower than the EC of defatted pumpkin seed powder (49.3%). In the case of the defatted melon seeds, the relatively high EC can probably be attributed to their protein and polysaccharide content; proteins and polysaccharides can promote the formation of stable oil/water interfaces due to the presence of both non-polar and polar groups (Fasasi et al., 2007; Ghanghas et al., 2020). Recent studies have suggested that some emulsifiers (e.g. carrageenan, polysorbate 80, carboxymethylcellulose) may have adverse effects on human health, such as gastrointestinal diseases and metabolic syndrome conditions (Chassaing et al., 2017; Cox et al., 2021). To this end, food without the use of artificial additives has attracted considerable attention by consumers over recent years. As a result, there is a considerable drive by the food industry for ‘clean label’ natural ingredients that can

be used to replace many synthetic ingredients (Li et al., 2023). Overall, these results indicate that that melon seed powders are natural good emulsifying agents and could be potentially used in bakery products.

Table 5.3. Functional properties of defatted Galia, Cantaloupe and Honeydew melon seeds, and pumpkin seeds.

Sample	WAC (g/g)	OAC (g/g)	FC (%)	FS (%)	EC (%)
Galia	1.6 ± 0.0 ^{c}	2.1 ± 0.1 ^{a}	11.5 ± 0.6 ^{a}	76.3 ± 4.4 ^{a}	49.7 ± 0.6 ^{ab}
Cantaloupe	1.9 ± 0.1 ^{b}	1.9 ± 0.0 ^{a}	4.1 ± 0.4 ^{c}	80.7 ± 4.4 ^{a}	50.7 ± 1.3 ^{a}
Honeydew	1.8 ± 0.1 ^{bc}	2.0 ± 0.1 ^{a}	7.8 ± 0.2 ^{b}	80.8 ± 1.4 ^{a}	47.5 ± 1.3 ^{b}
Pumpkin	2.5 ± 0.1 ^{a}	2.0 ± 0.1 ^{a}	9.0 ± 2.3 ^{ab}	78.3 ± 1.9 ^{a}	49.3 ± 1.3 ^{ab}

Data represent means ± standard deviation (n = 3). Values with the different lowercase letters in the same column are significantly different ($p < 0.05$). WAC - Water absorption capacity; OAC - Oil absorption capacity; FC - Foaming capacity; FS - Foaming stability; EC - Emulsifying capacity.

5.3.5. Total phenolic content and antioxidant capacity

5.3.5.1. Total phenol content (TPC)

The total phenolic acid content (TPC) of the three phenolic fractions, namely the free phenolics fraction (FP), conjugated phenolics fraction (CP) and bound phenolics fraction (BP) of the three varieties of defatted melon seeds and of the defatted pumpkin seeds (control) are shown in **Table 5.4**. Free phenolics are soluble in polar organic solvents (e.g. methanol) and can be extracted by a simple step; conjugated and bound phenolics to the cell wall structural components, such as cellulose, protein, and lignin, require more complex extraction steps (e.g. acid or alkaline hydrolysis) in order to be released (Gao et al., 2017; Robbins, 2003). To the best of our knowledge, this is the first report on the phenolic profile of defatted melon seeds. The total TPC (free + conjugated + bound) of defatted melon seeds ranged from 1.2 to 1.3 g GAE/kg, with Cantaloupe having the highest amount. Additionally, compared with the total TPC of defatted pumpkin seed (2.2 g GAE/kg), all three defatted melon seeds had lower TPC than defatted pumpkin seed. For all defatted seeds in this study, the order was as follows: FP > CP > BP.

5.3.5.2. Antioxidant capacity

Data in **Table 5.4** show the results of antioxidant activity by DPPH and FRAP assays for the three fractions of phenolic acids of the three varieties of defatted melon seed as well as of the defatted pumpkin seed (control). The DPPH and FRAP results for all four defatted seed also followed the order FP > CP > BP, in line with the TPC results. Previous

reports indicated that antioxidant capacity correlated well with the total phenolics content for rice brans and pitahaya peel (Pang et al., 2018; Tang et al., 2021). Similar good correlation, i.e of DPPH and FRAP with TPC, was observed in the present study (**Table 5.4**) (R^2 = 0.92, R^2 = 0.96, respectively). It is important to note that for the free phenolics fraction of the three defatted melon seeds, Honeydew exhibited the highest antioxidant capacity using the DPPH assay but lowest antioxidant capacity using the FRAP assay. A similar result was observed by de Oliveira Schmidt et al. (2020) for the antioxidant capacity of feijoa and cherry fruit, indicating that the differences in the results from the DPPH and the FRAP assay could be associated with different phenolics profiles. The antioxidant action of phenolic compounds depends on their chemical structures and the number of functional groups, and the reaction mechanisms of each antioxidant assay is different, hence phenolic compounds can be seen to behave differently in various antioxidant assays (de Oliveira Schmidt et al., 2020; Pang et al., 2018; Zhong et al., 2022).

Table 5.4. The total phenolic content (TPC) and antioxidant capacity (DPPH and FRAP) of defatted Galia, Cantaloupe and Honeydew melon seeds, and pumpkin seeds.

Sample	FP	CP	BP	Total
Total phenolic acid content (TPC) (g GAE/kg of DW)				
Galia	0.9 ± 0.0 [b]	0.2 ± 0.0 [b]	0.1 ± 0.0 [b]	1.2
Cantaloupe	1.0 ± 0.0 [b]	0.2 ± 0.0 [b]	0.1 ± 0.0 [b]	1.3
Honeydew	1.0 ± 0.1 [b]	0.2 ± 0.0 [b]	0.1 ± 0.0 [b]	1.3
Pumpkin	1.7 ± 0.1 [a]	0.4 ± 0.1 [a]	0.2 ± 0.0 [a]	2.3
(2,2-Diphenyl-1-picrylhydrazyl) (DPPH) radical scavenging ability (mmol TE/kg of DW)				
Galia	2.5 ± 0.1 [b]	1.8 ± 0.1 [b]	1.5 ± 0.0 [c]	5.9
Cantaloupe	2.7 ± 0.0 [b]	2.1 ± 0.1 [a]	1.7 ± 0.0 [b]	6.4
Honeydew	2.8 ± 0.1 [b]	2.1 ± 0.1 [a]	1.6 ± 0.0 [c]	6.6
Pumpkin	4.4 ± 0.2 [a]	2.2 ± 0.1 [a]	1.9 ± 0.0 [a]	8.5
Ferric reducing antioxidant capacity (FRAP) (mmol AA/kg of DW)				
Galia	5.8 ± 0.5 [c]	1.7 ± 0.1 [b]	0.5 ± 0.1 [c]	8.0
Cantaloupe	7.8 ± 0.7 [b]	1.4 ± 0.2 [b]	1.2 ± 0.0 [b]	10.4
Honeydew	5.5 ± 0.2 [c]	1.7 ± 0.2 [b]	1.0 ± 0.2 [b]	8.2
Pumpkin	12.2 ± 1.2 [a]	2.3 ± 0.1 [a]	2.2 ± 0.2 [a]	16.7

Correlation (R^2)	DPPH	FRAP
TPC	0.92	0.96

Data represent means ± standard deviation (n = 3). Values with the different lowercase letters in the same column are significantly different ($p < 0.05$). FP - free phenolics fraction; CP - conjugated phenolics fraction; BP - bound phenolics fraction.

5.3.6. The profile of phenolic acids

Data in **Table 5.5** show the phenolic acid profile (free, conjugated, and bound) present in the defatted melon seeds and pumpkin seeds. Twelve phenolic compounds (gallic acid, protocatechuic acid, caffeic acid, catechin, syringic acid, epicatechin, sinapic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, ferulic acid, gentisic acid) were quantified in this study. Gallic acid (5.7 - 8.4 mg/100 g), protocatechuic acid (4.0 - 4.9 mg/100 g) and caffeic acid (1.9 - 3.9 mg/100 g) were found in the free phenolics fraction for all three defatted melon seeds. This result agrees with Mallek-Ayadi et al. (2019), Zeb (2016), and Kolayli et al. (2010) who demonstrated that gallic acid, protocatechuic acid and caffeic acid are the most common phenolic compounds in the free phenolics fraction of melon seeds. Catechin was only found in defatted Honeydew melon seeds (7.4 mg/100 g), which was higher than the observations by Zeb (2016) for Honeydew melon seeds (5.4 mg/100 g). Additionally, defatted Cantaloupe and Honeydew seeds contained high amount of epicatechin (20.6 mg/100 g and 20.5 mg/100 g, respectively), and these were slightly lower than in defatted pumpkin seeds (22.8 mg/100 g). Chlorogenic acid was found in defatted Cantaloupe and Honeydew seeds, but syringic acid and *p*-coumaric acid were only found in defatted Cantaloupe seeds. This could be attributed to differennces in variety, region, soil conditions, growing condition, harvest times, and degree of maturity at harvest (Wang et al., 2012; Zadernowski et al., 2009; Zhao et al., 2021). Moreover, high amount of gentisic acid was also found, but only in the defatted Cantaloupe seeds (18.5 mg/100 g); this was not significantly different ($p > 0.05$) to the amount found in defatted pumpkin seeds (19.0 mg/100 g).

In terms of the profiles of conjugated and bound phenolics in melon seeds, to our knowledge, this is first report detecting this. Regarding the conjugated phenolics, a small amount of caffeic acid was found in defatted Cantaloupe (1.3 mg/100 g) and Honeydew (3.4 mg/100 g) seeds. Moreover, protocatechuic acid, catechin, epicatechin and chlorogenic acid were only found in defatted Honeydew melon seeds. The defatted Galia seeds did not contain any conjugated phenolic compounds, highlighting the potential differences between varieties. Regarding the bound phenolics, none of the 12 phenolic compounds were detected in the defatted melon seeds. This result could suggest that the bound phenolics in defatted melon could include other phenolic compounds although further research is need to elucidate this. Overall, these results show that the main form of phenolics in defatted melon seeds are free phenolics, hence the seeds could be used as good food source of such bioactive compounds.

Table 5.5. Free, conjugated, bound phenolics content (mg/100 g of DW) in defatted Galia, Cantaloupe, Honeydew melon seeds, and pumpkin seeds.

Compound	Galia	Cantaloupe	Honeydew	Pumpkin
Free				
Gallic acid	5.7 ± 0.1 [b]	5.8 ± 0.0 [b]	8.4 ± 0.8 [a]	5.4 ± 0.0 [b]
Protocatechuic acid	4.0 ± 0.2 [b]	4.2 ± 0.2 [ab]	4.9 ± 0.8 [ab]	5.2 ± 0.0 [a]
Caffeic acid	2.4 ± 0.2 [a]	2.7 ± 0.5 [a]	1.9 ± 0.1 [a]	2.1 ± 0.7 [a]
Catechin	ND	ND	7.4 ± 0.8 [a]	9.7 ± 3.8 [a]
Syringic acid	ND	1.5 ± 0.1 [a]	ND	1.0 ± 0.5 [a]
Epicatechin	ND	20.6 ± 1.5 [a]	20.5 ± 2.4 [a]	22.8 ± 0.9 [a]
Sinapic acid	ND	ND	ND	ND
Chlorogenic acid	ND	5.3 ± 0.6 [a]	3.7 ± 0.5 [b]	ND
Cinnamic acid	ND	ND	ND	1.0 ± 0.0
P-coumaric acid	ND	1.2 ± 0.1	ND	ND
Ferulic acid	ND	ND	ND	ND
Gentisic acid	ND	18.5 ± 1.0 [a]	ND	19.0 ± 0.3 [a]
Conjugated				
Protocatechuic acid	ND	ND	1.6 ± 0.4 [a]	1.9 ± 0.1 [a]
Caffeic acid	ND	1.3 ± 0.0 [b]	3.4 ± 1.5 [a]	ND
Catechin	ND	ND	5.3 ± 2.0 [a]	5.9 ± 0.7 [a]
Epicatechin	ND	ND	9.4 ± 1.9	ND
Chlorogenic acid	ND	ND	2.2 ±0.5	ND
Bound				
Protocatechuic acid	ND	ND	ND	2.6 ± 0.1
Caffeic acid	ND	ND	ND	1.2 ± 0.1
Total Phenolics	12.1	61.1	68.7	77.8

Data represent means ± standard deviation (n = 3). Values with the different lowercase letters in the same column are significantly different ($p < 0.05$). ND: not detected.

5.4. Conclusions

Defatted melon seeds are a good source of protein, dietary fibre, minerals and GABA, and could be used for the fortification of foods. Moreover, free phenolic acids are the major phenolic acid component in defatted melon seeds, and are present at considerable amounts. Additionally, defatted melon seed powder has good water and oil absorption capacity as well as good emulsifying capacity, which could enhance its potential application and added value in food formulations. Although defatted melon seeds contain a relatively high level of phytic acid, which could limit nutrient absorption, this limiting factor could be potential improved through processing. Overall, this study contributed new knowledge on the composition and physicochemical properties of defatted melon seeds and supports the concept of their valorisation.

5.5 References

Agrawal, R., Kumar, A., Singh, S., & Sharma, K. (2022). Recent advances and future perspectives of lignin biopolymers. In *Journal of Polymer Research* (Vol. 29, Issue 6). https://doi.org/10.1007/s10965-022-03068-5

AOAC. (2005). Official Methods of Analysis of AOAC International. In *Association of Official Analysis Chemists International*.

Bessada, S. M. F., Barreira, J. C. M., & Oliveira, M. B. P. P. (2019). Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. In *Trends in Food Science and Technology* (Vol. 93). https://doi.org/10.1016/j.tifs.2019.08.022

Chassaing, B., Van De Wiele, T., De Bodt, J., Marzorati, M., & Gewirtz, A. T. (2017). Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation. *Gut*, *66*(8). https://doi.org/10.1136/gutjnl-2016-313099

Chen, D. W., & Zhang, M. (2007). Non-volatile taste active compounds in the meat of Chinese mitten crab (Eriocheir sinensis). *Food Chemistry*, *104*(3). https://doi.org/10.1016/j.foodchem.2007.01.042

Cheng, Y. F., & Bhat, R. (2016). Functional, physicochemical and sensory properties of novel cookies produced by utilizing underutilized jering (Pithecellobium jiringa Jack.) legume flour. *Food Bioscience*, *14*. https://doi.org/10.1016/j.fbio.2016.03.002

Cominelli, E., Pilu, R., & Sparvoli, F. (2020). Phytic acid and transporters: What can we learn from Low phytic acid mutants. In *Plants* (Vol. 9, Issue 1). https://doi.org/10.3390/plants9010069

Comino, E., Dominici, L., & Perozzi, D. (2021). Do-it-yourself approach applied to the valorisation of a wheat milling industry's by-product for producing bio-based material. *Journal of Cleaner Production*, *318*. https://doi.org/10.1016/j.jclepro.2021.128267

Cox, S., Sandall, A., Smith, L., Rossi, M., & Whelan, K. (2021). Food additive emulsifiers: A review of their role in foods, legislation and classifications, presence in food supply, dietary exposure, and safety assessment. In *Nutrition Reviews* (Vol. 79, Issue 6). https://doi.org/10.1093/nutrit/nuaa038

Curtis, T. Y., Powers, S. J., Balagiannis, D., Elmore, J. S., Mottram, D. S., Parry, M. A. J., Rakszegi, M., Bedö, Z., Shewry, P. R., & Halford, N. G. (2010). Free amino acids and sugars in rye grain: Implications for acrylamide formation. *Journal of Agricultural and Food Chemistry*, *58*(3). https://doi.org/10.1021/jf903577b

de Oliveira Schmidt, H., Rockett, F. C., Klen, A. V. B., Schmidt, L., Rodrigues, E., Tischer, B., Augusti, P. R., de Oliveira, V. R., da Silva, V. L., Flôres, S. H., & de O. Rios, A. (2020). New insights into the phenolic compounds and antioxidant capacity of feijoa and cherry fruits cultivated in Brazil. *Food Research International*, *136*. https://doi.org/10.1016/j.foodres.2020.109564

Diana, M., Quílez, J., & Rafecas, M. (2014). Gamma-aminobutyric acid as a bioactive compound in foods: A review. In *Journal of Functional Foods* (Vol. 10). https://doi.org/10.1016/j.jff.2014.07.004

Du, S. kui, Jiang, H., Yu, X., & Jane, J. lin. (2014). Physicochemical and functional properties of whole legume flour. *LWT*, *55*(1). https://doi.org/10.1016/j.lwt.2013.06.001

Dudonné, S., Vitrac, X., Coutiére, P., Woillez, M., & Mérillon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, *57*(5). https://doi.org/10.1021/jf803011r

Embaby, H. E., & Rayan, A. M. (2016). Chemical composition and nutritional evaluation of the seeds of Acacia tortilis (Forssk.) Hayne ssp. raddiana. *Food Chemistry*, *200*. https://doi.org/10.1016/j.foodchem.2016.01.019

Fasasi, O. S., Eleyinmi, A. F., & Oyarekua, M. A. (2007). Effect of some traditional processing operations on the functional properties of African breadfruit seed (Treculia africana) flour. *LWT - Food Science and Technology*, *40*(3). https://doi.org/10.1016/j.lwt.2005.11.009

Gao, Y., Ma, S., Wang, M., & Feng, X. Y. (2017). Characterization of free, conjugated, and bound phenolic acids in seven commonly consumed vegetables. *Molecules*, *22*(11). https://doi.org/10.3390/molecules22111878

Ghanghas, N., M. T, M., Sharma, S., & Prabhakar, P. K. (2020). Classification, Composition, Extraction, Functional Modification and Application of Rice (Oryza sativa) Seed Protein: A Comprehensive Review. In *Food Reviews International*. https://doi.org/10.1080/87559129.2020.1733596

Gómez-García, R., Campos, D. A., Aguilar, C. N., Madureira, A. R., & Pintado, M. (2021). Valorisation of food agro-industrial by-products: From the past to the present and perspectives. *Journal of Environmental Management*, *299*. https://doi.org/10.1016/j.jenvman.2021.113571

Grasso, S., Omoarukhe, E., Wen, X., Papoutsis, K., & Methven, L. (2019). The use of upcycled defatted sunflower seed flour as a functional ingredient in biscuits. *Foods*, *8*(8). https://doi.org/10.3390/foods8080305

Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. In *Journal of Food Science and Technology* (Vol. 52, Issue 2). https://doi.org/10.1007/s13197-013-0978-y

Hao, W., Zhu, H., Chen, J., Kwek, E., He, Z., Liu, J., Ma, N., Ma, K. Y., & Chen, Z. Y. (2020). Wild Melon Seed Oil Reduces Plasma Cholesterol and Modulates Gut Microbiota in Hypercholesterolemic Hamsters. *Journal of Agricultural and Food Chemistry*, *68*(7). https://doi.org/10.1021/acs.jafc.9b07302

He, F. J., & MacGregor, G. A. (2008). Beneficial effects of potassium on human health. *Physiologia Plantarum*, *133*(4). https://doi.org/10.1111/j.1399-3054.2007.01033.x

Jia, W., Rodriguez-Alonso, E., Bianeis, M., Keppler, J. K., & van der Goot, A. J. (2021). Assessing functional properties of rapeseed protein concentrate versus isolate for food applications. *Innovative Food Science and Emerging Technologies*, *68*. https://doi.org/10.1016/j.ifset.2021.102636

Joshi, A. U., Liu, C., & Sathe, S. K. (2015). Functional properties of select seed flours. *LWT*, *60*(1). https://doi.org/10.1016/j.lwt.2014.08.038

Kolayli, S., Kara, M., Tezcan, F., Erim, F. B., Sahin, H., Ulusoy, E., & Aliyazicioglu, R. (2010).

Comparative study of chemical and biochemical properties of different melon cultivars: Standard, hybrid, and grafted melons. *Journal of Agricultural and Food Chemistry*, *58*(17). https://doi.org/10.1021/jf102408y

Lanham-New, S. A., Lambert, H., & Frassetto, L. (2012). Potassium. In *Advances in Nutrition* (Vol. 3, Issue 6). https://doi.org/10.3945/an.112.003012

Li, Z., Anankanbil, S., Pedersen, J. N., Nadzieja, M., & Guo, Z. (2023). Nanocellulose fractionated from TEMPO-mediated oxidation of cellulose as an energy-free ingredient for stabilizing Pickering emulsion. *Biochemical Engineering Journal*, *191*. https://doi.org/10.1016/j.bej.2022.108795

Lima, M. de A., Andreou, R., Charalampopoulos, D., & Chatzifragkou, A. (2021). Supercritical carbon dioxide extraction of phenolic compounds from potato (Solanum tuberosum) peels. *Applied Sciences (Switzerland)*, *11*(8). https://doi.org/10.3390/app11083410

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2018). Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. *Process Safety and Environmental Protection*, *113*. https://doi.org/10.1016/j.psep.2017.09.016

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2019). Phytochemical profile, nutraceutical potential and functional properties of Cucumis melo L. seeds. *Journal of the Science of Food and Agriculture*, *99*(3). https://doi.org/10.1002/jsfa.9304

Megazyme. (2017). *Phytic Acid (phytate)/Total Phosphorus*. Megazyme. https://www.megazyme.com/documents/Booklet/K-PHYT_DATA.pdf

Mokhtar, S. M., Swailam, H. M., & Embaby, H. E. S. (2018). Physicochemical properties, nutritional value and techno-functional properties of goldenberry (Physalis peruviana) waste powder concise title: Composition of goldenberry juice waste. *Food Chemistry*, *248*. https://doi.org/10.1016/j.foodchem.2017.11.117

Nikmaram, N., Dar, B. N., Roohinejad, S., Koubaa, M., Barba, F. J., Greiner, R., & Johnson, S. K. (2017). Recent advances in γ-aminobutyric acid (GABA) properties in pulses: an overview. In *Journal of the Science of Food and Agriculture* (Vol. 97, Issue 9). https://doi.org/10.1002/jsfa.8283

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., Oey, I., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. In *Food Control* (Vol. 79). https://doi.org/10.1016/j.foodcont.2017.03.027

Nwokocha, B. C., Chatzifragkou, A., & Fagan, C. C. (2023). Impact of Ultrasonication on African Oil Bean (Pentaclethra macrophylla Benth) Protein Extraction and Properties. *Foods*, *12*(8), 1627. https://doi.org/10.3390/foods12081627

Ofori, J., Tortoe, C., & Agbenorhevi, J. K. (2020). Physicochemical and functional properties of dried okra (Abelmoschus esculentus L.) seed flour. *Food Science and Nutrition*, *8*(8). https://doi.org/10.1002/fsn3.1725

Oh, S.-H., Moon, Y.-J., & Oh, C.-H. (2003). γ -Aminobutyric Acid (GABA) Content of Selected Uncooked Foods. *Preventive Nutrition and Food Science*, *8*(1). https://doi.org/10.3746/jfn.2003.8.1.075

Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y., & Bao, J. (2018). Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice.

Food Chemistry, *240*. https://doi.org/10.1016/j.foodchem.2017.07.095

Petkova, Z., & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (Cucumis melo L.) cultivated in Bulgaria. *Cogent Food and Agriculture*, *1*(1). https://doi.org/10.1080/23311932.2015.1018779

Poojary, M. M., Dellarosa, N., Roohinejad, S., Koubaa, M., Tylewicz, U., Gómez-Galindo, F., Saraiva, J. A., Rosa, M. D., & Barba, F. J. (2017). Influence of Innovative Processing on γ-Aminobutyric Acid (GABA) Contents in Plant Food Materials. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5). https://doi.org/10.1111/1541-4337.12285

Raes, K., Knockaert, D., Struijs, K., & Van Camp, J. (2014). Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. In *Trends in Food Science and Technology* (Vol. 37, Issue 1). https://doi.org/10.1016/j.tifs.2014.02.002

Rehman, Z. U., & Shah, W. H. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry*, *91*(2). https://doi.org/10.1016/j.foodchem.2004.06.019

Robbins, R. J. (2003). Phenolic acids in foods: An overview of analytical methodology. In *Journal of Agricultural and Food Chemistry* (Vol. 51, Issue 10). https://doi.org/10.1021/jf026182t

Rodríguez García, S. L., & Raghavan, V. (2022). Green extraction techniques from fruit and vegetable waste to obtain bioactive compounds—A review. In *Critical Reviews in Food Science and Nutrition* (Vol. 62, Issue 23). https://doi.org/10.1080/10408398.2021.1901651

Rodríguez-Miranda, J., Hernández-Santos, B., Herman-Lara, E., Vivar-Vera, M. A., Carmona-García, R., Gómez-Aldapa, C. A., & Martínez-Sánchez, C. E. (2012). Physicochemical and functional properties of whole and defatted meals from Mexican (Cucurbita pepo) pumpkin seeds. *International Journal of Food Science and Technology*, *47*(11). https://doi.org/10.1111/j.1365-2621.2012.03102.x

Sá, A. G. A., Pacheco, M. T. B., Moreno, Y. M. F., & Carciofi, B. A. M. (2022). Cold-pressed sesame seed meal as a protein source: Effect of processing on the protein digestibility, amino acid profile, and functional properties. *Journal of Food Composition and Analysis*, *111*, 104634. https://doi.org/10.1016/j.jfca.2022.104634

Sahin, E., Erem, E., Güzey, M., Kesen, M. S., Icyer, N. C., Ozmen, D., Toker, O. S., & Cakmak, H. (2022). High potential food wastes: Evaluation of melon seeds as spreadable butter. *Journal of Food Processing and Preservation*, *46*(10). https://doi.org/10.1111/jfpp.16841

Shawrang, P., Sadeghi, A. A., Behgar, M., Zareshahi, H., & Shahhoseini, G. (2011). Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chemistry*, *125*(2). https://doi.org/10.1016/j.foodchem.2010.09.010

Shewry, P., & Ward, J. (2010). HEALTHGRAIN Methods: Analysis of Bioactive Components in Small Grain Cereals. In *HEALTHGRAIN Methods: Analysis of Bioactive Components in Small Grain Cereals*.

Shi, M., Hlaing, M. M., Ying, D. Y., Ye, J. H., Sanguansri, L., & Augustin, M. A. (2019). New food ingredients from broccoli by-products: physical, chemical and technological properties.

International Journal of Food Science and Technology, *54*(4). https://doi.org/10.1111/ijfs.14111

Silva, M. A., Albuquerque, T. G., Alves, R. C., Oliveira, M. B. P. P., & Costa, H. S. (2020). Melon (Cucumis melo L.) by-products: Potential food ingredients for novel functional foods? In *Trends in Food Science and Technology* (Vol. 98). https://doi.org/10.1016/j.tifs.2018.07.005

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of structural carbohydrates and lignin in Biomass - NREL/TP-510-42618. In *National Renewable Energy Laboratory*.

Sridaran, A., Karim, A. A., & Bhat, R. (2012). Pithecellobium jiringa legume flour for potential food applications: Studies on their physico-chemical and functional properties. *Food Chemistry*, *130*(3). https://doi.org/10.1016/j.foodchem.2011.07.062

Tang, W., Li, W., Yang, Y., Lin, X., Wang, L., Li, C., & Yang, R. (2021). Phenolic compounds profile and antioxidant capacity of pitahaya fruit peel from two red-skinned species (Hylocereus polyrhizus and hylocereus undatus). *Foods*, *10*(6). https://doi.org/10.3390/foods10061183

Teigiserova, D. A., Hamelin, L., & Thomsen, M. (2019). Review of high-value food waste and food residues biorefineries with focus on unavoidable wastes from processing. *Resources, Conservation and Recycling*, *149*. https://doi.org/10.1016/j.resconrec.2019.05.003

Teixeira, G. L., Ávila, S., Hornung, P. S., Barbi, R. C. T., & Ribani, R. H. (2018). Sapucaia nut (Lecythis pisonis Cambess.) flour as a new industrial ingredient: Physicochemical, thermal, and functional properties. *Food Research International*, *109*. https://doi.org/10.1016/j.foodres.2018.04.071

Villalobos, M. del C., Serradilla, M. J., Martín, A., Ordiales, E., Ruiz-Moyano, S., & Córdoba, M. de G. (2016). Antioxidant and antimicrobial activity of natural phenolic extract from defatted soybean flour by-product for stone fruit postharvest application. *Journal of the Science of Food and Agriculture*, *96*(6). https://doi.org/10.1002/jsfa.7327

Wang, C., Zhao, J., Chen, F., Cheng, Y., & Guo, A. (2012). Separation, Identification, and Quantitation of Phenolic Acids in Chinese Waxberry (Myrica Rubra) Juice by HPLC-PDA-ESI-MS. *Journal of Food Science*, *77*(2). https://doi.org/10.1111/j.1750-3841.2011.02563.x

Weaver, C. M. (2013). Potassium and health. *Advances in Nutrition*, *4*(3). https://doi.org/10.3945/an.112.003533

Wyllie, S. G., Leach, D. N., Wang, Y., & Shewfelt, R. L. (1995). Key aroma compounds in melons: their development and cultivar dependence. In *Fruit Flavors: Biogenesis, Characterization, and Authentication.* (Vol. 596).

Yanty, N. A. M., Lai, O. M., Osman, A., Long, K., & Ghazali, H. M. (2008). Physicochemical properties of cucumis melo var. inodorus (honeydew melon) seed and seed oil. *Journal of Food Lipids*, *15*(1). https://doi.org/10.1111/j.1745-4522.2007.00101.x

Yasir, M., Sultana, B., Nigam, P. S., & Owusu-Apenten, R. (2016). Antioxidant and genoprotective activity of selected cucurbitaceae seed extracts and LC-ESIMS/MS identification of phenolic components. *Food Chemistry*, *199*. https://doi.org/10.1016/j.foodchem.2015.11.138

Zadernowski, R., Czaplicki, S., & Naczk, M. (2009). Phenolic acid profiles of mangosteen fruits

(Garcinia mangostana). *Food Chemistry*, *112*(3). https://doi.org/10.1016/j.foodchem.2008.06.030

Zeb, A. (2016). Phenolic profile and antioxidant activity of melon (Cucumis melo l.) seeds from pakistan. *Foods*, *5*(4). https://doi.org/10.3390/foods5040067

Zhao, L., Zhao, X., Xu, Y., Liu, X., Zhang, J., & He, Z. (2021). Simultaneous determination of 49 amino acids, B vitamins, flavonoids, and phenolic acids in commonly consumed vegetables by ultra-performance liquid chromatography–tandem mass spectrometry. *Food Chemistry*, *344*. https://doi.org/10.1016/j.foodchem.2020.128712

Zhong, J., Wang, Y., Li, C., Yu, Q., Xie, J., Dong, R., Xie, Y., Li, B., Tian, J., & Chen, Y. (2022). Natural variation on free, esterified, glycosylated and insoluble-bound phenolics of Rubus chingii Hu: Correlation between phenolic constituents and antioxidant activities. *Food Research International*, *162*. https://doi.org/10.1016/j.foodres.2022.112043

Chapter 6. Effect of defatted melon seed residue on dough development and bread quality

Abstract

The aim of this study was to investigate the effect of replacing wheat flour with defatted melon seed (*Cucumis melo L.*) residue (DMSR) on the dough properties and bread nutritional quality and physical characteristics. Adding DMSR did not affect the water absorption of dough, but it made the dough weaker and less extensible. Considering the physical characteristics of breads, DMSR decreased the bread specific volume and the cell number in the crumb, whereas the average cell size increased resulting in a heterogeneous and compact cell crumb structure. Compared with the control bread, DMSR breads exhibited a darker crust, a yellowish crumb, and a firmer texture. DMSR improved the nutritional quality of bread; the protein, lipid, fibre, and ash contents increased, whereas the starch content decreased. At 10% wheat flour replacement with DMSR, the fibre content increased more than five-fold compared to control bread. Overall, although DMSR had a negative impact on dough rheology and on certain physical characteristics of bread, overall, it exhibited considerable potential to fortify bread and improving it nutritional quality.

6.1. Introduction

Bread is a popular staple food in many countries, with refined wheat flour commonly used in most white bread formulations. However, especially in terms of fibre content, this makes white bread a high glycaemic index (GI) food, which is associated with health issues including diabetes and obesity (Lal et al., 2021; Zhu, 2019). Nowadays, with increasing health consciousness, consumers generally tend to purchase high nutritional value foods (Dhen et al., 2018; Hsieh et al., 2017). This trend is also reflected in the bakery market, where products containing ingredients that have beneficial effects on health are attracting consumers' attention (Sajdakowska et al., 2021). In the UK, nearly 11 million loaves are sold each day and they are significant contributors to UK nutrients intake, which provides 11% -12% of energy, 10% - 12% of protein, and 17% - 21% of fibre (Lockyer & Spiro, 2020; Steer et al., 2008). Consequently, bread is an important vehicle for nutrients and a key part of a healthy and balanced diet.

Melon (*Cucumis melo L.*) production was over 28 million tonnes in 2020 in the world (FAOSTAT, 2021). Melon seed is a by-product from melon supply chain, and can represent up to 10% of the total melon weight. Previous studies showed that melon seeds are good source of protein (22% - 39% w/w), lipid (30% - 45% w/w), fibre (19% - 34% w/w), and minerals (rich in potassium) (Mallek-Ayadi et al., 2018; Mian-Hao & Yansong, 2007; Wang et al., 2019). Research on melon seed valorisation has primarily focused on oil extraction, due to its high linoleic acid content (Mallek-Ayadi et al., 2018; Wang et al., 2019). After oil extraction, defatted melon seed residue (DMSR) is produced as a by-product, consisting of a high amounts of protein and fibre. Consequently, considering its high fibre and protein content, DMSR could be used as an ingredient for developing fortified foods. Previous studies have shown that

vegetable or fruit by-products have great potential for being re-utilised as functional ingredients to increase the nutritional value of bakery products (Ahmad et al., 2018; Chareonthaikij et al., 2016; Sardabi et al., 2021; Zarzycki et al., 2022). In addition, from a sustainable development perspective, re-introducing food by-products into the food chain as ingredients can improve the resource utilisation efficiency and reduce waste, which are key for transitioning to more sustainable consumption and production patterns (Difonzo et al., 2022). da Cunha et al (2020) demonstrated the possibility of using melon seed flour in cake production and indicated that 10% wheat flour replacement with melon seed flour was the most acceptable level for consumers in terms of sensory perception. However, to date, there is insufficient information in the literature about the utilisation of DMSR and its application for bread production. Therefore, the aim of this study was to investigate the effect of DMSR on dough properties and bread quality, to evaluate the possibility of utilising DMSR into bread production. These data could provide useful information to achieve a complete valorisation of melon seeds, reduce food waste, and develop nutritionally fortified bread. Besides that, DMSR was collected after cold-pressed oil processing rather than Soxhlet, in order to assurance edibility.

6.2. Materials and Methods

6.2.1. Materials

Honeydew melons (due to higher yield of seeds than Galia and Cantaloupe variety) were selected in this study and were purchased from Sainsbury (produced in Brazil, Reading, UK). The seeds were collected manually from the fresh melons. All collected seeds were washed with tap water to remove any flesh attached on the seeds' surface and then dried at 50 ^{o}C in

a tray dryer (APEX Construction LTD, England) for 24 h. Dried melon seeds were pressed using a cold-pressed oil machine (KK 20F SPEZ, oil press GmbH & Co, KG, Germany) to extract the oil. The defatted melon residue (DMSR) was collected and grounded with a food grinder (Caterlite, CK686, Bristol, UK) for 30 s, sieved through 1000 µm mesh sieves, and then stored at -20 ^{o}C until further use. The composition of DMSR was evaluated through preliminary work following by AOAC methods (AOAC, 2005): moisture 9.60 g/100 g, protein 34.13 g/100 g, fat 16.44 g/100 g, fibre 35.13 g/100 g, and ash 4.41 g/100 g. Other ingredients used for bread production were strong wheat flour (Marks & Spencer, Reading, UK; moisture 13.8 g/100 g, protein 13.5 g/100 g, fibre 1.6 g/100 g, fat 0.6 g/100 g, salt 0.03 g/100 g), instant dried yeast (Borwick's, UK), salt (Sainsbury's table salt, Sainsburys, Reading, UK) and baking fat (Marks & Spencer, Reading, UK; 75 g/100g vegetable fat; fat 75 g/100 g of which 28 g are saturated, salt 1.38 g/100 g).

6.2.2. Dough development characteristics

6.2.2.1. Farinographic analysis

Wheat flour was used to produce the control dough whereas two more doughs were formulated by replacing wheat flour with DMSR at different percentages: 5% and 10% (DMSR5 and DMSR10, respectively). A farinograph (Brabender Farinograph® FA/R-2 810105, Duisburg, Germany) with a 300 g bowl was used to determine the effect of DMSR flour on the dough development. The AACC 54-21.01 constant dough weight procedure was followed (AACC International, 2010). Briefly, the farinograph thermostat was maintained at 30 ^{o}C. The flour sample (14% moisture basis) without water addition was mixed in a 300 g bowl for 1 min. After that, the water was added to the flour sample and mixed at 63 rpm for 20 min. Results including flour water absorption to yield dough consistency of 500 Brabender Units (BU)

(WA; %), dough development time (time needed for the curve to reach maximum dough consistency which is usually the highest point on the curve when the curve is centered on the 500 B.U. line, DDT; min), dough stability time (time that dough consistency remains at 500 BU,DST; min), and mixing tolerance index (consistency difference between height at peak and that after 5 min, MTI; FU) were calculated. Each dough was assessed in triplicate.

6.2.2.2. Dough uniaxial extensibility

The extensibility of bread dough was determined using a Kieffer extensibility rig assembled on a Texture Analyser (TA-XT2, Stable Micro Systems, Surrey, UK) with a 5 kg load cell. The dough samples were each prepared using a 300 g mixing bowl of farinograph (Brabender Farinograph® FA/R-2 810105, Duisburg, Germany). 300 g of wheat flour (control) or composite flour (DMSR5: 95% wheat flour and 5% DMSR; DMSR10: 90% wheat flour and 10% DMSR) were added to the mixing bowl. Water addition varied depending on the water absorption results for each dough sample (**Table 6.1**) from the farinographic analysis (**Section 6.2.2.1**): control (187.7 g), DMSR5 (190.0 g), and DMSR10 (188.3 g). From the dough formed in the farinograph, 20 g were moulded into a cylinder and placed in a press lubricated with parafin oil, and then was compressed for 40 min. The press was sealed to reduce the moisture emission of the sample. Two dough strips were used for each dough replicate. The test conditions were as followed: pre-test speed at 2.0 mm/s, test speed at 3.3 mm/s, post-test speed of 10.0 mm/s, distance at 75 mm, and trigger force of 0.05 N. The resistance to extension (R/E; N) and extensibility (E; mm) were determined.

6.2.3. Bread baking procedure

The formulation of breads was as followed: 1000 g flour (control: 100% wheat flour; DMSR5: 95% wheat flour and 5% DMSR; DMSR10: 90% wheat flour and 10% DMSR), 7 g bakery fat, 15

g salt, 14 g dry yeast, 0.2 g ascorbic acid, and 600 mL water. The water content was constant in all the samples. Bread dough was prepared using the Z-blade mixer (Morton Mixers, UK). All ingredients were mixed at 48 rpm for 130 s at low speed, and then mixed high speed, i.e. at 111 rpm for 100 s. Afterwards, the dough was hand-moulded into three 460 g pieces and placed on a baking tray for an initial proving period (proofing oven ARM/93 proof oven, Salva, Lezo, Spain) at 40 ^{o}C for 10 min. Then, the dough pieces were moulded in a mono mini moulder (Mono Equipment, Swansea, UK) and transferred into a baking tin (17 x 7.5 x 8 cm). Doughs were proved for another 20 min, and then baked in a deck oven (3STA 4676, Polin Stratos, Verona, Italy) at 230 ^{o}C for 20 min. After baking, the loaves were removed from the tins, left to cool down to room temperature, and then sealed in polypropylene bags. Three bread loaves per replicate were obtained. Analyses were carried out during the following 24 h. Each bread formulation was prepared in triplicate.

6.2.4. Proximate composition analysis of bread

The moisture, protein (conversion factor x 6.25), lipid, fibre, and ash of control bread and DMSR breads were determined by the AOAC method (AOAC, 2005). Starch was determined using the Total Starch Assay Kit (Megazyme, Ireland). Samples were analysed in triplicate.

6.2.5. Bread physical characteristics

Weight loss (WL; %) of bread during baking was calculated according to Rodríguez-García et al. (2013) methodology. The bread specific volume was determined by using Volscan Profiler (VSP 600C, Stable Micro Systems, UK). Cell crumb structure of bread was determined according to Lau et al. (2022) methodology with minor modifications. Briefly, the image of bread slice was scanned using a flatbed scan (HP Scanjet G2710, Hewlett-Packard, United States). Afterwards, the image was analysed using Image J software (National Institute of

Health, USA). The image was cropped at the centre of the slice to produce a 5 cm x 4.5 cm crumb image, and then was split into colour channel (grey, red, and blue), and blue was selected because the produced picture was more clearly than the other two. The image was binarized; the number of cells and the area of the air cells were measured. Two slices per bread sample were measured.

6.2.6. Bread texture profile analysis

The texture properties of bread were determined using a Texture Analyser (TAX-Plus, Stable Micro Systems, Surrey, UK) with a 5 kg load cell, and analysed following Dhen et al. (2018) description with some modifications. Briefly, bread samples were sliced into 15 mm thick slices. The two middle bread slices of each bread were used for texture analysis. A two-cycle crumb compression test was performed using a 20 mm diameter cylindrical probe (p/20); samples were compressed 40% of their original height at a speed of 1.7 mm/s with 5 s waiting time between the two cycles. The results of hardness (N), springiness, cohesiveness, and chewiness (N) were calculated by the software Exponent (Version 6.1.18.0, Stable Micro Systems, Surrey, UK). Three measurements per replicate were performed, in each of the three loaves obtained.

6.2.7. Colour measurement of crumb and crust

A chroma meter (CR-400, Minolta, Japan) was used to measure the colour of the bread crust and crumb. The colour of the crust was measured in three points in the centre of the loaf. The colour of the crumb was measured at three points in the central part of a slice. Measurements were performed in the three breads loafs produced per batch. The results were expressed in accordance with the CIELAB system (illuminant C and 10° viewing angle). The measurements were made with an 8 mm diameter diaphragm inset with optical glass. The parameters

measured were L* (L* = 0 [black], L* = 100 [white]), a* (–a* = greenness and +a* = red) and b* (–b* = blueness and +b* = yellow). The total colour difference (ΔE*) between the control sample and each of the breads containing DMSR was calculated using **Equation 6.1** (Francis & Clydesdale, 1975):

Equation 6.1: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

The values used to determine whether the total colour difference was visually obvious were the following (Bodart et al., 2008): ΔE* < 1 colour differences are not obvious for the human eye; 1 < ΔE* < 3 minor colour differences could be appreciated by the human eye depending of the hue; ΔE* > 3 colour differences are obvious for the human eye.

6.2.8. Statistical analysis

One-way analysis of variance (ANOVA) was performed using Minitab (version 20, State College, USA) software package. Turkey's HSD test was used to compare the mean values ($p < 0.05$) among samples.

6.3. Results and Discussion

6.3.1. Dough mixing properties

The dough mixing properties are presented in **Table 6.1**. Dough water absorption (WA) was not significantly different ($p > 0.05$) between doughs when wheat flour was replaced by DMSR. Adding DMSR significantly increased ($p < 0.05$) dough development time (DDT), but no difference ($p > 0.05$) was observed between 5% and 10% enrichment. DDT increase could be due to the fibre content increasing in DMSR dough (DMSR proximate composition in **Section 6.2.1**). Fibre in DMSR competes for water with wheat flour components hindering the hydration of gluten proteins, thereby more time is required to develop the gluten network,

increasing the DDT (Gökşen & Ekiz, 2016; Wirkijowska et al., 2020). Dough stability time (DST) and mixing tolerance index (MTI) indicate dough strength and tolerance to mixing; a strong dough is characteristed by a high DST and low MTI values (Chisenga et al., 2020; Guardianelli et al., 2021). Adding DMSR significantly decreased DST and increased MTI ($p < 0.05$), indicating that the replacement of wheat flour with DMSR resulted in a weaker and less stable dough. These results could be attributed to gluten dilution and increased fibre content which induces greater disruption of the gluten network (Chisenga et al., 2020; Pasqualone et al., 2019). Similar results were observed in previous works, in which Moldavian dragonhead seed residue or Flaxseed addition resulted in softer bread doughs (Wirkijowska et al., 2020; Zarzycki et al., 2022).

6.3.2. Dough uniaxial extensibility

Extensibility reflects the extension capacity of dough and relates to bread final volume (Burešová et al., 2014; Coțovanu & Mironeasa, 2021). Dough extensibility properties are presented in **Table 6.1**. The resistance to extension (R/E) and extensibility (E) of dough decreased significantly ($p < 0.05$) when DMSR proportion increased, indicating that the DMSR dough became weaker and softer. A specific ratio of gliadin (determines dough extensibility) to glutenin (determines the dough elasticity and strength) fractions is important for dough extensibility (Barak et al., 2013; Lu et al., 2018). The presence of non-gluten proteins from DMSR could have reduced the possibility of gliadins and glutenins interacting, thereby reducing dough extensibility.

Table 6.1. Mixing properties and uniaxial extensibility of the different dough samples.

Dough	Mixing properties				Unaxial extension	
	WA (%)	**DDT (min)**	**DST (min)**	**MTI (FU)**	**R/E (N)**	**E (mm)**
Control	62.57 ± 0.32 [a]	3.57 ± 0.11 [b]	9.17 ± 0.29 [a]	16.33 ± 1.53 [c]	0.45 ± 0.02 [a]	70.27 ± 2.45 [a]
DMSR5	63.33 ± 0.35 [a]	4.27 ± 0.25 [a]	5.57 ± 0.21 [b]	27.67 ± 2.08 [b]	0.16 ± 0.01 [b]	40.35 ± 4.94 [b]
DMSR10	62.77 ± 0.25 [a]	4.57 ± 0.11 [a]	2.33 ± 0.15 [c]	123.67 ± 4.04 [a]	0.08 ± 0.02 [c]	18.25 ± 1.86 [c]

Mean ± standard deviation values (n = 3) in the same column with different superscript letters are significantly different ($p < 0.05$) according to the Tukey's HSD Test; WA- water absorption; DDT- dough development time; DST- dough stability time; MTI- Mixing tolerance index; R/E - resistance to extension; E - extensibility. Control - 100% wheat flour, DMSR5 - 5% wheat flour replaced by defatted melon seed residue, DMSR10 - 10% wheat flour replaced by defatted melon seed residue.

6.3.3. Proximate compositions of bread

The proximate composition of breads is presented in **Table 6.2**. The moisture content decreased as DMSR content increased in breads; DMSR10 had a significantly lower moisture content ($p < 0.05$) than control bread. This could be attributed to the initial moisture content difference between wheat flour (13.80 g/100 g) and DMSR (9.60 g/100 g). DMSR breads had significantly higher ($p < 0.05$) lipid, protein, and fibre content than control bread. Especially, the fibre content in DMSR10 bread (3.41 g/100 g) was more than 5-fold higher compared to the control bread (0.57 g/100 g). According to the European regulation for nutrition and health claims on foods, products that claim to be 'source of fibre' and 'high fibre' should contain at least 3 g and 6 g of fibre per 100 g product, respectively (The Council of European Union, 2007). Therefore, DMSR10 bread could be labelled as 'source of fibre'. In contrast, DMSR breads contained lower starch content as compared to control bread. Luo & Zhang (2018) indicated that increasing fibre or decreasing starch content is essential to develop low-Glycaemic index (GI) bread, which might have potential health benefits for preventing hyperglycemia related diseases. In terms of ash content, a significant increase ($p < 0.05$) was observed in DMSR10 bread as compared to control bread. Previous studies reported that melon seed is rich in potassium (1148 - 2082 mg/100 g) (Mallek-Ayadi et al., 2018; Morais et al., 2017); thus, DMSR10 bread could contribute to an increased dietary intake of potassium.

Table 6.2. Proximate composition (g/100 g) of bread samples.

Sample	Moisture	Lipid	Protein	Ash	Starch	Fibre
Control	38.07 ± 0.30 ^{a}	0.42 ± 0.04 ^{c}	9.71 ± 0.12 ^{c}	1.69 ± 0.03 ^{b}	49.37 ± 0.43 ^{a}	0.56 ± 0.15 ^{c}
DMSR5	37.71 ± 0.12 ^{ab}	0.71 ± 0.06 ^{b}	9.96 ± 0.03 ^{b}	1.69 ± 0.01 ^{b}	48.05 ± 0.29 ^{b}	1.71 ± 0.10 ^{b}
DMSR10	37.23 ± 0.24 ^{b}	1.02 ± 0.04 ^{a}	10.29 ± 0.03 ^{a}	1.79 ± 0.05 ^{a}	44.93 ± 0.30 ^{c}	3.41 ± 0.38 ^{a}

Mean ± Satndard deviation values (n = 3) in the same column with different superscript letters are significantly different ($p < 0.05$) according to the Tukey's HSD Test. Control - 100% wheat flour; DMSR5 - 5% wheat flour replaced by defatted melon seed residue; DMSR10 - 10% wheat flour replaced by defatted melon seed residue.

6.3.4. Physical characteristics of bread

Table 6.3 shows the physical characteristics of control bread and DMSR breads. Weight loss (WL) values were not significantly different ($p > 0.05$) among the three bread formulations. However, as mentioned in section 6.3.3 (**Table 6.1**) DMSR breads had lower moisture content than the control. These results could be due to the lower moisture content of DMSR (9.6 g/100 g) over wheat flour (13.8 g/100 g). The decreasing or increasing effects on WL may depend on the substitution ingredient used due to their different chemical compositions and molecular structures (Alinovi et al., 2022; Lazou et al., 2022; Sardabi et al., 2021). Specific volume is an important parameter as it relates with the total gas phase retained in the final bread. As expected, a significant decrease in bread specific volume ($p < 0.05$) was observed with increasing DMSR (**Table 6.3 and Figure 6.1**). The addition of DMSR reduced the dough uniaxial extensibility (**Table 6.1**), thus decreasing the expansion capacity of dough during fermentation and baking and resulting in a lower volume of bread.

In terms of crumb structure, the number of cells decreased with increasing amount of DMSR (**Table 6.3**). In contrast, the average cell size increased when the DMSR ratio increased. These findings could be explained by the dough mixing properties discussed in **Section 6.3.1**. As the DMSR proportion increased, the dough strength decreased, giving place to a dough in which gas phase destabilization phenomena, such as flocculation and coalescence of bubbles, took place, resulting in bigger cells and less cells numbers. These results are in agreement with other studies, where it is reported that addition of high fibre ingredients reduced the strength of gluten network and the stability of gas cell walls; this resulted in broken gas cell walls and coalescence of cells into larger ones (Bigne et al., 2018; Han et al., 2019; Ni et al., 2020; Saka et al., 2022).

Table 6.3. Physical characteristics of bread samples.

Sample	WL (%)	Specific volume (ml/g)	Number of cells	Average cell size (mm^2)
Control	8.72 ± 0.33 [a]	3.02 ± 0.08 [a]	1017.00 ± 54.29 [a]	0.77 ± 0.15 [c]
DMSR5	8.80 ± 0.41 [a]	2.67 ± 0.04 [b]	742.33 ± 11.15 [b]	1.17 ± 0.06 [b]
DMSR10	8.80 ± 0.16 [a]	2.48 ± 0.04 [c]	535.67 ± 42.36 [c]	1.93 ± 0.06 [a]

Mean ± Standard deviation values (n = 6) in the same column with different superscript letters are significantly different ($p < 0.05$) according to the Tukey's HSD Test. WL – weight loss; Control - 100% wheat flour; DMSR5 - 5% wheat flour replaced by defatted melon seed residue; DMSR10 - 10% wheat flour replaced by defatted melon seed residue.

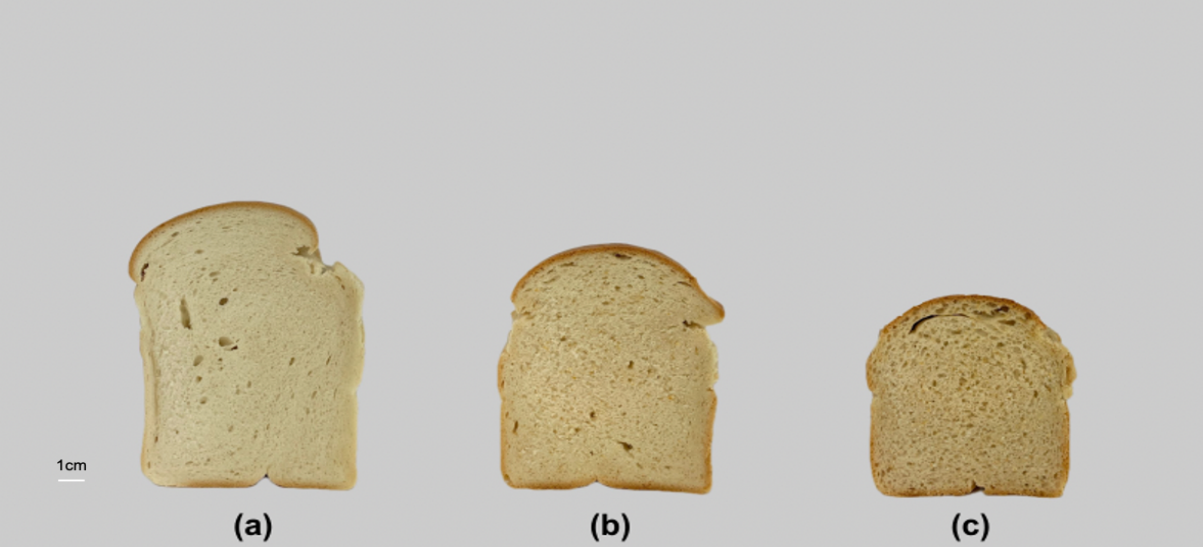

Figure 6.1. Scanned images of bread slices; (a) Control bread - 100% wheat flour; (b) DMSR5 - 5% replacing level of wheat flour; (c) DMSR10 - 10% replacing level of wheat flour.

6.3.5. Texture properties of bread

Texture properties of all formulation breads are presented in **Table 6.4**. The hardness of bread increased significantly ($p < 0.05$) with increasing the DMSR ratio. As it was discussed in the previous section, breads with higher DMSR content presented lower volumes and a more compact cell crumb structure, thus giving place to firmer crumbs, this is supported by the PCA plot **(Figure 6.2)**, where DMSR breads were positively associated with hardness and average cell size, but were negatively associated with volume and cell number. This was attributed to the increased level of fibre leading to a weaker and less extensible dough, with lower gas retention ability during fermentation and baking, thereby resulting in a compact structure (Ahmad et al., 2018; Dhen et al., 2018; Ma et al., 2019). These results were in line with previous studies, where fruit or vegetable by-products were added into bread formulations to increase dietary fibre (Ahmad et al., 2018; Ni et al., 2020; Zarzycki et al., 2022). Moreover, the lower moisture content in DMSR breads may have also been another factor to the increased hardness value. Water is the most common plasticizer in bread, which is related to hardness, thus, a lower moisture content could result in a firmer structure (Alinovi et al., 2022; Das et al., 2015; Mastromatteo et al., 2013). Additionally, a significant increase in chewiness ($p < 0.05$) was observed when increasing the percentage of DMSR in bread formulation. Chewiness reflects the extent of difficulty in food mastication before swallowing, and harder foods having higher chewiness (Xin et al., 2022).

Springiness and cohesiveness reflect the elasticity of bread and the resistance of its

internal structure, respectively (Dhen et al., 2018; Ulziijargal et al., 2013). The springiness and cohesiveness values of both of DMSR breads were significantly lower ($P < 0.05$) than control bread, indicating that DMSR breads were less elastic and with a weaker cell crumb structure than control bread. These results could be attributed to the dilution of the gluten content. As mentioned before, when wheat flour was replaced by DMSR the inter-molecular interaction between gluten proteins for the formation of the network were disrupted, which led to a less elastic and fragile crumb structure in DMSR breads. Previous studies in bread, in which wheat flour was replaced by wheat by-products and broad bean hull also observed that substituted breads presented the characteristics of lower springiness and cohesiveness (Ni et al., 2020; Pasqualone et al., 2017).

Table 6.4. Texture properties of bread samples.

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
Control	13.66 ± 0.52 ^{c}	0.91 ± 0.01 ^{a}	0.71 ± 0.02 ^{a}	8.43 ± 0.15 ^{c}
DMSR5	19.76 ± 0.40 ^{b}	0.83 ± 0.02 ^{b}	0.61 ± 0.02 ^{b}	9.51 ± 0.24 ^{b}
DMSR10	22.69 ± 0.41 ^{a}	0.81 ± 0.01 ^{b}	0.55 ± 0.01 ^{c}	10.51 ± 0.18 ^{a}

Mean ± Standard deviation values (n = 9) in the same column with different superscript letters are significantly different ($p < 0.05$) according to the Tukey's HSD Test. Control - 100% wheat flour; DMSR5 - 5% wheat flour replaced by defatted melon seed residue; DMSR10 - 10% wheat flour replaced by defatted melon seed residue.

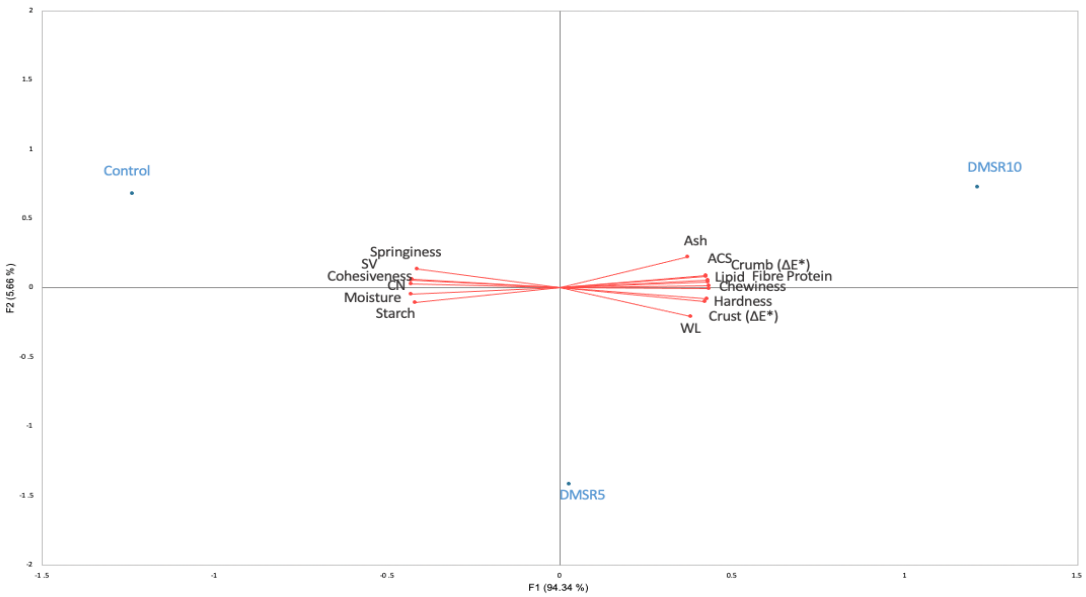

Figure 6.2. Principal Components Analysis (PCA) of bread samples. Control - 100% wheat flour; DMSR5 - 5% replacing level of wheat flour; DMSR10 - 10% replacing level of wheat flour; SV - specific volume; CN - cell number; ACS - average cell size; WL - weight loss.

6.3.6. Colour of bread crust and crumb

The crust and crumb colour of all formulation breads are presented in **Table 6.5** and in **Figure 6.1**. Bread crust became significantly ($p < 0.05$) darker (lower L*) when DMSR ratio increased in bread. This result could be associated with the dark colour of DMSR. Previous studies reported similar results when dark colour ingredients were used in bread production (Alinovi et al., 2022; Mikulec et al., 2019). In addition, the DSMR bread crusts colour presented lower values of the red component (a*) and yellow component (b*) than the control bread crust. These colour changes in the crust could be attributed to Maillard reaction due to higher protein content in DMSR than wheat (34.13 g/100 g and 13.5 g/100 g, respectively). These results are in line with previous studies in which a* and b* values decreased when wheat flour substitution by roasted

flaxseed flour/defatted hemp flour increased in bread (Lazou et al., 2022; Marpalle et al., 2014).

In terms of bread crumb colour, similar trends as in the crusts were observed. The lightness (L*) of the crumb decreased significantly ($p < 0.05$) with increasing DMSR. In contrast, a significant increase in b* (yellowness) and decrease in a* (greenness) ($p < 0.05$) were observed in the breads with increasing DMSR. Crumb colour changes could be mainly attributed to the originally colour of DMSR, rather than to chemical reactions. During baking, the centre of the crumb cannot reach temperature above 100 °C. In addition, due to its higher moisture content, Maillard and caramelization reactions do not take place or are slower in comparison to the crumb, thereby they could not produce a significant impact on crumb colour (Lau et al., 2022; Purić et al., 2020).

The total colour differences (ΔE*) for crust and crumb in all DMSR breads were higher than 3 as compared to control bread, indicating that the differences in colour between DMSR breads and control bread were obvious to the human eye.

Table 6.5. Crust and crumb colour parameters of bread samples.

Crust	**L***	**a***	**b***	**ΔE***
Control	59.76 ± 1.78 [a]	13.79 ± 0.58 [a]	36.77 ± 0.64 [a]	0
DMSR5	53.56 ± 1.35 [b]	12.4 ± 0.22 [b]	23.14 ± 0.95 [b]	15.14
DMSR10	47.13 ± 0.3 [c]	12.29 ± 0.21 [b]	20.07 ± 0.16 [c]	21.02
Crumb				
Control	77.11 ± 1.01 [a]	-1.60 ± 0.06 [c]	12.60 ± 0.29 [c]	0
DMSR5	72.16 ± 0.61 [b]	-1.24 ± 0.09 [b]	14.98 ± 0.11 [b]	3.83
DMSR10	66.02 ± 0.25 [c]	-0.58 ± 0.08 [a]	16.17 ± 0.34 [a]	11.70
Ingredient				
WF	94.02 ± 0.20	-0.59 ± 0.04	9.93 ± 0.14	-
DMSR	69.96 ± 0.19	4.98 ± 0.17	26.62 ± 0.60	-

Mean ± Standard deviation values (n = 9) in the same column with different superscript letters are significantly different ($p < 0.05$) according to the Tukey's HSD Test; WF - wheat flour. Control - 100% wheat flour; DMSR5 - 5% wheat flour replaced by defatted melon seed residue; DMSR10 - 10% wheat flour replaced by defatted melon seed residue.

6.4. Conclusions

Replacement of 10% of wheat flour by DMSR resulted in a bread that could be considered 'source of fibre'. Moreover, DMSR addition enhanced protein and lipid content, improving bread nutritional quality. The reduction of starch content and the increase of fibre in DMSR breads could lead to a lower glycaemic index (GI) food than control bread. To this end, further work to evaluate the starch digestibility, blood glucose response and sensory profiling of DMSR breads should be carried out. DMSR addition reduced dough strength and extensibility, and had a negative effect in bread volume, hardness and springiness. Future work to reduce the negative effect of DMSR on bread physical properties will be carried out by using processing technologies to

modify the technological properties of DMSR, such as thermal treatment, micro-milling, and fermentation. Overall, in the present work, DMSR was re-introduced into the food chain and incorporated into bread production as a complementary ingredient to wheat flour, helping in reducing food waste and contributing to a more sustainable food system.

6.5. References

AACC International. (2010). AACC International Approved Methods. In *AACC International Approved Methods*. https://doi.org/10.1094/aaccintmethods

Ahmad, B. S., Talou, T., Straumite, E., Sabovics, M., Kruma, Z., Saad, Z., Hijazi, A., & Merah, O. (2018). Protein bread fortification with cumin and caraway seeds and by-product flour. *Foods*, *7*(3). https://doi.org/10.3390/foods7030028

Alinovi, M., Rinaldi, M., Paciulli, M., Littardi, P., & Chiavaro, E. (2022). Chestnut peels and wheat bran at different water level influence the physical properties of pan bread. European Food Research and Technology, 248(5). https://doi.org/10.1007/s00217-022-03959-3

AOAC. (2005). Official Methods of Analysis of AOAC International. In *Association of Official Analysis Chemists International*.

Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. *LWT*, *51*(1). https://doi.org/10.1016/j.lwt.2012.09.011

Bigne, F., Puppo, M. C., & Ferrero, C. (2018). Mesquite (Prosopis alba) flour as a novel ingredient for obtaining a “panettone-like” bread. Applicability of part-baking technology. *LWT - Food Science and Technology*, *89*. https://doi.org/10.1016/j.lwt.2017.11.029

Bodart, M., de Peñaranda, R., Deneyer, A., & Flamant, G. (2008). Photometry and colorimetry characterisation of materials in daylighting evaluation tools. *Building and Environment*, *43*(12). https://doi.org/10.1016/j.buildenv.2007.12.006

Burešová, I., Kráčmar, S., Dvořáková, P., & Středa, T. (2014). The relationship between rheological characteristics of gluten-free dough and the quality of biologically leavened bread. *Journal of Cereal Science*, *60*(2). https://doi.org/10.1016/j.jcs.2014.07.001

Chareonthaikij, P., Uan-On, T., & Prinyawiwatkul, W. (2016). Effects of pineapple pomace fibre on physicochemical properties of composite flour and dough, and consumer acceptance of fibre-enriched wheat bread. *International Journal of Food Science and Technology*, *51*(5). https://doi.org/10.1111/ijfs.13072

Chisenga, S. M., Workneh, T. S., Bultosa, G., Alimi, B. A., & Siwela, M. (2020). Dough rheology and loaf quality of wheat-cassava bread using different cassava varieties and wheat substitution levels. *Food Bioscience*, *34*. https://doi.org/10.1016/j.fbio.2020.100529

Coțovanu, I., & Mironeasa, S. (2021). Impact of different amaranth particle sizes addition level on wheat flour dough rheology and bread features. *Foods*, *10*(7). https://doi.org/10.3390/foods10071539

da Cunha, J. A., Rolim, P. M., da Silva Chaves Damasceno, K. S. F., de Sousa, F. C., Nabas, R. C., & Seabra, L. M. A. J. (2020). From seed to flour: Sowing sustainability in the use of cantaloupe melon residue (Cucumis melo L. Var. Reticulatus). *PLoS ONE*, *15*(1). https://doi.org/10.1371/journal.pone.0219229

Das, L., Raychaudhuri, U., & Chakraborty, R. (2015). Effects of hydrocolloids as texture improver in coriander bread. Journal of Food Science and Technology, 52(6). https://doi.org/10.1007/s13197-014-1296-8

Dhen, N., ben Rejeb, I., Boukhris, H., Damergi, C., & Gargouri, M. (2018). Physicochemical and sensory properties of wheat- Apricot kernels composite bread. *LWT*, *95*. https://doi.org/10.1016/j.lwt.2018.04.068

Difonzo, G., Grassi, S., & Paciulli, M. (2022). Upcycling of Agro-Food Chain By-Products to Obtain High-Value-Added Foods. Foods, 11(14), 2043. https://doi.org/10.3390/foods11142043

FAOSTAT. (2021). Crops and livestock products. *Food and Agriculture Organization of the United Nations (FAO), Statistics Division, Rome Italy. Http://Www.Fao.Org/Faostat/En/#data/QCL*.

Francis, F. J., & Clydesdale, F. M. (1975). *Food colorimetry: theory and applications*. AVI Publishing Co. Inc.

Gökşen, G., & Ekiz, H. İ. (2016). Effect of Prunus mahaleb Seed Powder on Dough Rheology and Bread Quality. *Journal of Food Quality*, *39*(5). https://doi.org/10.1111/jfq.12220

Guardianelli, L., Puppo, M. C., & Salinas, M. v. (2021). Influence of pistachio by-product from edible oil industry on rheological, hydration, and thermal properties of wheat dough. *LWT*, *150*. https://doi.org/10.1016/j.lwt.2021.111917

Han, W., Ma, S., Li, L., Zheng, X., & Wang, X. (2019). Impact of wheat bran dietary fiber on gluten and gluten-starch microstructure formation in dough. *Food Hydrocolloids*, *95*. https://doi.org/10.1016/j.foodhyd.2018.10.033

Hsieh, P. H., Weng, Y. M., Yu, Z. R., & Wang, B. J. (2017). Substitution of wheat flour with wholegrain flours affects physical properties, sensory acceptance, and starch digestion of Chinese steam bread (Mantou). *LWT - Food Science and Technology*, *86*. https://doi.org/10.1016/j.lwt.2017.08.051

Lal, M. K., Singh, B., Sharma, S., Singh, M. P., & Kumar, A. (2021). Glycemic index of starchy crops and factors affecting its digestibility: A review. In *Trends in Food Science and Technology* (Vol. 111). https://doi.org/10.1016/j.tifs.2021.02.067

Lau, T., Clayton, T., Harbourne, N., Rodriguez-Garcia, J., & Oruna-Concha, M. J. (2022). Sweet corn cob as a functional ingredient in bakery products. *Food Chemistry: X*, *13*. https://doi.org/10.1016/j.fochx.2021.100180

Lazou, A., Anastasiadis, G., Provata, T., Koliou, Z., & Protonotariou, S. (2022).

Utilization of industrial hemp by-product defatted seed flour: effect of its incorporation on the properties and quality characteristics of ‘tsoureki’, a rich-dough baked Greek product. Journal of the Science of Food and Agriculture. https://doi.org/10.1002/jsfa.12351

Lockyer, S., & Spiro, A. (2020). The role of bread in the UK diet: An update. In *Nutrition Bulletin* (Vol. 45, Issue 2). https://doi.org/10.1111/nbu.12435

Lu, X., Brennan, M. A., Serventi, L., & Brennan, C. S. (2018). Incorporation of mushroom powder into bread dough—effects on dough rheology and bread properties. *Cereal Chemistry*, *95*(3). https://doi.org/10.1002/cche.10043

Luo, K., & Zhang, G. (2018). Nutritional property of starch in a whole-grain-like structural form. *Journal of Cereal Science*, *79*. https://doi.org/10.1016/j.jcs.2017.09.006

Ma, J., Kaori, F., Ma, L., Gao, M., Dong, C., Wang, J., & Luan, G. (2019). The effects of extruded black rice flour on rheological and structural properties of wheat-based dough and bread quality. *International Journal of Food Science and Technology*, *54*(5). https://doi.org/10.1111/ijfs.14062

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2018). Chemical composition and bioactive compounds of Cucumis melo L. seeds: Potential source for new trends of plant oils. *Process Safety and Environmental Protection*, *113*. https://doi.org/10.1016/j.psep.2017.09.016

Marpalle, P., Sonawane, S. K., & Arya, S. S. (2014). Effect of flaxseed flour addition on physicochemical and sensory properties of functional bread. *LWT*, *58*(2). https://doi.org/10.1016/j.lwt.2014.04.003

Mastromatteo, M., Guida, M., Danza, A., Laverse, J., Frisullo, P., Lampignano, V., & del Nobile, M. A. (2013). Rheological, microstructural and sensorial properties of durum wheat bread as affected by dough water content. Food Research International, 51(2). https://doi.org/10.1016/j.foodres.2013.01.004

Mian-Hao, H., & Yansong, A. (2007). Characteristics of some nutritional composition of melon (Cucumis melo hybrid ’ChunLi’) seeds. *International Journal of Food Science and Technology*, *42*(12). https://doi.org/10.1111/j.1365-2621.2006.01352.x

Mikulec, A., Kowalski, S., Sabat, R., Skoczylas, Ł., Tabaszewska, M., & Wywrocka-Gurgul, A. (2019). Hemp flour as a valuable component for enriching physicochemical and antioxidant properties of wheat bread. LWT, 102. https://doi.org/10.1016/j.lwt.2018.12.028Morais, D. R., Rotta, E. M., Sargi, S. C., Bonafe, E. G., Suzuki, R. M., Souza, N. E., Matsushita, M., & Visentainer, J. v. (2017). Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. *Journal of the Brazilian Chemical Society*, *28*(2). https://doi.org/10.5935/0103-5053.20160178

Ni, Q., Ranawana, V., Hayes, H. E., Hayward, N. J., Stead, D., & Raikos, V. (2020).

Addition of broad bean hull to wheat flour for the development of high-fiber bread: Effects on physical and nutritional properties. *Foods*, *9*(9). https://doi.org/10.3390/foods9091192Pasqualone, A., de Angelis, D., Squeo, G., Difonzo, G., Caponio, F., & Summo, C. (2019). The effect of the addition of apulian black chickpea flour on the nutritional and qualitative properties of durum wheat-based bakery products. *Foods*, *8*(10). https://doi.org/10.3390/foods8100504

Pasqualone, A., Laddomada, B., Centomani, I., Paradiso, V. M., Minervini, D., Caponio, F., & Summo, C. (2017). Bread making aptitude of mixtures of re-milled semolina and selected durum wheat milling by-products. *LWT*, *78*. https://doi.org/10.1016/j.lwt.2016.12.032

Purić, M., Rabrenović, B., Rac, V., Pezo, L., Tomašević, I., & Demin, M. (2020). Application of defatted apple seed cakes as a by-product for the enrichment of wheat bread. *LWT*, *130*. https://doi.org/10.1016/j.lwt.2020.109391

Rodríguez-García, J., Laguna, L., Puig, A., Salvador, A., & Hernando, I. (2013). Effect of Fat Replacement by Inulin on Textural and Structural Properties of Short Dough Biscuits. *Food and Bioprocess Technology*, *6*(10). https://doi.org/10.1007/s11947-012-0919-1

Sajdakowska, M., Gębski, J., Jeżewska-Zychowicz, M., & Królak, M. (2021). Consumer choices in the bread market: The importance of fiber in consumer decisions. *Nutrients*, *13*(1). https://doi.org/10.3390/nu13010132

Saka, İ., Baumgartner, B., & Özkaya, B. (2022). Usability of microfluidized flaxseed as a functional additive in bread. *Journal of the Science of Food and Agriculture*, *102*(2). https://doi.org/10.1002/jsfa.11378

Sardabi, F., Azizi, M. H., Gavlighi, H. A., & Rashidinejad, A. (2021). The effect of Moringa peregrina seed husk on the in vitro starch digestibility, microstructure, and quality of white wheat bread. *LWT*, *136*. https://doi.org/10.1016/j.lwt.2020.110332

Steer, T., Thane, C., Stephen, A., & Jebb, S. (2008). Bread in the diet: consumption and contribution to nutrient intakes of British adults. *Proceedings of the Nutrition Society*, *67*(OCE8). https://doi.org/10.1017/s0029665108000372

The Council of European Union. (2007). Regulation on nutrition and health claims made on foods. *Official Journal*, *12*(3).

Ulziijargal, E., Yang, J. H., Lin, L. Y., Chen, C. P., & Mau, J. L. (2013). Quality of bread supplemented with mushroom mycelia. *Food Chemistry*, *138*(1). https://doi.org/10.1016/j.foodchem.2012.10.051

Wang, D. H., Wang, Z., Le, K. P., Cortright, J. R., Park, H. G., Tobias, H. J., & Brenna, J. T. (2019). Potentially High Value Conjugated Linolenic Acids (CLnA) in Melon Seed Waste. *Journal of Agricultural and Food Chemistry*, *67*(37). https://doi.org/10.1021/acs.jafc.9b04744

Wirkijowska, A., Zarzycki, P., Sobota, A., Nawrocka, A., Blicharz-Kania, A., & Andrejko,

D. (2020). The possibility of using by-products from the flaxseed industry for functional bread production. *LWT*, *118*. https://doi.org/10.1016/j.lwt.2019.108860

Xin, T., Tang, S., Su, T., Huang, Z., Huang, F., Zhang, R., Dong, L., Deng, M., Shen, Y., & Su, D. (2022). Impact of replacing wheat flour with lychee juice by-products on bread quality characteristics and microstructure. *LWT*, *165*, 113696. https://doi.org/10.1016/j.lwt.2022.113696

Zarzycki, P., Wirkijowska, A., Nawrocka, A., Kozłowicz, K., Krajewska, M., Kłosok, K., & Krawęcka, A. (2022). Effect of Moldavian dragonhead seed residue on the baking properties of wheat flour and bread quality. *LWT*, *155*. https://doi.org/10.1016/j.lwt.2021.112967

Zhu, F. (2019). Glycemic control in Chinese steamed bread: Strategies and opportunities. In *Trends in Food Science and Technology* (Vol. 86). https://doi.org/10.1016/j.tifs.2019.02.038

Chapter 7. General discussion and future work

7.1. General discussion

The overall aim of the thesis was to explore valorisation strategies for melon seeds, targeting the recovery of high value-added compounds, and evaluating melon seeds as food ingredient. More specifically, the work carried out in this thesis generated useful knowledge in relevance to both academic and food industry stakeholders on: (1) the nutritional value of melon seed and its oil-extraction by-products (referred to as defatted meal/residue); (2) the impact of different processing methods melon seeds chemical composition; (3) melon seed oil quality and how it can be influenced using different extraction technologies; and (4) the use of defatted melon seed meal as wheat flour substitution in bread making.

In **Chapter 3**, the impact of three processing methods, namely soaking, boiling, and roasting, on the nutritional and anti-nutritional compounds in melon seed was evaluated. Generally, processing can improve food palatability and digestibility as well as ensure food safety (Zhao et al., 2019). During processing, the texture, sensory, and nutritional quality of the food matrix can significantly change (Feizollahi et al., 2021). Soaking and boiling reduced tannins content up to 20% and 26%, respectively; this could be attributed to the leaching effect of tannins, as water-soluble components, into the water phase. In contrast, roasting did not reduce tannins content. Tannins, are anti-nutritional compounds which can bind to proteins and form insoluble complexes; the latter decreases protein digestibility (Nikmaram et al., 2017). It was suggested that soaking and boiling could represent promising processing methods to improve the nutrient availability of melon seeds. In addition, boiling decreased protein content (by

approximately 6%), and concurrently increased oil content (2% - 5%). It was suggested that boiling could be used as a pre-treatment method, especially before oil extraction, to improve the oil yield. Notably, it was found that boiling caused minor decrease in linoleic acid (C18:2) content, which is associated with the oxidation of unsaturated fatty acids at high temperature. Therefore, in cases whereby boiling is considered as pre-treatment method for melon seed oil extraction, time and temperature as key parameters should be considered further to maintain oil nutritional value and oil yield.

In **Chapter 4**, the impact of three oil extraction methods, (Soxhlet, cold-pressed, and aqueous enzymatic extraction), on the physicochemical properties, content of bioactive compounds, and oxidative stability of melon seed oil was evaluated. An appropriate oil extraction method is important to assure the quality and nutritional value of the oil. Apart from food applications, high-quality oils could be used in other sectors (e.g. cosmetics and pharmaceuticals), which could further increase their added value. Melon seed oils were found to be rich in linoleic acid (53.6% - 70.8%), as well as in β-sitosterol (119.5 - 291.9 mg/100 g), and squalene (101.1 - 164.7 mg/100 g), which support its suggested use as alternative edible oil. In addition, the choice of extraction method did not influence fatty acid profile of melon seed oil, but impacted on physicochemical properties, content of bioactivity compounds, and oxidative stability. Specifically, AEE and CPE extracted melon seed oil showed higher saponification values than SE. According to Akintayo & Bayer (2002), high saponification value in oils denotes their suitability for the production of liquid soaps or shampoos; as such, CPE and AEE extracted melon seed oil have potential application prospects in personal care. Besides that, among the three extraction methods, Soxhlet and aqueous enzymatic extraction oil samples exhibited better oxidative stability compared to oil samples obtained by cold-pressed extraction. The

low oxidative stability in cold-pressed oil could be attributed to its higher composition in pro-oxidant factors (e.g. metal ions) that are more likely to cause oil oxidation.

Defatted melon seed, a by-product of melon seed oil extraction (often referred to as meal/residue), could be considered as functional food ingredient in food formulations. As such, in **Chapter 5**, the research work focused on evaluating the nutritional value, functional properties, and phenolic acid distribution of defatted melon seed. The meal contained considerable amounts of protein (51.1% - 54.2%, w/w), fibre (29.4% - 33.2%, w/w), minerals such as potassium and magnesium, and GABA (γ-aminobutyric acid; 1.4 - 4.3 mmol/kg), all of which supported its proposition as valuable food ingredient. However, it also exhibited relatively high phytic acid content (5.0% - 5.8%, w/w), which could interfere with nutrient bioavailability. In addition, defatted melon seeds showed satisfactory functional properties, including water/oil absorption capacity, and emulsifying capacity, comparable to similar materials such as defatted pumpkin seeds. All the above indicated defatted melon seeds potential as ingredient as wheat flour or fat replacer, in bakery and meat product formulations, respectively. With regards to phenolics, gallic acid, syringic acid, p-coumaric acid, and gentisic acid were primarily found in free form; protocatechuic acid, caffeic acid, catechin, epicatechin, and chlorogenic were found in free and conjugated form; no phenolics were found in bound form. The antioxidant properties of phenolics have been linked to many health benefits (Rockenbach et al., 2011; Sandhu & Gu, 2010). The above indicated that defatted melon seeds could be a good natural source of antioxidants. Currently, products without artificial ingredients, also referred to as ‘clean label’ have received increasing attention among consumers and the food industry, often driving the agenda in food development activities.

Based on the results from **Chapter 5**, defatted melon seed was used as wheat flour substitute in **Chapter 6** for bread production, to assess its effect on dough development and bread quality. Bread is a traditional staple product, consumed on a daily basis across many parts of the world (especially Europe and North America) and plays an important part of the diet. As such, it was considered an appropriate prototype product to be reformulated with defatted melon seeds. Wheat flour was substituted by defatted melon seeds at 5% and 10%. The melon seed meal addition reduced dough strength and extensibility, which in turn made dough softer and weaker. These observations were attributed to gluten dilution and increased fibre content, both of which induce disruption of the gluten network. The latter had a negative effect on bread quality, as indicated by reduction in bread volume, hardness and springiness in the formulated samples. However, in terms of the bread's nutritional quality, compared with control bread (100% wheat flour), defatted melon seed breads had higher protein, lipid, and fibre content. More specifically, with regards to fibre content, 10% defatted melon seed breads could be labelled as 'source of fibre', containing 3.4 g/100 g, which represented more than five-fold increase in fibre content compared to control bread (about 0.6 g/100 g). In addition, defatted melon seed breads had lower starch content than control. Increasing fibre or decreasing starch content is essential to develop low-Glycaemic index (GI) bread, which might have potential health benefits for preventing hyperglycemia related diseases (Luo & Zhang, 2018).

7.2. Limitations and future work

In **Chapters 3** and **6**, melon seed was processed by roasting method to convert it as food resource and defatted melon seed was used as wheat flour substitution to develop bread, respectively. Both approaches are commonly used in domestic cooking and food industry

processing; during roasting and baking processing, high temperature can induce Maillard reactions. The latter depend on the presence of reducing sugars and amino acids and lead the formation of volatile compounds that contribute to flavour and aroma development (Bi et al., 2022). However, acrylamide, a processing contaminant in the Maillard reaction, classified as "probably carcinogenic to humans", would be also generated during these processing conditions. Previous studies showed that the major precursors for acrylamide formation are free asparagine and reducing sugars (principally glucose, fructose, and maltose) (Muttucumaru et al., 2017; Xu et al., 2016). In our study, free asparagine was detected in melon seeds. According to Oddy et al. (2022), the targeting point should focus on reducing acrylamide content in roasted or baked goods as low as possible, since absence of acrylamide is not achievable. Recently, the European Commission is expected to set maximum levels of acrylamide in food products, as an attempt to protect public health (European Commission, 2023). As such, future work resulting from this thesis should focus on monitoring acrylamide formation in roasted melon seeds and reformulated bread, to quantify the levels of acrylamide formation and suggest preventative processing measures (moisture content, temperature, duration) to keep acrylamide to minimum levels in the finished products.

Another aspect of the thesis that could be followed up in future work is the potential of AEE (aqueous enzymatic extraction in **Chapter 4)** as means of melon seed oil extraction. The major challenge in AEE is oil yield, thus, improving oil yield is key work in the future. Other extraction technologies, such as ultrasonication and microwave, could be combined with AEE to improve the efficiency of enzyme hydrolysis and maximise oil yields (Mwaurah et al., 2020). On the other hand, as with similar aqueous based methods, during AEE, emulsification of the extracted oil was observed, which represents a limitation as it negatively influenced oil yield

and recovery (de Souza et al., 2020; Fang et al., 2016; Mat Yusoff et al., 2015). Therefore, in order to improve oil yield, a number of de-emulsification methods could be investigated to recover oil from the emulsion phase. Heating, freeze-thawing, pH, have been reported as efficient ones and could be explored in future work (Zhang et al., 2013). Besides, apart from the efficiency of de-emulsification methods, the quality of the recovered oil from emulsion phase is also worth investigating in the future work.

Based on the work presented in **Chapter 6**, defatted melon seed could successfully substitute wheat flour up to 10%. Sensory evaluation and flavour analysis could be conducted in the future, to identify any differences in attributes in the reformulated bread products. Consumer acceptance is an important determinant of the successful commercialization of either newly developed or reformulated products (Yang et al., 2020). Besides that, defatted melon seed breads exhibited improved nutritional value in terms of fibre content, and their potential as low-Glycaemic index food. These first findings could be followed up by human intervention studies, aiming to evaluate (1) postprandial glycaemic response after meal; (2) insulin response after meal; as well as (3) subjective appetite rating after meal (Binou et al., 2021, 2022; Zhao et al., 2023). The above suggested studies can provide useful information to assess this novel enriched bread potential as low-Glycaemic index food, and contribute to further develop and utilise defatted melon seed as valuable ingredient in food formulation.

7.3. References

Akintayo, E. T., & Bayer, E. (2002). Characterisation and some possible uses of Plukenetia conophora and Adenopus breviflorus seeds and seed oils. *Bioresource Technology*, *85*(1). https://doi.org/10.1016/S0960-8524(02)00073-1

Bi, S., Niu, X., Yang, F., Xu, Y., Dai, Y., Liu, Y., & Zhou, Q. (2022). Roasting pretreatment of walnut (Juglans regia L.) kernels: improvement of the oil flavor profile and correlation with the chemical composition. *Food and Function*, *13*(21). https://doi.org/10.1039/d2fo01990f

Binou, P., Stergiou, A., Kosta, O., Tentolouris, N., & Karathanos, V. T. (2022). Positive postprandial glycaemic and appetite-related effects of wheat breads enriched with either α-cyclodextrin or hydroxytyrosol/α-cyclodextrin inclusion complex. *European Journal of Nutrition*, *61*(7). https://doi.org/10.1007/s00394-022-02913-z

Binou, P., Yanni, A. E., Stergiou, A., Karavasilis, K., Konstantopoulos, P., Perrea, D., Tentolouris, N., & Karathanos, V. T. (2021). Enrichment of bread with beta-glucans or resistant starch induces similar glucose, insulin and appetite hormone responses in healthy adults. *European Journal of Nutrition*, *60*(1). https://doi.org/10.1007/s00394-020-02265-6

de Souza, T. S. P., Dias, F. F. G., Koblitz, M. G. B., & de Moura Bell, J. M. L. N. (2020). Effects of enzymatic extraction of oil and protein from almond cake on the physicochemical and functional properties of protein extracts. *Food and Bioproducts Processing*, *122*. https://doi.org/10.1016/j.fbp.2020.06.002

European Commission. (2023). *Acrylamide*. https://food.ec.europa.eu/safety/chemical-safety/contaminants/catalogue/acrylamide_en

Fang, X., Fei, X., Sun, H., & Jin, Y. (2016). Aqueous enzymatic extraction and demulsification of camellia seed oil (Camellia oleifera Abel.) and the oil's physicochemical properties. *European Journal of Lipid Science and Technology*, *118*(2). https://doi.org/10.1002/ejlt.201400582

Feizollahi, E., Mirmahdi, R. S., Zoghi, A., Zijlstra, R. T., Roopesh, M. S., & Vasanthan, T. (2021). Review of the beneficial and anti-nutritional qualities of phytic acid, and procedures for removing it from food products. *Food Research International*, *143*. https://doi.org/10.1016/j.foodres.2021.110284

Luo, K., & Zhang, G. (2018). Nutritional property of starch in a whole-grain-like structural form. *Journal of Cereal Science*, *79*. https://doi.org/10.1016/j.jcs.2017.09.006

Mat Yusoff, M., Gordon, M. H., & Niranjan, K. (2015). Aqueous enzyme assisted oil extraction from oilseeds andemulsion de-emulsifying methods: A review. In *Trends in Food Science and Technology* (Vol. 41, Issue 1). https://doi.org/10.1016/j.tifs.2014.09.003

Muttucumaru, N., Powers, S. J., Elmore, J. S., Dodson, A., Briddon, A., Mottram, D. S., & Halford, N. G. (2017). Acrylamide-forming potential of potatoes grown at different locations, and the ratio of free asparagine to reducing sugars at which free asparagine becomes a limiting factor for acrylamide formation. *Food Chemistry*, *220*. https://doi.org/10.1016/j.foodchem.2016.09.199

Mwaurah, P. W., Kumar, S., Kumar, N., Attkan, A. K., Panghal, A., Singh, V. K., & Garg, M. K. (2020). Novel oil extraction technologies: Process conditions, quality parameters, and

optimization. *Comprehensive Reviews in Food Science and Food Safety*, *19*(1), 3–20. https://doi.org/10.1111/1541-4337.12507

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., Oey, I., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. In *Food Control* (Vol. 79). https://doi.org/10.1016/j.foodcont.2017.03.027

Oddy, J., Raffan, S., Wilkinson, M. D., Elmore, J. S., & Halford, N. G. (2022). Understanding the Relationships between Free Asparagine in Grain and Other Traits to Breed Low-Asparagine Wheat. In *Plants* (Vol. 11, Issue 5). https://doi.org/10.3390/plants11050669

Rockenbach, I. I., Gonzaga, L. V., Rizelio, V. M., Gonçalves, A. E. de S. S., Genovese, M. I., & Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (Vitis vinifera and Vitis labrusca) pomace from Brazilian winemaking. *Food Research International*, *44*(4), 897–901. https://doi.org/10.1016/j.foodres.2011.01.049

Sandhu, A. K., & Gu, L. (2010). Antioxidant Capacity, Phenolic Content, and Profiling of Phenolic Compounds in the Seeds, Skin, and Pulp of *Vitis rotundifolia* (Muscadine Grapes) As Determined by HPLC-DAD-ESI-MS ^{n}. *Journal of Agricultural and Food Chemistry*, *58*(8), 4681–4692. https://doi.org/10.1021/jf904211q

Xu, F., Oruna-Concha, M. J., & Elmore, J. S. (2016). The use of asparaginase to reduce acrylamide levels in cooked food. In *Food Chemistry* (Vol. 210). https://doi.org/10.1016/j.foodchem.2016.04.105

Yang, Q., Shen, Y., Foster, T., & Hort, J. (2020). Measuring consumer emotional response and acceptance to sustainable food products. *Food Research International*, *131*. https://doi.org/10.1016/j.foodres.2020.108992

Zhang, S. B., Liu, X. J., Lu, Q. Y., Wang, Z. W., & Zhao, X. (2013). Enzymatic demulsification of the oil-rich emulsion obtained by aqueous extraction of peanut seeds. *JAOCS, Journal of the American Oil Chemists' Society*, *90*(8). https://doi.org/10.1007/s11746-013-2265-5

Zhao, C., Liu, Y., Lai, S., Cao, H., Guan, Y., San Cheang, W., Liu, B., Zhao, K., Miao, S., Riviere, C., Capanoglu, E., & Xiao, J. (2019). Effects of domestic cooking process on the chemical and biological properties of dietary phytochemicals. In *Trends in Food Science and Technology* (Vol. 85). https://doi.org/10.1016/j.tifs.2019.01.004

Zhao, W., Liu, Z., Fan, Z., Wu, Y., Lou, X., Liu, A., & Lu, X. (2023). Apple preload increased postprandial insulin sensitivity of a high glycemic rice meal only at breakfast. *European Journal of Nutrition*. https://doi.org/10.1007/s00394-022-03079-4

Appendix:

Appendix 1. List of conference, seminars, and publications

Conference/seminars:

1. Oral Presentation at 3rd FNS Research Symposium on 3rd November 2020 in University of Reading, under title “Valorisation of melon seed”.

2. Poster presentation at 4th FNS Research Symposium on 2nd November 2021 in University of Reading, under title “Valorisation of melon seed”.

3. Oral Presentation at Department of Food and Nutritional Sciences Seminar on 2nd February 2022 in University of Reading, under title “Evaluation of defatted melon seeds as functional food ingredient”.

4. Poster presentation at Total Food 2022 Conference on 13th July 2022 in University of Nottingham, under title “Evaluation of defatted melon seeds as functional food ingredient”.

Publications:

Chapter 6 of this thesis has been published as “Zhang, G., Chatzifragkou, A., Charalampopoulos, D., & Rodriguez-Garcia, J. (2023). Effect of defatted melon seed residue on dough development and bread quality. LWT, 183, 114892.”

Appendix 2. Extensibility curve of control and DMSR doughs.