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Upcycling of melon seed (*Cucumis melo* L.) oil processing by-product: evaluation of functional properties and nutritional components as novel ingredient

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Abstract

Background Defatted melon seed, a major by-product from melon oil processing chain, is scarcely utilised. However, it has high potential value and can be used as novel ingredient in food products production. In line with zero waste policy and food sustainability, exploring and utilisation of this oil processing by-product can reduce food waste, and is key to moving towards a more sustainable food system. Therefore, this study aimed to assess the nutritional profile and functional properties of three varieties of defatted melon seeds (Galia, Cantaloupe, and Honeydew), and then compare them with defatted pumpkin seeds (as control group).

Results In this study, three varieties of melon seeds (Galia, Cantaloupe, and Honeydew) and pumpkin seeds (as control group) were defatted using Soxhlet extraction with petroleum ether; subsequently, their functional properties and nutritional components were assessed. 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 in water/oil absorption capacity and emulsifying capacity. The phenolics were mainly free phenolics (FP) fraction (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 followed the same sequence (FP > CP > BP).

Conclusion Considering the nutritional composition, functional properties, and the presence of potentially bio-active compounds, defatted melon seeds have considerable potential to be used as a functional food ingredient for the reformulation of foods.

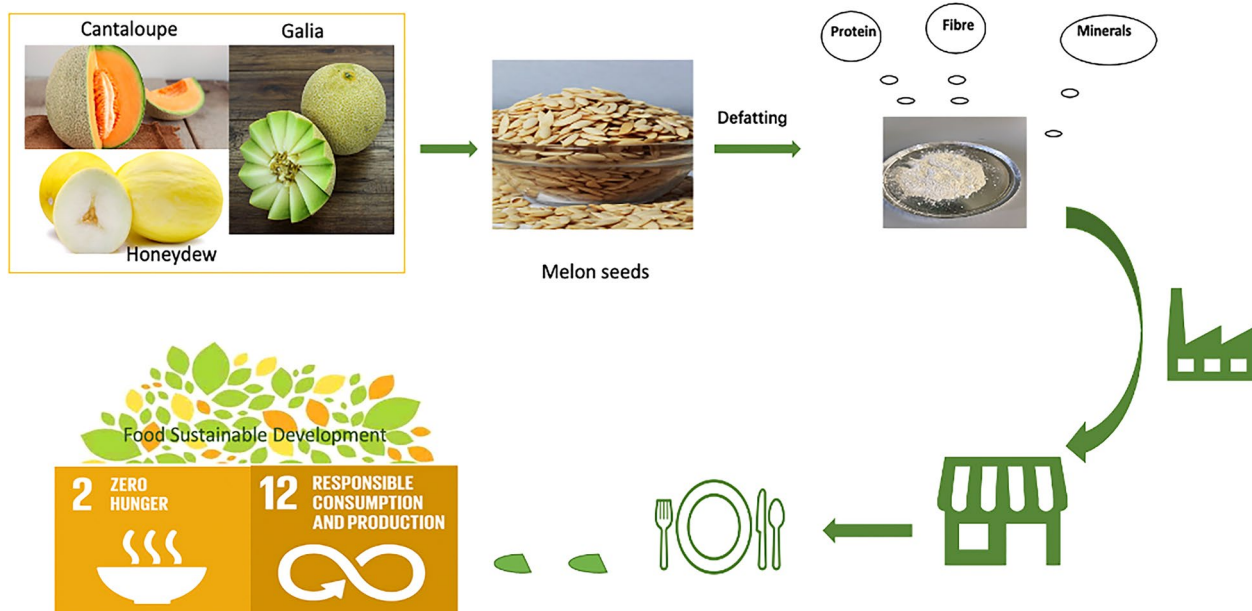
Keywords Defatted melon seed, Food by-product, Physicochemical properties, Functional food ingredient, Clean label, Food sustainable development

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Graphical Abstract



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 [1]. Despite of their potential nutritional value (e.g. protein, polysaccharide, and oil), generally, they are scarcely utilised for higher value applications, and are disposed to landfill due to the lack of sustainable management strategies [2, 3]. 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 [4]. 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 sector and other sectors (e.g. personal care, packaging, chemical and energy) [3, 5].

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

[6–8]. 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) [2, 9, 10]. The variation in the nutritional composition of melon seeds might be associated with the variety of melons, growing conditions, and seasonal variation of harvest [9, 11]. 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 [2, 9, 12–14]. Defatted melon seed 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 [15]. Many studies have been demonstrated that oil processing by-products have great potential value and can be re-utilised as novel and sustainable plant-based protein source and/or as potential ingredient in food applications, such as bakery products and meat products [15–18]. However, to date, there are insufficient 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).

Materials and methods

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-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate ($\geq 99\%$), ascorbic acid (analytical reagent grade), ferric chloride (ACS reagent), oxalate (99%), xylose ($\geq 99\%$, GC grade), arabinose ($\geq 99\%$), glucose ($\geq 99.5\%$, GC grade), vanillin (99%), catechin ($\geq 98\%$, HPLC grade), caffeic acid ($\geq 98\%$, HPLC grade), epicatechin ($\geq 90\%$, HPLC grade), p-coumaric acid ($\geq 98.0\%$, HPLC grade), protocatechuic acid (99.7%), cinnamic acid ($\geq 9\%$, HPLC grade), ferulic acid, gallic acid, syringic acid (98%), sinapic ($\geq 98\%$), and gentisic acid (98%) were purchased from Sigma Aldrich (UK).

Materials and preparation process

The Galia melon (from Honduras), Honeydew melon (from Brazil), Cantaloupe melon (from Brazil) were obtained from Sainsbury's Supermarket (Reading, UK). 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 using 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.

Proximate analysis

The proximal composition of grounded seed powder was determined by the AOAC standard methods [19]. More specifically, ash was determined by the AOAC 923.03 method, lipid by the AOAC 930.90 method, and protein ($\text{N} \times 6.25$ 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 carbohydrate composition was determined using the protocol by the National Renewable Energy Laboratory, NREL/TP-510-42618 [20]. 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 $\times$ 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. The acid-soluble lignin was measured using filtered acid hydrolysed sample with a UV-Vis spectrometer at 320 nm. The acid-insoluble lignin was measured by gravimetric analysis and was calculated as [the solid residue after hydrolysis – (ash of solid residue after hydrolysis + protein content of sample)].

Anti-nutritional compounds

Phytic acid

The phytic acid content was determined using a phytic acid kit (Megazyme, Ireland) and following the manufacturer's assay procedure [21]. 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 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. The phytic acid content was calculated by the following equation (provided by Megazyme).

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

Tannins

The tannins content of sample was determined according to Shawrang et al. [22] described with certain modifications. 0.5 g of sample was extracted with 10 mL of methanol at room temperature for 12 h. Then, 1.5 mL of extract was collected and centrifuged at 13,000 rpm for 10 min. After this step, 1 mL of supernatant was mixed with 5 mL of freshly vanillin-HCl reagent (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. Catechin was used for constructing a calibration curve. The tannins content was expressed as mg of CE (catechin equivalent)/100 g of dry weight (DW).

Free amino acids

The amino acid composition was determined according to Curtis et al. [23]. Briefly, 0.5 g of sample was mixed with 10 mL of 0.01 M of HCL for 15 min at room temperature and then was centrifuged at 7,200 rpm for 15 min. 100 μ L 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 $\times$ 0.25 $\times$ 0.25). The analytical conditions were as follows: the oven temperature was held initially at 110 $^{\circ}$ C for 1 min, then increased at a rate of 30 $^{\circ}$ C/min to 310 $^{\circ}$ C; the temperature of the transfer line and ion source were kept 320 $^{\circ}$ C and 230 $^{\circ}$ 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 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.

Functional properties

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. [24] with slight modifications. Briefly, 0.5 g of seed powder was added into a weighted centrifuge tube (as m1) and then 5 mL distilled water were added for WAC and 3 mL olive oil for OAC, respectively. The mixture was vortexed for 1 min and left standing for 30 min; it was then centrifuged for 20 min at 3,000 rpm. The upper layer was removed, and the residue was weighted (as 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:

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

where, m2 is the weight of residue plus centrifuge tube; m1 is the weight of the centrifuge tube; Ws is the sample weight.

Foaming capacity and stability

The foaming capacity (FC) and foaming stability (FS) were measured according to Embaby & Rayan [25] with some modifications. 25 mL distilled water were added to 0.5 g sample 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:

$$\text{FC (\%, v/v)} = \frac{(V2 - V1)}{V1} \times 100\%$$

V1: volume of mixture before homogenisation; V2: volume of mixture plus the foam; The foaming stability (FS) was calculated as the ratio of the foam volume change after 30 min

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

Emulsifying capacity

The emulsifying capacity (EC) was determined according to Shi et al. [26] with some modifications. 5 mL distilled water were added to 0.5 g sample 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 $\times$ g and the volume of the emulsion was measured (V2). The emulsifying capacity (% v/v) was calculated by:

$$\text{EC (\%, v/v)} = \frac{V2}{V1} \times 100\%$$

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

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

Extraction of free phenolics

The extraction of the free phenolics fraction was conducted as described by Shewry & Ward [27] with slight modifications. Briefly, 0.025 g sample powder was treated three times with 1 mL of 80% (v/v) aqueous methanol in an ultrasonic bath for 10 min. Each time, the mixture was centrifuged for 15 min at 5,000 rpm and then the

supernatant collected and pulled together; the residue was also collected and stored at -20°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°C until further analysis.

Extraction of conjugated phenolics

The extraction of the conjugated phenolics fraction was conducted as described by Shewry & Ward [27]. 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 for 5 min at 13,200 rpm, and then the supernatant collected and pulled 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°C for further analysis.

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 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 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°C for further analysis [27].

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 Zhang et al. [28] with slightly modifications. Briefly, 200 μL of each phenolic fraction were mixed with 1 mL of Folin-Ciocalteu reagent (diluted tenfold 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. Gallic acid standards (0–10 mg/L) were used to construct a standard curve. The total phenolic acid content was expressed

as g of gallic acid equivalent (GAE)/kg of dry weight (DW).

Antioxidant activity

DPPH radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted as described by Yasir et al. [29] with slight modifications. Briefly, 100 μL of each phenolics fraction were mixed with 1.5 mL of 0.1 mM DPPH 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. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standards were used to construct a standard curve and the DPPH radical scavenging activity values were expressed as mmol of TE (Trolox equivalent) per kg of dry weight (DW) sample.

Ferric reducing antioxidant potential (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was conducted as described by Dudonné et al. [30] 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. Ascorbic acid standards were used to construct a standard curve. The result was expressed as mmol of AA (ascorbic acid)/kg of dry weight (DW) sample.

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 $\times$ 4.6 mm), according to Lima et al. [31]. The mobile phase included two solvents: (A) 1% (v/v) 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, protocatechuic acid, caffeic acid, catechin, syringic acid, epicatechin, sinapic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, ferulic acid, gentisic acid) were identified and quantified by comparing to the retention times and areas of individual standards.

Statistical analysis

All the experiments were carried out in triplicate unless otherwise stated. Results are expressed as mean $\pm$ 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.

Results and discussion

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 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 melon seeds [32]. 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%) [17, 33–35]. 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 high amounts in 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 slightly lower than defatted pumpkin seeds (12.2% w/w). 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 [36]. Besides, lignin has received much attention

Table 1 Chemical composition of defatted Galia, Cantaloupe, Honeydew melon seeds and defatted pumpkin seeds

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

Data represent mean $\pm$ standard deviation (n = 3). Values with the different superscript letters in the same row are significantly different ($p < 0.05$)

and exhibited promise in the applications of polymeric materials, such as nanocomposites, biosorbents, and hydrogels [37]. Overall, it was suggested that defatted melon seeds could be considered as a relatively good source of lignin and its prospects in the field of lignin could be further investigated and evaluated.

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 [13]. 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 [38, 39]. 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) [38, 40]. 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.

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 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 of minerals, and can therefore reduce their bioavailability, for example for calcium, iron, and zinc [41]. 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) [42]. Overall, it could be considered that the contents are relatively high and could interfere the bioavailability of

melon seed's minerals, but could be effectively reduced through processing such as thermal treatment, fermentation, and soaking [43, 44].

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 [45, 46]. From Table 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) [44] indicating that the defatted melon seeds contain relatively low amount of tannins, which is beneficial to its nutritional quality and protein digestibility.

Free amino acids

The free amino acids of the three defatted melon seed varieties and the defatted pumpkin seeds (control) are presented in Table 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. Free amino acids are important to ingredient flavour, indicating that all defatted melon seeds flavour could have a lighter 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 [47]. Glycine, serine, and alanine can contribute sweet taste, whereas glutamic acid can provide umami taste to the defatted melon seeds [48]. It was suggested that defatted melon seeds could be used as potential flavour (e.g. sweet and umami) ingredient in food development.

Table 2 Amino acid profile (mmol/kg of DW) of defatted Galia, Cantaloupe, Honeydew, and defatted 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 mean ± standard deviation (n = 3). Values with the different superscript letters in the same row are significantly different ($p < 0.05$)

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 [49–51]. According to Table 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. [52] reported the GABA contents in commercial cereals including corn (199 nmol/g dry weight), barley (190 nmol/g dry weight), and brown rice (123 nmol/g dry weight). The GABA contents of all defatted melon seeds were higher than Oh et al. [52] report, indicating that the defatted melon seeds could be used as a good source of GABA in everyday diet.

Functional properties

The results from the analysis of the functional properties of defatted melon seeds are presented in Table 3.

Table 3 Functional properties of defatted Galia, Cantaloupe and Honeydew melon seeds, and defatted 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 mean ± standard deviation (n = 3). Values with the different superscript 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.

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) [53]. 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 [32, 54]. Joshi et al. [55] and Rodríguez-Miranda et al. [16] reported that defatting could improve the water absorption capacity, especially for seed powders with high lipid contents; 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 [56].

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) [55]. The higher OAC is most likely associated with the number of nonpolar sites in protein [16]; in addition, the second structure of protein could also affect the OAC [57]. The OAC can be an important parameter because it can influence flavour

and texture [58]. 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).

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 [59]. 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 [16]. The high foaming stability of defatted melon seeds are expected to contribute key functionalities when the seeds are incorporated in bakery and confectionery products.

Emulsifying capacity (EC)

The emulsifying capacity (EC) of food ingredients is an important property for food applications such as ice cream and bakery [58]. 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 [56, 60]. Recently, 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 [61, 62]. 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 [63]. Overall, these

results indicate that that melon seed powders are natural good emulsifying agents and could be potentially used in bakery products [2, 8].

Total phenolic content and antioxidant capacity

Total phenolics 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 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 [64, 65]. To the best of our knowledge, this is the first report on the three fractions of 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, indicating that defatted melon seeds could be considered as a relatively good source of phenolics. For all defatted seeds in this study, the order was as follows: FP > CP > BP.

Antioxidant capacity

Table 4 shows the results of antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) 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 [66, 67]. Similar high positive correlation, i.e. of DPPH and FRAP with TPC, was observed in the present study (Table 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. [68] 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

Table 4 The total phenolic content (TPC) and antioxidant capacity of defatted Galia, Cantaloupe and Honeydew melon seeds, and defatted pumpkin seeds

Sample	FP	CP	BP	Total
Total phenolic acid content (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
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 (R²)		DPPH		FRAP
TPC		0.92		0.96

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

groups, and the reaction mechanisms of each antioxidant assay is different, hence phenolic compounds can be seen to behave differently in various antioxidant assays [67–69].

The profile of phenolic acids

Table 5 shows 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. [70], Zeb [71] and Kolayli et al. [11] 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 [71] 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 differences in variety, region, soil conditions, growing condition, harvest times, and degree of maturity at harvest [72–74]. 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 demonstrating 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 needed 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 Free, conjugated, bound phenolics content (mg/100 g of DW) in defatted Galia, Cantaloupe, Honeydew melon seeds, and defatted 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	ND
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	75.7

Data represent mean ± standard deviation (n = 3). Values with the different superscript letters in the same row are significantly different (p < 0.05). ND: not detected

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. Future work will be conducted on the reduction of phytic acid content in defatted melon seeds through processing methods, such as fermentation, soaking, thermal

(e.g. extrusion and roasting) and non-thermal methods (e.g. cold plasma pulsed electric fields).

Supplementary Information

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Supplementary file 1.

Author contributions

GZ: Conceptualization, Methodology, Investigation, Resources, Writing—original draft, Writing—review & editing. ZL: Methodology, Writing—original draft, Writing—review & editing. DC: Conceptualization, Methodology, Writing—original draft, Writing—review & editing.

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Competing interests

The authors declare that they have no competing or interests.

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