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Journal of Agriculture and Food Research 16 (2024) 101182

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Comparative extraction of melon seed (*Cucumis melo* L.) oil by conventional and enzymatic methods: Physicochemical properties and oxidative stability

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ARTICLE INFO

Keywords:
Melon seed oil
Linoleic acid
Squalene
By-products
Food waste valorisation
Extraction method

ABSTRACT

Oil was extracted from melon seeds with three different methods (Soxhlet, cold-pressed, aqueous enzymatic extraction), aiming to evaluate its physicochemical properties and oxidative stability. The melon seed oil contained high levels of linoleic acid (53.6 %–70.8 %, w/w), squalene (101.1–164.7 mg/100 g), and β-sitosterol (119.5–291.9 mg/100 g). Results showed that the choice of the extraction method did not alter the fatty acid composition, but impacted on the physicochemical properties, the content of bioactive compounds and oxidative stability of the oil. Specifically, melon seed oil obtained by aqueous enzymatic extraction (AEE) exhibited higher tocopherol content and better oxidative stability compared to the oil obtained by other two extraction methods. Overall, AEE is a promising oil extraction method and could be an alternative to conventional oil extraction methods that could be implemented for the production of high-quality melon seed oil.

1. Introduction

Edible oil is an important ingredient in daily diet, providing nutritional and phytochemical compounds associated with energy and health benefits [1]. With the increasing world population, the identification of sustainable alternative oil sources with high nutritional quality is necessary to satisfy the growing food demand. On the basis of developing resilient food systems, oil recovery from agricultural residues or by-products has recently attracted research interest [2,3].

Melon (*Cucumis melo* L.) belongs to the *Curcurbitaceae* family and is one of the most important commercial tropical fruits cultivated in the world. Melon seeds represent 10 % of the total melon weight; currently, they are underutilised and are usually discarded as waste [4]. Recent studies have shown that melon seeds contain considerable amounts of oil (30.7 %–44.5 %, w/w) [5,6], and is rich in unsaturated fatty acids and bioactive compounds, such as linoleic acid and tocopherols, respectively [7,8].

Conventional solvent and cold-pressed extraction methods are widely used in the oil industry. Conventional solvent extraction uses large amounts of organic or nonpolar solvents, and has limitations due to reduced oil quality [9]. In contrast, cold-pressed extraction takes place in the absence of organic solvents and can maintain oil quality, but may cause lipid oxidation issues [10]. The limitations of these traditional extraction methods have prompted the development of novel, environmentally friendly, and sustainable extraction processes that yield edible oil of high quality. Aqueous enzymatic extraction (AEE) has received much attention in this regard [11]. Compared with traditional extraction methods, the use of water as extraction solvent and the potential of enzyme reusability are major advantages in AEE [12]. Nevertheless, low oil recovery is one of the major challenges for AEE; recent studies have shown that its combination with other extraction technologies such as ultrasonication and microwave, could improve oil yield [13,14]. Studies have also reported that oil quality and the recovery nutritional components can vary depending on extraction methods [7,9]. However, to date, there is scarce information regarding the quality and nutritional value of melon seed oil obtained by different extraction methods.

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

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https://doi.org/10.1016/j.jafr.2024.101182
Received 15 March 2024; Received in revised form 6 April 2024; Accepted 24 April 2024
Available online 24 April 2024

2. Materials and methods

2.1. Chemicals and standards

Methanol (HPLC grade), 2-propanol (Laboratory reagent grade), n-hexane (HPLC grade), petroleum ether (laboratory reagent grade), and acetonitrile (HPLC grade) were purchased from Fisher Scientific (UK). Tri-sil HTP reagent was purchased from Thermo Scientific (UK). Fatty acid methyl esters (FAME) standard mixture (C4–C24) and isooctane (used for gas chromatography) were purchased from Supelco (UK). Protease (from *Bacillus amyloliquefaciens*), cellulase (from *Trichoderma reesei*), 5α-cholestan-3β-ol (≥95 %), sodium methoxide solution (0.5 M, ACS grade), β-sitosterol (≥95 %), cholesterol (≥99 %, analytical grade), squalene (≥98 %), campesterol (~65 %), stigmasterol (~95 %), α-tocopherol (≥96 %, HPLC grade), γ-tocopherol (≥96 %, HPLC grade), and δ-tocopherol (≥90 %) standards were purchased from Sigma Aldrich (UK).

2.2. Melon seed preparation

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

2.3. Soxhlet extraction (SE)

Melon seed powder (30 g, grounded and passed through 600 μm sieve) was weighted and extracted in a Soxhlet apparatus with petroleum ether for 6 h at 40 °C. After the extraction process, a rotary evaporator (R-144, BUCHI, UK) was used to remove the residual solvent from the extracted oil. The melon seed oils were stored in the freezer at −18 °C until further analysis.

2.4. Cold-pressed extraction (CPE)

Melon seeds (200 g) were pressed at room temperature using a cold-press machine (KK 20F SPEZ, oil press GmbH & Co, KG, Germany). After pressing, the oils were centrifuged at 1107×*g* for 15 min (ST 8 Centrifuge, Thermo) at room temperature to separate the oil from the residue; then the cold-pressed oil was collected and stored in a freezer (−18 °C) for future analysis.

2.5. Aqueous enzymatic extraction (AEE)

Aqueous enzymatic extraction of melon seed oil was based on the method reported by Ref. [15]. Briefly, 5 g of melon seed powder (grounded and passed through 600 μm of sieve) were mixed with 25 cm^3 of distilled water, at a liquid/solid ratio of 5:1. The pH was adjusted to pH 6 with using 1.0 mol dm^{-3} of HCl/NaOH, and 3 % (v/w) of protease and cellulase, in a ratio of 3:1, were added to the mixture. Then, the mixture was incubated in a water bath at 50 °C and 150 rpm for 6 h. After extraction, the suspension was centrifuged at 1107×*g* for 20 min (ST 8 Centrifuge, Thermo). The oil (upper layer) was collected using micropipette and stored at −18 °C for further analysis.

2.6. Oil physicochemical properties

The following parameters were evaluated based on the respective AOAC method [16]acid value (AOAC method, 969.17), iodine value (AOAC method, 993.20), saponification value (AOAC method, 920.160), and peroxide value (AOAC method, 965.33).

2.7. Fatty acid composition analysis

The fatty acid composition was determined according to Milinsk et al. [17] with some modifications. Briefly, 50 mg of melon seed oil samples were added to 2 cm^3 of 0.5 mol dm^{-3} of sodium methoxide solution in methanol and mixed for 5 min for methyl esterification. Then, 1 cm^3 of isooctane and 5 cm^3 of saturated sodium chloride solution were added and stirred vigorously for 15 min. The upper layer was collected and transferred into a GC vial, and was analysed by GC (7690B, Agilent, USA) equipped a flame ionisation detector (FID). The analysis of fatty acid methyl esters (FAME) was conducted using a fused silica capillary column HP-88 (100 x 0.25 x 0.2). The oven temperature program was held 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 carrier gas was helium at a constant column flow rate of 1.5 cm^3/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 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 (%).

2.8. Determination of sterols and squalene content

Sterols and squalene content were determined according to the method described by Liu et al. [18] with some modifications. Briefly, 0.2 g of oil sample was mixed with 20 cm^3 of 1.0 mol dm^{-3} of KOH in ethanol and 1 cm^3 of 1.0 mol dm^{-3} of internal standard (5α-cholestan-3β-ol), and heated at 90 °C for 1 h in a reflux condenser. After that, 10 cm^3 of distilled water and 5 cm^3 of n-hexane were added to the mixture, followed by vigorous mixing for 30 s. Then, samples were left to rest for 5 min and the n-hexane layer was collected with a micropipette. The extraction with n-hexane was repeated three times and all extracts were combined and evaporated under N_2 flow at 40 °C until dryness. The residue was derivatised by using 0.5 cm^3 of Tri-sil HTP reagent for 30 min at 60 °C in a water bath. Afterwards, the derivatised sample was transferred into GC vials, and was analysed by GC (7690B, Agilent, USA) equipped with a flame ionisation detector (FID) using a HP-5ms column (30 m × 0.25 mm x 0.25 μm, J&W Scientific, Folsom, CA, USA). The column temperature was initially held at 200 °C for 0.5 min, then increased to 270 °C at a rate of 10 °C/min, and then held at 270 °C for 25 min. The FID detector temperature was set at 290 °C and the temperature of injection port was set at 280 °C. Helium was used as a carrier gas and the flow rate was kept 1.0 cm^3/min. The split ratio was 20:1. Sterols and squalene were quantified according to calibration curves constructed with known concentrations of external standards.

2.9. Analysis of tocopherol content

The tocopherol content of melon seeds oil was determined according to Martakos et al. [19] with slight modifications. Briefly, 100 mm^3 of oil sample were dissolved into 900 mm^3 of 2-propanol, mixed, filtered (0.20 μm filter), and analysed by HPLC coupled with a diode array detector (DAD) (Agilent 1260, Agilent Technologies, Stockport, UK) in a Zorbax SB-C18 column (150 × 4.6 mm, Agilent, UK). An isocratic method was applied using (A) methanol (50 %) and (B) acetonitrile (50 %) as mobile phase. The flow rate was 1.0 cm^3/min, and the DAD was set at 295 nm. Each tocopherol compound was quantified according to calibration curves constructed with known concentrations of external standard.

2.10. Determination of oil oxidation stability

The determination of oxidation stability of melon seeds oil was carried out as described by Kiralan et al. [20] with some modifications.

Briefly, 10 g of oil sample in a glass tune were placed in an air oven (dark condition) at 60 °C for 30 days. The peroxide value was measured according to the AOAC method (965.33), at three-day-intervals for a total of 30 days storage duration.

2.11. Statistical analysis

All samples were analysed in triplicate. The data were analysed by Minitab (version 20) statistical analysis software. One-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) test were used to evaluate significant differences ($p < 0.05$) among sample mean values. Pearson's correlation coefficients were determined using Minitab (version 20) statistical analysis software.

3. Results and discussion

3.1. Physicochemical properties

The physicochemical parameters of melon seed oils samples are shown in Table 1. The acid value represents the free fatty acid content in oils, which reflects the degree of lipid rancidity and is associated with oil quality, since free fatty acids could denote triglyceride hydrolysis during storage; the lower is it, the better freshness and quality of the oil [21]. The acid value of melon seed oils ranged from 0.7 to 1.8 mg KOH/g. According to the Codex standard for edible oils, the maximum allowable limit of acid value for edible oil is 4 mg KOH/g, and over 4 mg KOH/g is regarded as unqualified [22]. In this study, the acid value of melon seed oil samples (0.7–1.8 mg KOH/g) was lower than the standard (4 mg KOH/g), suggesting that the oil has good quality. Comparing the acid values of oils extracted by three different methods, Soxhlet extracted oil had highest acid value among three methods, whereas no significant difference ($p > 0.05$) was observed between cold-pressed and aqueous enzymatic extraction. It was indicated that cold-pressed and aqueous enzymatic extracted oil had a relatively higher oil quality than Soxhlet extracted oil. The high acid value in Soxhlet extracted oil could be attributed to prolonged extraction processing with relatively high temperature, including oil extraction and solvent residue evaporation process [23]. According to Anconi et al. [24], long extraction process with the high temperature could promote the decomposition and oxidation of the triacylglycerol into glycerol and free fatty acids.

The peroxide value is an indicator of the degree of oil oxidation [23]. No significant differences were observed between extraction methods ($p > 0.05$) (Table 1). High peroxide value of oil may negatively affect the sensory perception of oils and human health [25]. Therefore, the Codex Alimentarius Commission has defined the maximum peroxide value (15 meq O_2/kg) for edible oils, over the maximum peroxide value is regarded as unqualified and unsafe for consumption [22]. The peroxide value of all melon seed oils ranged from 6.7 to 8.9 meq O_2/kg, consistently lower than the Codex standard for edible oils (up to 15 meq O_2/kg), indicating that melon seed oil has good quality and can be safe for consumption [22]. Besides, according to Mat Yusoff et al. [23], the peroxide value of oil products ranged from 5 (meq O_2/kg) to 10 (meq O_2/kg) is considered as having a moderate oxidation rate, over 10 (meq O_2/kg) is considered as having a high oxidation rate, indicating that all melon seed oils (6.7–8.9 meq O_2/kg) were at moderate oxidation rate.

The iodine value reflects the unsaturation degree of fatty acids. Iodine values between 117.0 and 147.3 g I_2/100 g were obtained in melon seed oils, which were higher than those reported for sunflower, olive, and rapeseed oil (80.0–107.5 g I_2/100 g), indicating the unsaturated nature of melon seed oil [26]. Results of fatty acid composition (Table 2) confirmed this fact, as the presence of high amounts of linoleic acid and oleic acid. Among three extraction methods, Soxhlet extracted oil showed significantly higher ($p < 0.05$) iodine value than cold-pressed and aqueous enzymatic extractions, except Honeydew variety. According to Khattab et al. [27], other non-triglyceride materials in the extracted oil, including partial esters, phospholipids, chlorophylls, and pigments, may affect the iodine value based on the number of double bonds in these molecules. It was indicated that the Soxhlet extracted oil could contained higher amounts of non-triglyceride lipids and/or non-lipid fractions than oils extracted by other two methods.

The saponification value reflects the average chain length of fatty acids; a higher saponification value indicates a shorter chain length of fatty acids [21]. The saponification value of melon seed oil ranged from 108.9 to 205.4 mg KOH/g, whereas the saponification values of melon seed oil obtained by cold-pressed and aqueous enzymatic extraction were higher than that of Soxhlet extracted oil. This indicated that the Soxhlet extracted oil contained longer fatty acids [28]. According to Górnaś et al. [29], high saponification value in oils indicates their suitability for soap production. As such, another avenue that could be explored based on this quality parameter is the utilisation of melon seed oil obtained by cold-pressed and aqueous enzymatic extraction for soap making.

Table 1
Physicochemical parameters of Galia, Cantaloupe, and Honeydew seed oil obtained using different extraction methods.

Variety	Extraction method	Acid value (mg KOH/g)	Peroxide value (meq O_2/kg)	Iodine value (g I_2/100 g)	Saponification value (mg KOH/g)
Galia	SE	1.8 ± 0.1^{a}	8.1 ± 0.4^{a}	147.2 ± 2.1^{a}	129.0 ± 1.4^{c}
	CPE	1.3 ± 0.1^{b}	7.8 ± 0.3^{a}	137.7 ± 2.1^{b}	190.2 ± 1.6^{b}
	AEE	1.2 ± 0.2^{b}	8.4 ± 0.5^{a}	132.6 ± 3.3^{b}	205.4 ± 1.9^{a}
Cantaloupe	SE	1.8 ± 0.3^{a}	8.9 ± 0.4^{a}	141.1 ± 2.6^{a}	108.9 ± 4.9^{b}
	CPE	1.1 ± 0.1^{b}	8.3 ± 0.4^{a}	133.6 ± 3.1^{b}	190.2 ± 0.9^{a}
	AEE	1.0 ± 0.2^{b}	6.7 ± 0.1^{b}	126.9 ± 1.4^{c}	183.9 ± 1.4^{a}
Honeydew	SE	1.4 ± 0.2^{a}	8.1 ± 0.4^{a}	124.2 ± 3.5 ^{ab}	123.0 ± 2.3^{c}
	CPE	0.7 ± 0.1^{b}	7.8 ± 0.4^{a}	129.7 ± 7.4^{a}	185.5 ± 0.9^{a}
	AEE	1.0 ± 0.2 ^{ab}	8.2 ± 0.5^{a}	117.0 ± 4.3^{b}	152.2 ± 1.2^{b}

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

3.2. Fatty acid profile

The fatty acid profile of extracted melon seed oils is presented in Table 2. In relation to the extraction methods, no significant differences ($p > 0.05$) were observed in the fatty acid composition of each variety. In terms of fatty acid profile, all samples were rich in unsaturated fatty acids (over 80 %, w/w), with linoleic acid representing the predominant one (53.59 %–70.79 %, w/w), followed by oleic acid (14.45 %–29.92 %, w/w). In terms of saturated fatty acids, palmitic acid (8.81 %–10.19 %, w/w) was found to be the main one, followed by stearic acid (4.53 %–6.05 %, w/w). The results of this study are in agreement with previous reports, highlighting the abundance of linoleic acid in melon seed oil [6,8]. With regards to fatty acid profile differences among the varieties, large differences were found in the linoleic and oleic acid contents, and less in the palmitic and stearic acid contents. Among the three varieties, Galia and Cantaloupe exhibited much higher linoleic acid content than Honeydew, whereas Honeydew exhibited higher oleic acid content compared to the other two varieties (Table 2). A similar result was reported by Górnaś et al. [30], who investigated the fatty acid composition in eight varieties of kenaf seed oil from South Africa; these differences could be due to genotypic variations and the impact of varying growth

Table 2
Fatty acid profile (%) of Galia, Cantaloupe, and Honeydew melon seed oil obtained using different extraction methods.

Fatty acid	Galia			Cantaloupe			Honeydew		
(%)	SE	CPE	AEE	SE	CPE	AEE	SE	CPE	AEE
Palmitic acid (C16:0)	9.56 ± 0.05^{a}	9.62 ± 0.03^{a}	8.81 ± 0.02^{b}	9.87 ± 0.01^{b}	9.88 ± 0.01^{b}	10.19 ± 0.16^{a}	9.36 ± 0.06^{a}	9.44 ± 0.03^{a}	9.43 ± 0.01^{a}
Stearic acid (C18:0)	4.53 ± 0.02^{c}	4.65 ± 0.01^{b}	4.79 ± 0.01^{a}	6.00 ± 0.02^{a}	6.05 ± 0.01^{a}	5.47 ± 0.06^{b}	5.91 ± 0.07^{b}	6.05 ± 0.02^{a}	5.73 ± 0.001^{c}
Oleic acid (C18:1)	14.87 ± 0.06^{b}	15.45 ± 0.05^{a}	14.45 ± 0.07^{c}	15.39 ± 0.02^{b}	15.78 ± 0.01^{a}	14.47 ± 0.06^{c}	29.10 ± 0.12^{b}	29.92 ± 0.07^{a}	28.22 ± 0.07^{c}
Linoleic acid (C18:2)	69.88 ± 0.12^{b}	69.27 ± 0.05^{c}	70.79 ± 0.09^{a}	67.44 ± 0.01^{b}	67.30 ± 0.02^{b}	68.77 ± 0.16^{a}	54.33 ± 0.09^{b}	53.59 ± 0.05^{c}	55.28 ± 0.09^{a}
α-Linolenic acid (C18:3)	0.204 ± 0.001^{b}	0.176 ± 0.001^{c}	0.28 ± 0.01^{a}	0.245 ± 0.008^{a}	0.212 ± 0.002^{b}	0.242 ± 0.009^{a}	0.155 ± 0.001^{a}	0.144 ± 0.003^{b}	0.153 ± 0.001^{a}
Arachidic acid (C 20:0)	0.175 ± 0.001^{a}	0.17 ± 0.02^{a}	0.200 ± 0.001^{a}	0.245 ± 0.001^{a}	0.238 ± 0.001^{b}	0.221 ± 0.003^{c}	0.235 ± 0.002^{a}	0.234 ± 0.001^{a}	0.221 ± 0.001^{b}
Gondoic acid (C20:1)	0.14 ± 0.01^{a}	0.143 ± 0.003^{a}	0.15 ± 0.01^{a}	_	_	_	0.144 ± 0.001^{a}	0.144 ± 0.001^{a}	0.138 ± 0.001^{a}
Tricosanoic acid (C 23:0)	0.14 ± 0.01^{b}	0.148 ± 0.004^{b}	0.187 ± 0.001^{a}	0.179 ± 0.001^{a}	0.185 ± 0.002^{a}	0.151 ± 0.004^{b}	0.160 ± 0.001^{a}	0.154 ± 0.001^{b}	0.138 ± 0.002^{c}
SFA	14.405	14.588	13.987	16.294	16.353	16.032	15.665	15.878	15.519
MUFA	15.01	15.593	14.6	15.39	15.78	14.47	29.244	30.064	28.358
PUFA	70.084	69.446	71.07	67.685	67.485	69.012	54.485	53.734	55.433
Unknown	0.501	0.373	0.343	0.631	0.409	0.486	0.606	0.324	0.69

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

parameters (e.g. climate, soil, and year of harvest) [30–32].

Overall, the considerably high levels of unsaturated fatty acids (mainly due to linoleic and oleic acid) render melon seed oil a nutritionally valuable oil as compared to other commercial vegetable oils, such as olive (80 %) and sunflower oil (85 %) [33].

3.3. Tocopherol content

Tocopherols (Vitamin E) are oil-soluble natural antioxidants, which play an important role in oil oxidative stability and human health [9, 34]. The tocopherol content of the three melon seed oil varieties extracted with different methods is presented in Table 3 γ-Tocopherol was the major tocopherol in melon seed oils, ranging from 6.8 to 67.3 mg/100 g. α-Tocopherol was not detected in any of the oils, whereas δ-tocopherol was only detected in low amounts in the Cantaloupe seed oil (0.87–1.19 mg/100 g). This indicated that γ-tocopherol was the dominant tocopherol in melon seed oil, in agreement with previous studies [6,7]. The highest γ-tocopherol content was found in Galia melon seed oil (Table 3). Compared with previous studies, Rabadán et al. [6] and Zhang et al. [7] detected low concentrations of α-tocopherol (1.2–7.4 mg/100 g) and δ-tocopherol (0.9–2.7 mg/100 g) in melon seed oil. In contrast, this study confirmed the prevalence of γ-tocopherol in melon seed oils. Tocopherols are the chain-breaking antioxidants, that inhibit the lipid oxidation during the initiation phase or the propagation phase by donating hydrogen atoms to lipid radicals [35]. According to Horn et al. [35] and Player et al. [36], γ-tocopherol has a higher antioxidative activity than α-tocopherol in the oil, since the α-tocopherol is more likely to participate in side-reactions that γ-tocopherol, resulting in high degradation rate and efficiency loss. Fernández-Cuesta et al. [37] assessed the oil stability index of the high γ-tocopherol safflower lines and high α-tocopherol safflower lines using a Rancimat Instrument at 110 °C, and reported that the induction time of oxidation in high γ-tocopherol safflower oil (121 h) is three times longer than that of high α-tocopherol safflower oil (32 h). Overall, it was suggesting that the presence of γ-tocopherol can contribute to the oxidative stability of melon seed oil. Notably, the tocopherol content varied significantly among the three melon varieties in this study; previous studies reported that tocopherol content in plant materials is mostly influenced by agronomical factors including variety, region, soil, and climate [34,38]. For example, Ghosh et al. [39] assessed the tocopherol content in 1151 Chinese soybean accessions from different regions of China and found that the α-tocopherol content and γ-tocopherol content showed the significant positive correlation with latitude ($r = 0.62$ and $r = 0.36$, respectively), while the δ-tocopherol content showed the significant negative correlation with latitude ($r = -0.49$). Considering the total tocopherol content of melon seed oils, significant differences on tocopherol content were observed with different extraction methods ($p < 0.05$); the ranking in terms of effectiveness was AEE > CPE > SE, except for the Galia variety. It can be seen that the extraction method affects the tocopherol content. The high tocopherol content of AEE

Table 3
Tocopherol content (mg/100 g) of Galia, Cantaloupe, and Honeydew melon seed oil obtained using different extraction methods.

Variety	Extraction	α-tocopherol	γ-tocopherol	δ-tocopherol	Total
Galia	SE	ND	52.4 ± 0.4^{b}	ND	52.4
	CPE	ND	47.4 ± 0.3^{c}	ND	47.4
	AEE	ND	67.3 ± 0.2^{a}	ND	67.3
Cantaloupe	SE	ND	28.6 ± 0.4^{c}	1.19 ± 0.01^{a}	29.8
	CPE	ND	32.5 ± 0.1^{b}	0.87 ± 0.03^{b}	33.37
	AEE	ND	46.9 ± 0.3^{a}	ND	46.9
Honeydew	SE	ND	6.8 ± 0.2^{c}	ND	6.8
	CPE	ND	14.62 ± 0.03^{b}	ND	14.62
	AEE	ND	15.0 ± 0.1^{a}	ND	15.0

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

extracted oil could be attributed to enzymatic hydrolysis that breaks down the plant cell walls and oil seed membrane structure, thereby allowing easier access of oil bodies out of seed cells and enhancing the tocopherol release [14,40].

3.4. Sterol and squalene content

Sterols are commonly present in the unsaponifiable part of vegetable oils. Numerous studies have reported that sterols have anti-inflammatory activity, inhibit cholesterol absorption, and may also exhibit antioxidant and anti-bacterial effects [28,41].

The sterol content of the melon seed oils obtained with different extraction methods is presented in Table 4. The results showed that β-sitosterol was the major sterol (119.5–291.9 mg/100 g), followed by campesterol (83.6–133.2 mg/100 g); cholesterol and stigmasterol were not detected. The abundance of β-sitosterol in melon seed oil has led to the suggestion of utilising this sterol as an identification marker for melon seed oil [8,42,43]. As shown in Table 4, the amount of individual sterols and total sterols seemed to be influenced by the oil extraction method. Li et al. [44] and Zhang et al. [45] reported similar results for sea buckthorn and milk thistle seed oils obtained by different extraction methods. Sterols are located in monolayers and bilayers of various cell organelles, such as oil bodies, chloroplasts, and chromoplasts, forming protective layers on seed surfaces, or are components of the cell membranes [46,47]. During oil extraction, the extraction of these compounds requires the disruption of the membrane matrix and interaction with the extraction solvent, followed by release into the oil [46]. Therefore, the final concentration of these compounds in oil can be affected by their physicochemical properties (e.g. polarity, structure, partition coefficient) and the extraction method as well as the extraction conditions (e.g. temperature, pressure, polarity of solvent) [44,47,48].

Squalene is a compound of high nutritional value with potential health benefits, including anti-photooxidative and anti-atherosclerotic effects [49,50]. The squalene content of the melon seed oils ranged from 101.1 to 164.7 mg/100 g (Table 4). Compared with other commercial vegetable oils, the squalene content of melon seed oil was higher than that of rapeseed sunflower, corn, and palm oil, all of which have been reported to contain squalene in the range of 11.9–43.7 mg/100 g [1,51].

3.5. Oil oxidative stability

Oil oxidative stability is an important index to evaluate oil quality and shelf life [52]. The oxidative stability of melon seed oils is shown in Fig. 1, which depicts the peroxide value changes during storage at 60 °C. At the end of the storage period (30th day), AEE-derived melon seed oils (Galia 77.7 meq O_2/kg, Cantaloupe 91.2 meq O_2/kg, and Honeydew 134.5 meq O_2/kg) had the lowest peroxide value compared to SE (Galia 131.6 meq O_2/kg, Cantaloupe 157.9 meq O_2/kg, and Honeydew 171.6 meq O_2/kg) and CPE (Galia 153.8 meq O_2/kg, Cantaloupe 325.1 meq O_2/kg, and Honeydew 428.7 meq O_2/kg), indicating that the

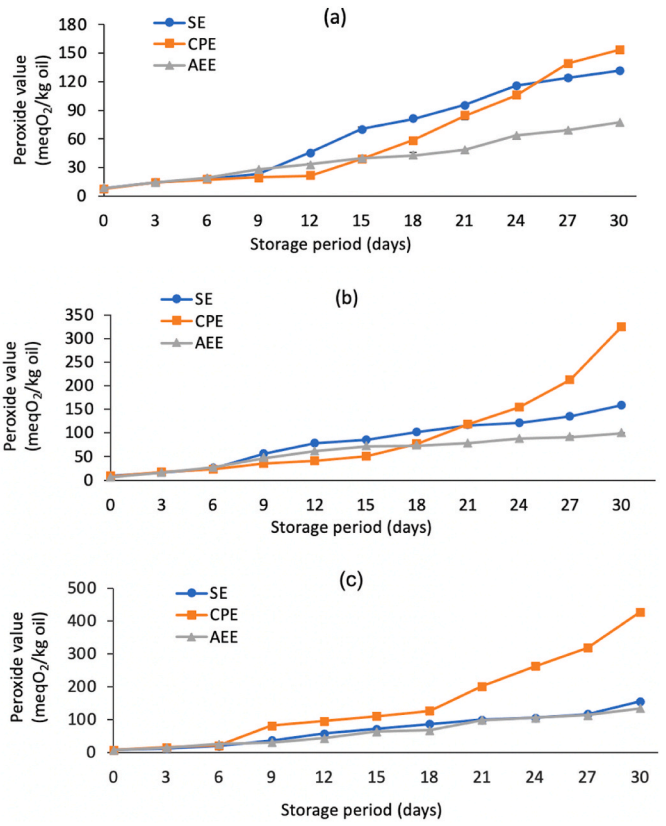

Fig. 1. Peroxide value change of melon seed oils, obtained using different extraction methods, during storage at 60 °C. (a) Galia variety; (b) Cantaloupe variety; (c) Honeydew variety; SE- Soxhlet extraction; CPE - cold-pressed extraction; AEE - aqueous enzymatic extraction. Error bars are shown in the symbol.

AEE-derived melon seed oil had better oxidative stability. Furthermore, under the same extraction method, the Honeydew seed oil showed the lowest oxidative stability compared to the other two varieties. This could be attributed to the tocopherol content of the extracted oil (Table 3), since the presence of tocopherol has positive effects on oil oxidative stability. In contrast, although CPE extracted oil had a higher tocopherol content than SE extracted oil (Table 3), its oxidative stability was lower than SE extracted oil, indicating that other compounds (e.g. sterols and squalene) also play an important role in oil oxidative stability. Micera et al. [53] and Cui et al. [54] demonstrated that squalene and carotenoids are effective quenchers of singlet oxygen and can break lipid peroxidation chains during the initial or the propagation phases, thus preventing lipid oxidation. In addition, as shown by the Pearson correlation analysis depicted in Fig. 3, the peroxide value at the end of storage (30th day) depends on many factors, but none of them showed a

Table 4
Sterols and squalene content (mg/100 g) of Galia, Cantaloupe, and Honeydew melon seed oil obtained using different extraction methods.

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

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

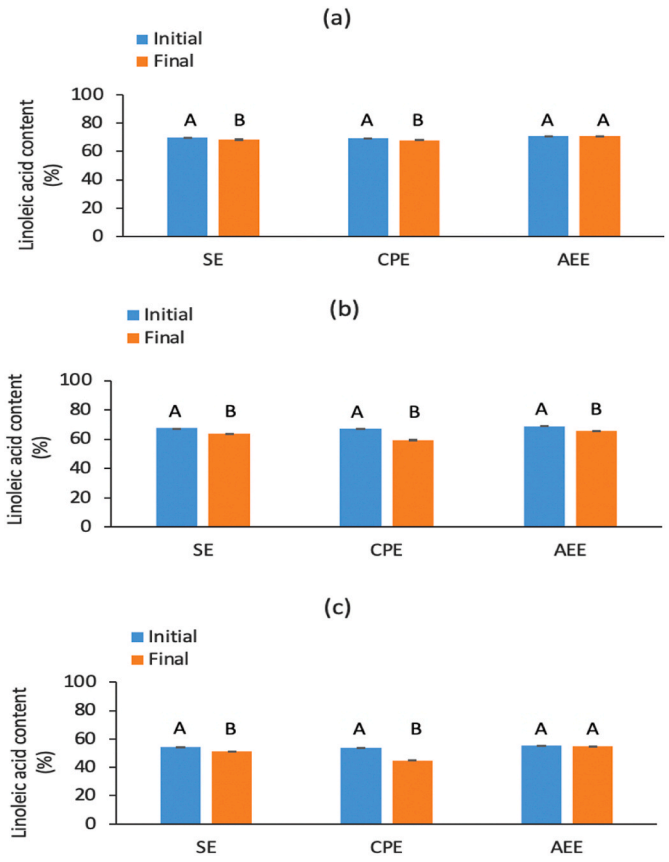

Fig. 2. Linoleic acid (C18:2) content of melon seed oils, obtained using different extraction methods, before (A) and after storage at 60 °C for 30 days (B). (a) Galia variety; (b) Cantaloupe variety; (c) Honeydew variety; SE - Soxhlet extraction; CPE - cold-pressed extraction; AEE - aqueous enzymatic extraction.

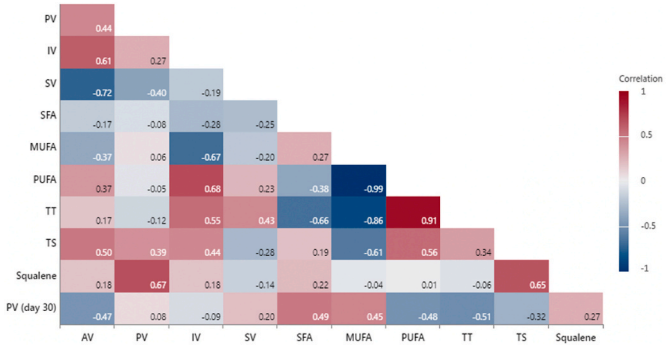

Fig. 3. Analysis of Pearson correlation. Correlation values that are close to 1, −1, and 0 indicate a strong positive correlation, a strong negative correlation, and a weak or no linear relationship, respectively. PV - peroxide value; AV - acid value; IV - iodine value; SV - saponification value; SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; TT - total tocopherol content; TS - total sterol content; PV (day 30) - peroxide value at end of storage (30th day).

high correlation. It has been reported that synergistic effects between tocopherol and other antioxidants (e.g. phenolic acids, carotenoids, sterols, squalene as well as some Maillard reaction products) could play an important role in enhancing the oxidative stability of oils [55,56]. In addition, many studies have been reported and demonstrated that multicomponent antioxidants present together have higher antioxidant activity than when used alone [35,57,58]. For example, Naziri et al. [58] reported that tocopherol had synergistic effects with squalene to decrease the autooxidation of pumpkin seed oil. On the other hand, the oxidative stability of oil depends on the level of unsaturated fatty acid as well as the content of the oil in pro/antioxidant components [59]. According to Zhou et al. [60] and Grajzer et al. [61], cold-pressed oil could contain more pro-oxidant factors (e.g. metal ions), and the pro-oxidative mechanism of metal ions is the deprivation of hydrogen atoms directly, promoting the formation of free radicals and decomposition of lipid hydroperoxides. During the oil pressing, the pressed oils are exposed to transition metal due to contact with pressed machine [61]. The highest oxidative stability in AEE extracted oil could be attributed to the extraction of other antioxidant compounds (e.g. phenolics) during enzymatic hydrolysis; during enzymatic hydrolysis, the plant cell wall is hydrolysed, making the bioactivity compounds more susceptible to extract and release from the plant matrix [62,63]. In addition, many previous studies have been demonstrated and reported that AEE is an effective method in bioactivity compounds (e.g. phenolics) extraction [62–64]. Although further research is needed to elucidate the above, overall, in this study, it was shown that CPE and AEE extracted oils had the lowest and highest oxidative stability.

Data shown in Fig. 2 show the difference in the linoleic acid (C18:2) content of melon seed oils between day 0 and day 30. The melon seed oil obtained by CPE showed a higher loss rate of linoleic acid compared to AEE and SE. Especially for the Cantaloupe and Honeydew varieties, the linoleic acid content decreased by up to 9 %. A high loss rate of unsaturated fatty acids indicates a high degree of oxidation and as a consequence a reduction of the oil nutritional quality [65]. In contrast, the loss rate of linoleic acid for the melon seed oils obtained by SE and AEE were lower than CPE, further indicating that the SE and AEE extracted oil had better oxidative stability than the CPE extracted oil. Therefore, taken together, these results suggest that melon seed oils obtained by SE and AEE had better oxidative stability and relatively higher linoleic acid retention, with the oils obtained by AEE performing best.

4. Conclusions

Melon seed oil was found to be rich in linoleic acid, phytosterols, and squalene; due to such composition it could be used for a wide range of applications in the food, cosmetics, and pharmaceutical sectors. In relation to the extraction method, the choice of the extraction method did not affect the fatty acid composition but impacted on the physicochemical properties, the content of bioactivity compounds, and the oxidative stability of the oils. Melon seed oil obtained by the aqueous enzymatic extraction (AEE) method exhibited a high tocopherol content and oxidative stability. Overall, this study could provided comparable extraction data that can be utilised to develop effective methods for the production high-quality melon seed oil. Future work will be conducted on the oil oxidative stability to provide a more understanding the effect of different extraction methods as well as their main mechanisms.

Funding

This research did not receive any specific grant from any funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Guoqiang Zhang: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Ziqian Li:** Writing – review & editing, Methodology, Investigation. **Zheng Guo:** Writing – review & editing. **Dimitris Charalampopoulos:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing or interests.

Data availability

Data will be made available on request.

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