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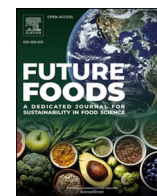
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Effect of processing methods on the phytochemical content of melon seeds (*Cucumis melo* L.)

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ABSTRACT

This study aimed to investigate and evaluate the effect of processing (soaking, boiling, and roasting) on the phytochemical content of melon seeds. Two varieties of melon seeds (Galia and Cantaloupe) were processed by three processing 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 lipid, 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 tannins by 40 % - 114 % and phytic acid contents by 3 % - 5 %. Of the three processing methods, roasting was most effective in remaining the nutritional value of melon seeds, and boiling was most effective in reducing tannins content. Overall, this study could guide the development of appropriate melon seed processing method to retain high nutritional value.

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 (Namet et al., 2023; Zhang et al., 2023). Melons are increasingly cultivated and consumed due to their sweet flesh and attractive aroma, with the global production being about 28.5 million tons in 2022 (FAOSTAT et al., 2022). In the UK, the most consumed melon varieties are Galia, honeydew, and Cantaloupe, with the annual fresh melon consumption can reach around 220,000 tons (Frankowska et al., 2019). Melon seeds (accounts for 10 % of melon weight), is one of the major by-products 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 values, attributed to their high levels of proteins (15 % - 45 %), lipids (25 % - 45 %), dietary fibre (19 % - 25 %), and minerals (rich in potassium), indicating that they could be a potential valuable food ingredient (De Melo et al., 2000; Mallek-Ayadi et al., 2018; Mian-Hao and Yansong, 2007; Petkova

and Antova, 2015; Rabadán et al., 2020). Besides, It has been shown that melon seeds are rich in various valuable bioactivity compounds, such as phenolics, tocopherols, and phytosterols, which are beneficial to human health (Namet et al., 2023; Mallek-Ayadi et al., 2019).

Despite their rich nutritional profile, melon seeds are not generally included in culinary or food formulations and have hardly been used as food ingredient in most countries, since melon seeds are still regarded as food waste in their concept. 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). The above evidence shows the possibility of melon seeds as an edible food ingredient and offers a way to upcycle melon seeds.

Nevertheless, melon seeds are still as an uncommon ingredient, and are even regarded as waste in most of countries, therefore, melon seeds need to be processed to improve their edibility as well as safety before consumption (Tenyang et al., 2017). In general, fermentation, roasting, soaking, boiling, popping, and extrusion are common processing technologies used in food production to improve food safety and edibility. In

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terms of processing technologies, soaking, boiling, and roasting are commonly used in both domestic cooking and food industry, indicating that these methods are important edible processing technologies and are widely accepted by consumers (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). Babiker et al. (2021) found that the gallic acid content and iron content in hemp seeds (*Cannabis sativa* L.) increased significantly ($p < 0.05$) after 14 min roasting (160 °C), from 5.0 mg to 22.0 mg/100 g and 10.0 to 12.3 mg/100 g, respectively. In contrast, processing could result in nutritional value loss, for example through leaching of minerals and lipid oxidation (Yang et al., 2014; Zhao et al., 2019). For example, Tonfack Djikeng et al. (2018) reported that the calcium content decreased significantly ($p < 0.05$) from 1119.35 to 568.00 mg/100 g after 60 min boiling of walnut seeds (*Tetracarpidium conophorum*). Additionally, Jain et al. (2016) studied the roasting effect on the fatty acid profile of garden cress seeds (*Lepidium sativum*) and found that the unsaturated fatty acid content decreased significantly ($p < 0.05$) after roasting for 3 min at 150 °C, from 83.60 % to 79.02 %.

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, since it can convert food waste to edible ingredient and could result to products with good nutritional quality that are suitable for human consumption (Roobab et al., 2022; Zia et al., 2023). Furthermore, it provides an opportunity to food by-products to re-introduce into the food chain, thereby promoting their value-added development as novel sustainable foods and extending the mass of edible foods for human consumption (Maletti et al., 2022; Zia et al., 2023; Zhang and Li, 2024). Above all, this can advocate for food industries to develop sustainable food in production and help consumers to easily to access the healthy and nutritionally sustainable food in their daily diet, thereby strengthening the sustainability and resilience of the food system and achieving the sustainable development goals 12 (ensure food sustainable production and consumption) (Zhang et al., 2023). For example, Maletti et al. (2022) recovered watermelon rinds and processed it through candying processing, and demonstrated that the watermelon rinds can be converted into edible candied. However, to date, there is no available study on the effect of processing on the phytochemical content 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. To better reduce melon seed waste in the UK and convert it to sustainable food, two of the most consumed varieties of melons in the UK (Galia and Cantaloupe) were selected and their seeds were collected as this study object. This study 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 as sustainable food.

2. Materials and methods

2.1. Chemical 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).

2.2. Melon seed preparation

Two most consumed melons in the UK (Galia and Cantaloupe) were purchased from Waitrose Supermarket (Reading, UK). Melon fruits 1 - 1.5 kg with uniform size. 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 2.3. A portion of the raw melon seeds (control group) was dried at 50 °C for 16 h in a tray dryer (Model No. U0P8, Armfield, Ringwood, England), grounded in a food grinder (Caterlite, CK686, Bristol, UK), passed through a 600 μ m standard sieve, and then sealed in a plastic container and stored at -20 °C until further analysis (Zhang et al., 2023). This dried melon seeds without any processing was used as control group for comparison with processed groups.

2.3. Processing methods

2.3.1. Soaking

The soaking method was followed Sahni & Sharma (2020) description with slight modifications. Briefly, 100 g 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 soaking, the seeds were dried at 50 °C for 16 h in a tray dryer (Model No. U0P8, Armfield, England), grounded, and then passed through a 600 μ m standard sieve. The seed powder was sealed in a plastic container and stored at -20 °C until further analysis.

2.3.2. Boiling

The boiling method was followed Choe et al. (2022) description with slight modifications. Briefly, 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 to achieve the soft texture to consumption. 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 (Model No. U0P8, Armfield, England), grounded, and then passed through a 600 μ m standard sieve. The seed powder was sealed in a plastic container and stored at -20 °C until further analysis.

2.3.3. Roasting

The roasting method was followed Tenyang et al. (2022) description with slight modifications. Briefly, 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 to achieve crispy but not burnt effect. The roasted melon seeds were grounded and then passed through a 600 μ m standard sieve. The seed powder was sealed in a plastic container and stored at -20 °C until further analysis.

2.4. Proximate analysis

The moisture content was determined using a moisture analyser (MA37 - 1, Satorius, Goettingen, Germany). The protein (conversion factor used was 6.25), lipid, and ash content were determined according to AOAC method (AOAC et al., 2005).

The carbohydrate composition was determined according to Sluiter et al. (2008) description. Briefly, melon seed powders (300 mg) were hydrolysed with 3 mL of (72 %, v/v) H₂SO₄ and incubated in a water bath at 30 °C for 1 h. Afterwards, the mixture was diluted by adding 84 mL distilled water and autoclaved (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, UK) with an Aminex HPX-87H column (300×7.8 mm); the operating conditions were as follows: 0.005 M sulphuric acid was used as mobile phase and the flow

rate was 0.6 mL/min, column temperature was at 65 °C. Calibration standard curves were constructed 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 following the formulation, as list below. The calorific values (kcal/100 g) were calculated using Atwater general factor system (energy values of 4 kcal/g for carbohydrate, 4 kcal/g for protein, and 9 kcal/g for lipid).

$$W_{\text{acid-insolublelignin}} = W_{\text{solidafterhydrolysis}} - (W_{\text{ashsolidafterhydrolysis}} + W_{\text{pos}})$$

Where,

$W_{\text{solid after hydrolysis}}$ = the solid residue after hydrolysis (g); $W_{\text{ash solid after hydrolysis}}$ = ash of solid residue after hydrolysis (g); W_{pos} = amount of protein present in the solid residue after hydrolysis (g)

2.5. Mineral content

The mineral content (calcium, zinc, magnesium, potassium, and iron) was analysed according to Mbuma et al. (2022) description with slight modifications. Briefly, melon seed sample (1 g) was ashed. The ash was digested with 5 mL of concentrated hydrochloric acid (36 %) on a hot plate (100 °C) for 30 min. After digestion, the sample was diluted to 50 mL using water (HPLC grade), and then determined the minerals using an atomic absorption spectrophotometer (Nov AA 350, Analytik Jena GmbH, Germany). Potassium was determined using a flame photometer (PFP7, Janway, UK).

2.6. Anti-nutritional compounds analysis

2.6.1. Phytic acid

The phytic acid content was measured using a Phytic Acid Assay Kit (Megazyme, Ireland) and following manufacturer's assay procedure (Megazyme, 2017). Briefly, melon seed sample (1 g) 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 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 (provided by Megazyme).

$$\text{Phyticacidcontent} = \frac{\text{Phosphorus (g/100)}}{0.282}$$

2.6.2. Tannins

The tannins content was determined according to Shawrang et al. (2011) description with slight modifications. Melon seed sample (0.5 g) was extracted with 10 mL of methanol at room temperature for 12 h. 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 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), and then was incubated for 20 min at room temperature. Afterwards, 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.

2.6.3. Oxalate

The oxalate content was determined following Israr et al. (2013) description. Briefly, melon seed sample (1 g) was added into 50 mL of 1 M of H₂SO₄ and incubated in a water bath at 15 °C for 15 min. After incubation, the mixture was made up to 100 mL with 1 M of H₂SO₄. 1 mL of the mixture was centrifuged at 3000 rpm for 15 min and the supernatant was filtered and then analysed by HPLC. Agilent 1260 series (Agilent technologies, UK) was used with an Aminex ion exclusion HPX-87H (300×7.8 mm) analytical column. The analytical conditions as follow: 0.005 M H₂SO₄ was used as mobile phase solution, flow rate was

at 0.6 mL/min, column temperature at 65 °C, and detector was set at 210 nm. A calibration curve with oxalate standards was constructed for quantification.

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, melon seed sample (0.1 g) was mixed with 6 M HCL in a sealed tube with nitrogen flushed; the suspension was hydrolysed at 110 °C for 24 h. Afterwards, the hydrolysate was used for its amino acid content analysis 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 (100×0.25×0.25). The analytical conditions were as follows: oven temperature was initially at 110 °C (held for 1 min), then increased up to 310 °C at a rate of 30 °C/min; the temperature of the transfer line and ion source were kept 320 °C and 230 °C, respectively; the carrier gas was helium, flow rate at 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 in the EZ-Faast Kit. Analysis was performed in duplicate.

2.8. Fatty acid composition analysis

The fatty acid composition was determined according to Milinsk et al. (2008) description with slight modifications. 50 mg melon seed oils (obtained by Soxhlet extraction) were added to 2 mL of 0.5 M sodium methoxide solution 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 for 15 min. After that, the upper layer was collected and then 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 (100×0.25×0.2). The carrier gas was helium; the flow rate was 1.5 mL/min; split injection system with a splitting ratio 1:50; the temperature of the injection and detector were kept at 250 °C and 280 °C, respectively. The oven temperature program was initially at 120 °C (held for 1 min), then increased up to 175 °C at 10 °C/min and held for 10 min, increased to 210 at 5 °C/min and held for 5 min, and finally to 230 °C at the same rate and held for 10 min. The compositions of fatty acids were identified by comparison of retention time of FAME mixture standards. The individual fatty acid composition was expressed as a relative percentage of total fatty acids identified (%).

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. Results and discussion

3.1. Proximate composition

Table 1 shows 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 and Yansong, 2007; Yanty et al., 2008) who reported a range of 25 % - 35% w/w for lipid and

Table 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 deviations (*n* = 3). Values with different lowercase letters in the same row within each variety are significantly different (*p* < 0.05).

15 % - 29% w/w for protein. These differences regarding the lipid and protein contents 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). 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, 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 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, roasted melon seeds showed significantly lower (*p* < 0.05) moisture content than the control melon seeds. This could be associated with water evaporation in the high temperature. 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. According to Li et al. (2022), water migration is related to the chemical components and structure characteristics of seeds, different types of seeds and the uneven distribution of chemical components result in different structural characteristics. Therefore, this heterogeneity affects the immigration rate of water, leading to different changes after processing. A significant decrease (*p* < 0.05) was observed in protein content for both of varieties of melon seeds after boiling. This result was in line with previous studies, which reported a decrease in protein content was observed in 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). Additionally, according to DeVries et al. (2017) and Gao et al. (2015), the principle of Kjeldahl method is to measure 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, a significant increase (*p* < 0.05) in lipid content 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 structure change of oil body, after boiling, the oil body lipoprotein membranes could be altered thereby enhancing oil release from oil bodies and membranes, resulting in increased oil extractability (Cai et al., 2021). Besides, no significant changes (*p* > 0.05) were observed in ash content in both of varieties of melon seeds after the three processing methods. Regarding with carbohydrates, a significant increase (*p* < 0.05) in glucose content was observed for both varieties of melon seeds after roasting. It could be explained that roasting disrupted the crystalline structure of lignocellulosic component of the seeds, causing the release of the free cellulose from its lignin seal (McIntosh and Vancov, 2011).

3.2. Mineral content

Table 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)

Table 2
The mineral content of Galia and Cantaloupe seeds after different processing methods.

Galia	Minerals (mg/100 g DW)				
	Potassium (K)	Magnesium (Mg)	Calcium (Ca)	Iron (Fe)	Zinc (Zn)
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 deviations (*n* = 3). Values with different lowercase letters in the same column within each variety are significantly different (*p* < 0.05).

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 in melon seeds (Mallek-Ayadi et al., 2018; Morais et al., 2017). Compared to the above studies, the potassium content in 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 growing 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 et al., 2012), therefore, the high amount of potassium in the melon seeds, indicates that they could be potentially used as a potassium food source.

After processing, significant changes were observed in some cases. In terms of potassium, a significant reduction ($p < 0.05$) was observed in the Cantaloupe variety of boiled melon seeds compared to the control melon seeds. The decrease in potassium could be associated with leaching effect (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. In addition, the iron content showed a significant increase ($p < 0.05$) in roasted Cantaloupe melon seeds as compared to control Cantaloupe seeds. Tenyang et al. (2022) reported a similar result for sunflower seeds after roasting (at 120 °C for 30 min), as the zinc and iron content increased from 7.37 to 10.02 (mg/100 g) and 10.62 to 14.80 (mg/100 g), respectively. 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 and bound iron; in addition, this change varied depending on the structure and binding degree of complexes and processing conditions. In contrast, the magnesium and calcium contents showed no significant difference ($p > 0.05$) for both varieties of melon seeds after processing.

3.3. Fatty acid profile

Table 3 shows the fatty acid content of the two varieties of extracted melon seeds oil from control (without any processing) and processed melon seeds; 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 seed oil, 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). However, the linoleic acid content in this study was higher than that the linoleic acid content of the above studies (66.4 % - 69.2 %) (Mallek-Ayadi et al., 2018; Rabadán et al., 2020; Yanty et al., 2008), the reason could be related to the variety and climate conditions (Gonzalez et al., 2012; Zemour et al., 2021; Zhang et al., 2024). Comparing the linoleic acid content of melon seed oil (74.9 % - 75.7 %) 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 potentially 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 (UFA) and reducing the risk of cardiovascular disease (Bowen et al., 2019; Marangoni et al., 2020). Besides, it has been reported that the ratio of polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) is used to assess nutritional value of oil for human health, with the PUFA/SFA ratio above 0.45 considered acceptable (Tenyang et al., 2017; Xu et al., 2023). In this study, the PUFA/SFA ratios were 5.0 - 5.3, indicating that melon seeds oil is safe and more beneficial to human health. The presence of high content of UFA (around 85 %) and PUFA/SFA ratio (5.0 - 5.3) in melon seeds oil demonstrates its potential to be considered as a novel oil source into the human diet.

During processing, the levels of UFA was affected, primarily in linoleic acid content. After roasting, the linoleic acid content was significantly decreased ($p < 0.05$) for both varieties of melon seeds oil (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 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) 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 (from 74.9 % reduced to 68.3 %), while it did not significantly change ($p > 0.05$) in 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). Additionally, soaking processing resulted in

Table 3
The fatty acid composition of Galia and Cantaloupe seeds oil after different processing methods.

Fatty acids (%)	Control	Galia Soaking	Boiling	Roasting	Control	Cantaloupe 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
Stearate 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
PUFA/SFA	5.3	5.0	4.4	4.7	5.0	4.9	4.9	4.6

Data represented as mean ± standard deviations ($n = 2$). 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.

similar changes to boiling, a decrease in the linoleic acid content was observed only in the Galia variety (74.9 % reduced to 71.9 %). Overall, these results indicate that roasting has a more negative effect on the linoleic acid content compared to boiling and soaking.

3.4. Amino acid profile

Table 4 shows the amino acid profile of the two varieties of melon seeds without any processing (control), and after processing. 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 and 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, some proteins have bound amino acid side chains that can react chemically with each other or with other components (e.g. fat and polysaccharides) under appropriate conditions, resulting in a change in the 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).

3.5. Analysis of anti-nutritional compounds

Table 5 shows the anti-nutritional compounds of the two varieties of melon seeds without any processing (control), and after processing. Phytic acid, tannins, and oxalate 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, 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).

Tannins are water-soluble phenolic compounds, and their anti-nutritional effect is associated with protein digestibility. They can inhibit digestive enzymes or bind with proteins resulting in the formation of complexes, thus, interfering with protein digestibility (Nikmaram et al., 2017). The tannins content in melon seeds ranged from 7.8 - 9.8 (mg CE/100 g). Soaking and boiling resulted in significant tannins reduction ($p < 0.05$) in 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, tannins content was significantly increased ($p < 0.05$) after roasting in both varieties. Godrich et al. (2023) observed a similar result as tannins increased in chickpeas and red kidney beans after roasting (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.

Oxalate 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

Table 4
Amino acid composition (% in g/100 g seed DW) of Galia and Cantaloupe seeds after different processing methods.

Essential amino acids	Control	Galia Soaking	Boiling	Roasting	Control	Cantaloupe 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 deviations ($n = 2$). Values with different lowercase letters in the same row within each variety are significantly different ($p < 0.05$).

Table 5

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

Anti-nutritional compounds	Control	Galia Soaking	Boiling	Roasting	Control	Cantaloupe 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
Oxalate (mg/100 g DW)	ND	ND	ND	ND	ND	ND	ND	ND

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

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 foods. Comparing melon seeds with the above results, it can be suggested that melon seeds could be as low oxalate foods.

Conclusions

This study demonstrated that melon seeds are a good food source of protein, oil, minerals and dietary fibre. Additionally, the high content 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, 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); later could be unfavourable for the bioavailability of nutrients (e.g. mineral and protein). Soaking reduced the tannins content (by 13 - 20 %), although not as effectively as boiling. Overall, among the three processing methods in this study, roasting was most effective for retaining nutritional value, and boiling was most effective in reduction of anti-nutritional compounds (tannins). To this end, future work will aim to investigate the sensory characteristics (e.g. colour, flavour) and acceptability of melon seeds as well as the bioavailability of melon seed nutrients (e.g. protein and minerals) under processing methods. Besides, traditional processing methods involving high temperatures can result in unfavourable degradation of food nutritional quality (i.e. loss of linoleic acid, as mentioned earlier). Based on this point, in order to better maintain the seed nutritional quality, the effect of novel processing technologies on the nutritional quality of melon seed can be investigated. To this end, some promising non-thermal processing technologies, such as pulsed electric fields, pulsed light, and cold plasma, can be conducted in future work to overcome the limitations of the traditional processing technologies to provide a novel food ingredient with high nutritional quality.

Ethical statement

This work does not involve trials on any human or animals

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Guoqiang Zhang: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Ziqian Li:** Writing – review & editing. **Afroditi Chatzifragkou:** Writing

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Declaration of competing interest

The authors declare that they have no competing or interests.

Data availability

All data generated and analyzed during the current study are included in this published article.

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