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Characterization of Crude Watermelon Seed Oil by Two Different Extractions Methods

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Abstract The aim of this paper was to study the physical–chemical composition of the watermelon seed oil extracted by a mechanical process using an expeller and by a chemical process using hexane as the solvent. The watermelon seed oil had a high concentration of unsaturated fatty acids. The two primary sterols were stigmasterol and β-sitosterol, which corresponded to approximately 47 and 30% of the total phytosterols. The oil had a low tocopherol content (65.19 mg/kg for S and 73.19 mg/kg for E). Comparing the two extraction methods, extraction by expeller produced an oil of superior quality with respect to oxidative stability, carotenoids and Lovibond color. No significant differences were found between the two extraction methods with respect to the minor components of the oil considered as functional, such as phytosterols.

Keywords Watermelon seed oil · Solvent extraction · Expeller · Minor compounds · Unsaponifiables

Introduction

The food industry produces large volumes of solid and liquid waste resulting from the production, distribution, preparation and food consumption chain. These wastes generate potential problems for their treatment and disposal, resulting in pollution with a loss of biomass and valuable nutrients. Much research has been carried out to investigate the conversion of the waste from food processing into useful products or even its use as a raw material for other industries. In these is the use of fruit processing waste for the production of oil and functional ingredients [1, 2].

According to Dias and Rezende [3], the worldwide production of watermelon in 2007 was 93.17 million tons with an average yield of 25.86 t ha^{-1}. Brazil in 2006 produced 1.9 thousand tons with a yield of 20.9 t ha^{-1}. Watermelon is consumed almost exclusively as a fresh fruit, but it is also consumed in the form of juices, jellies, jams, sauces and salads. In some countries, the peel is pickled. China and various regions of Asia and the Middle East consume the seeds. In India, watermelon seed flour is used in bread making. In Southern Russia, a beer is produced with watermelon juice as an ingredient. Another use is to make candy with the white part [3].

Watermelon seeds (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) show a potential for use in the food industry as they remain intact after removing the pulp and peel when processing candies and juices. According to the results obtained by El-Adawy and Taha [1], watermelon seeds contain high levels of protein (35.66–36.47%) and oil (50.10–51.01%), presenting good potential for their use in food formulations. A characterization of the fatty acids in watermelon seed oil indicated high levels of unsaturated fatty acids (78.35%), due to the presence of linoleic acid (59.6%) and oleic acid (18.1%) [1]. It has been used as a cooking oil and as a food additive in Western countries and in the Middle East of Africa [4].

Organic solvents are commonly used to extract lipids from oil seeds, but solvent extraction show some drawbacks such as the possibility of thermal degradation of the unsaturated fatty acids and functional compounds, depending on the extraction conditions used and the need to eliminate residues of the organic solvent from the oil [5].

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The mechanical cold extraction of oils using an expeller is mainly used for fiber-rich sources with oil content above 20%, i.e., in the extraction of oil from copra, palm, peanut and cotton, among others, aiming at a pre-extraction or the preservation of compounds which might be degraded in the solvent extraction process. But the expeller usually results in the lower extraction yield than that obtained using solvent extraction (10–18% of the oil) [6].

The aim of the present study was to study the physical and chemical characteristics of the watermelon seed oil extracted by a mechanical process (expeller) and by hexane solvent extraction. The study was focused on the preservation of minor compounds such phytosterols, tocopherols and total carotenoids.

Materials and Methods

Raw Material

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) of the Top Gun Variety, was purchased at the Campinas city market and pulped, generating 4 kg of wet seeds. The wet seeds were dried in a forced air oven at 60 °C for 24 h, resulting in 1.5 kg of dry seeds.

Oil Extraction Methods

Part of the dry seeds (0.75 kg) were extracted mechanically (E-cold extraction) by compression using an expeller (Komet, model CA59G, Mönchengladbach, Germany), and the other part (0.75 kg) chemically using hexane as the solvent (S) according to the AOCS [7] method AC 3-44. The extraction yield in crude oil from the mechanical method was determined from the difference in oil content between the cake from the expeller extraction and the original seed, according to the AOCS [6] method AC 3-44.

Analytical Methods for the Oil

The moisture content of the oil was determined by Karl Fischer titration (Mettler Toledo, model DL31, Barueri, SP, Brazil), according to the AOCS [7] method Ca 2e-84. The peroxide value was determined according to the AOCS [7] method Cd 8b-90, the iodine value using the AOCS [7] method Cd 1c-85 and the saponification index using the AOCS [7] method Cd 3a-94, the latter two being calculated from the composition in fatty acids. The refractive indices of oils were determined at 25 °C using a Milton Roy Company (Ivyland, PA, USA) refractometer according to the AOCS [7] method Cc 7-25. The free fatty acid (FFA) content was determined using the AOCS [7] method Ca 5a-40 and expressed as % oleic acid. The acid value was determined mathematically by multiplying the value obtained for FFA by 1.99.

The tocopherol and tocotrienol contents of the samples were determined according to the AOCS [7] method Ce 8-89, using a HPLC equipped with a Perkin Elmer 200 isocratic pump and Perkin Elmer LC 240 fluorescence detector, using an excitation wavelength = 290 nm, emission wavelength = 330 nm. A flow rate of 1.0 mL/min, 250 × 4 mm Li Chrosorb Si 60* 5 μm analytical column (Merck). The mobile phase: hexanol/isopropanol 99:1 (HPLC grade solvents filtered and degassed for 10 min in an ultrasonic bath). Quantification was carried out by way of external standard curves of four tocopherols (Tocopherol Set, cat. n°. 613424, Calbiochem, Darmstadt, Germany) and daily reference of quantitative and qualitative tocopherol and tocotrienol standards.

The phytosterols were quantified according to the methodology described by Becker et al. [8] using capillary gas chromatography (CGC Agilent 6850 Series GC System). Oil samples were weighed (2.5 g) for the initial saponification stage, with posterior extraction of the unsaponifiable matter. Separations of the unsaponifiable components were realized and the sterol fraction isolated and extracted. The separation, identification and quantification of the sterols were using a gas chromatography with a capillary column (CGC Agilent 6850 Series GC System–LM-5, no 171200, 30 m × 0.25 mm × 0.3 μm) containing 5% phenyl, 95% methylpolysiloxane.

The total carotenoid content was obtained using the method described by PORIM [9]. The samples of S and E (0.8 g) were diluted in hexane (25 mL) and read at 446 nm in a dual bean spectrophotometer (UV/VIS Lambda 20—Perkin Elmer).

The oils oxidative stability index (OSI) was determined according to the AOCS [7] method Cd 12b—92 at a temperature of 110 °C. The Lovibond color of the oils obtained using different extraction methods was determined according to the AOCS [7] method Cc 13b-45.

The lipid classes in the oils were determined and quantified using high performance size exclusion chromatography (HPSEC) technique, using a Perkin Elmer 250 liquid chromatograph equipped with a Sicon Analytic refractive index detector (Hitachi High Technologies America, USA) and 500 and 100 Å Jordi Gel DVB 300 × 7.8 mm columns. The mobile phase was tetrahydrofuran (THF) with a flow rate of 1 mL/min. The sample was dissolved in 1% (v/v) tetrahydrofuran and an injection volume of 20.0 μL was used.

The fatty acid methyl esters composition was determined according to the AOCS [7] method Ce 1-62 by capillary gas chromatography—CGC, using an Agilent 6850 Series GC System equipped with a 60 m Agilent DB-23 capillary column (50% cyanopropyl–methylpolysiloxane), internal

diameter of 0.25 mm and 0.25 μm film. The conditions for the chromatographic operations were as follows: column flow = 1.0 mL/min; linear velocity 24 cm/s; detector temperature 280 °C; injector temperature 250 °C; oven temperature at 110 °C for 5 min, then rising from 110 to 215 °C at 5 °C/min, followed by 215 °C for 34 min; carrier gas of helium; volume injected 1.0 μL; 1:50 split. The fatty acid methyl esters were prepared by adapting the method described by Hartman and Lago [10] to a micro-scale.

The triacylglycerol composition was obtained assuming a random distribution with a computer program developed by Antoniosi Filho, Mendes and Lanças [11], based on the fatty acid composition.

Statistical Analysis

All analyses were carried out in triplicate and evaluated statistically using STATISTICA 7.0 (StaSoft, Inc.) software for the variance analysis (ANOVA) and Tukey test, at a level of significance of 95% ($p \leq 0.05$).

Results and Discussion

The mechanical method resulted in 21.64% oil, a result that is lower than the solvent extraction method, which resulted in 32.16% of seed oil. This fact was expected since the oil extraction yield by mechanical methods is often 10–18% less oil [6].

Table 1 shows the fatty acid composition of the crude watermelon seed oil. Palmitic, oleic and linoleic acids are the main fatty acids present in the oil, constituting more than 90% of the triacylglycerol portion with a predominance of linoleic acid (65.61%), in agreement with the values found by Nolte and Loesecke (65.85%) for the Cuban Queen variety [12]. The total proportion of unsaturated fatty acids of 82.11%, was close to the value of 78.36% cited by El-Adawy and Taha [1], although they found a lower proportion of linoleic acid (59.61%), oleic acid (18.07%) and palmitic acid (11.3%). These differences can probably be explained based on the different varieties of watermelon used in each study. Dubois et al. [13] obtained results similar to those of Adawy and Taha1 for the fatty acid composition. The results are comparable to the findings of Baboli and Kordi [14]: linoleic (68.3%), oleic (13.3%), palmitic (11.4%), and stearic (7%) acids.

Due to the presence of large amounts of linoleic and oleic acids, and the almost complete absence of fatty acids with three unsaturated bonds (C18:3 *cis*), watermelon oil could be suitable for culinary purposes, for the manufacture of margarines and especially as a salad oil since it also provides a desirable flavor and characteristic aroma, also observed by Nolte and Loesecke [12].

Table 1 Fatty acid composition, iodine value and saponification index for crude watermelon seed oil

Fatty acid[a]	%(w/w)
(C14:0) Myristic acid	0.05 ± 0.00
(C16:0) Palmitic acid	10.06 ± 0.11
(C16:1) Palmitoleic acid	0.07 ± 0.00
(C18:0) Stearic acid	7.31 ± 0.01
(C18:1 *cis*) Oleic acid	16.08 ± 0.15
(C18:2 *cis*) Linoleic acid	65.61 ± 0.07
(C18:3 *cis*) Linolenic acid	0.18 ± 0.03
(C20:0) Arachidic acid	0.33 ± 0.00
(C20:1) Gadoleic acid	0.10 ± 0.01
(C22:0) Behenic acid	0.06 ± 0.02
(C22:1) Erucic acid	0.07 ± 0.04
(C24:0) Lignoceric acid	0.08 ± 0.00
Saturated fatty acids	17.90
Monounsaturated fatty acids	16.31
Polyunsaturated fatty acids	65.79
Total unsaturated fatty acids	82.11
Calculated saponification index	*192*
Calculated iodine value	*128*

[a] The results represent the averages of four determinations

The iodine value reflects the active sites of unsaturation in the fatty acids constituting the oil [15]. The values found for the iodine value (128 g of iodine/100 g of oil) and saponification index (192 mg of KOH/g of oil) were close to the values found by Nolte and Loesecke [12], 134 g of iodine/100 g of oil, and 197 mg of KOH/g of oil, respectively for the *Cuban Queen Variety*. However the values found by El-Adawy and Taha [1] and Baboli and Kordi [14] for the iodine value (115 and 156 g of iodine/100 g of oil, respectively) and saponification index were different (201 and 200 mg of KOH/g of oil, respectively).

Table 2 shows the triacylglycerol composition obtained using a computer program. It can be seen that the predominant triacylglycerols were constituted of LLL, OLL, SLL and PLL, indicating a low melting point for the watermelon seed oil. No data on the triacylglycerol composition of watermelon seed oil is available in the literature for comparative purposes.

The lipid groups found in the samples extracted by solvent (S) and expeller (E) were respectively: 95.86 and 97.77% of triacylglycerols, 1.75 and 1.22% of diglycerides and 1.88 and 0.91% of monoacylglycerols. According El-Adawy and Taha [1], crude oil from watermelon seeds contained 94.9% of triacylglycerols, 0.35% of diacylglyceols, 0.98% of monoacylglycerols and 1.41% free fatty acids as determined from thin-layer chromatography. Seven lipids groups were identified in total, including hydrocarbons, sterols and phospholipids.

Table 2 Theoretical triacylglycerol composition of watermelon oil

Triacylglycerol composition[a]	%
PLP (50:2)	2.06
POS (52:1)	0.74
PLS (52:2)	3.81
POL (52:3)	6.62
PLL (52:4)	13.50
SLS (54:2)	1.68
SOL (54:3)	5.26
SLL (54:4)	15.16
OLL (54:5)	*21.68*
LLL (54:6)	*29.49*

[a] Where: *L* linoleic acid; *S* stearic acid; *P* palmitic acid; *O* oleic acid

Table 3 shows the physical–chemical characteristics of the crude watermelon seed oils extracted using a solvent (S) and an expeller (E). The effects of the extraction method on the oil stability and acidity were apparent, from the differences in the peroxide value, oxidative stability index and acid value.

The peroxide value indicates the primary oxidation state of an oil or fat, which is influenced by factors such as the fatty acid constituents, time and storage conditions [15]. The peroxide values observed for the solvent extraction—S (9,29 mequiv $O_2.kg^{-1}$) and *expeller* extraction—E (0,27 mequiv O_2 kg^{-1}) oils were within the limit established by Brazilian legislation [16] and by Codex Alimentarius [17]—FAO/OMS (max. 10 mequiv O_2 kg^{-1}). However, the expeller extraction method reflected better conservation of watermelon seed oil with greater preservation of the natural antioxidants due to the lower operating temperature.

Table 3 Physical-chemical characteristics of the crude watermelon oils extracted by solvent and by expeller

Determination	Solvent (S)[a]	Expeller (E)[a]
Refractive index	$1.4745^{A} \pm 0.00$	$1.4744^{A} \pm 0.00$
Peroxide value (mequiv O_2 kg^{-1})	$9.29^{A} \pm 0.40$	$0.27^{B} \pm 0.00$
Acid value (mg KOH/g)	$0.51^{A} \pm 0.06$	$0.19^{B} \pm 0.04$
FFA (% as oleic acid)	$0.25^{A} \pm 0.03$	$0.10^{B} \pm 0.02$
Moisture (%)	$0.04^{B} \pm 0.00$	$0.11^{A} \pm 0.03$
Carotenoids (mg/kg)	$30.55^{B} \pm 3.86$	$39.14^{A} \pm 0.84$
Tocopherols (mg/kg)		
Alpha	$1.68^{A} \pm 0.29$	$1.43^{A} \pm 0.38$
Gamma	$62.83^{A} \pm 10.12$	$71.07^{A} \pm 11.15$
Delta	$0.69^{A} \pm 0.05$	$0.69^{A} \pm 0.07$
Total	$65.20^{A} \pm 5.69$	$73.19^{A} \pm 6.61$
Lovibond color (1″)	$70.0Y/9.9R^{B}$	$70.0Y/10.6R^{A}$

[a] The results represent the averages of three determinations. Samples followed by the same letters in the same line do not differ significantly ($p \leq 0.05$) according to the Tukey test

The oxidative stability data for the E and S oils (6.70 and 5.78 h at 110 °C, respectively) are considered low values, which is to be expected due to their fatty acid compositions, with higher contents of unsaturated (82.11%) fatty acids. These data corroborate with Baboli and Kordi [14] who reported values of 5.41 h at 110 °C for the watermelon seed oil extracted with hexane. Another factor to be considered is the low content of tocopherols (65.19 mg/kg for S and 73.19 mg/kg for E), since these help preserve the oil. The tocopherol content represents a vitamin E content of 21.46 UI/mg (S) and 23.56 UI/mg (E).

According to Huang, Frankel and German [18], good antioxidant activity can be observed for α-tocopherol at concentrations from 100 to 250 mg/kg and for γ-tocopherol between 250 and 500 mg/kg. Jung and Min [19] reported good antioxidant activity for δ-tocopherol at concentrations between 500 and 1,000 mg/kg, and Huang, Frankel and German [18] reported antioxidant activity for mixtures of α + γ-tocopherol at a concentration of 250 mg/kg.

The free fatty acid contents of the two oils were statistically different from each other ($p \leq 0.05$), being 0.25 and 0.10% as oleic acid. These values were different from those mentioned in the literature, which were up to six times higher. Such differences being related to the quality of the raw material as a result of the storage time and degradation of the oil due to heating during extraction [1, 12].

The appearance of an oil is often the first barrier to its acceptance by consumers [15]. Visually, watermelon oil presents a red–orange color, the intensity being greater in oil E. Table 3 shows the Lovibond color values found. Higher values found for the readings of R (red) and Y (yellow) translate as higher pigment concentrations. The present study showed that the oil E presented statistically higher ($p \leq 0.05$) values for red than for the oil S, confirmed by the higher values for carotenoids found in this oil.

The average values found for total phytosterols for S and E were 247.83 and 205.88 mg/100 g of oil, respectively, which were not statistically different from each other ($p > 0.05$). These values are lower than the values found by Normén et al. [20] for soybean oils (335 mg/100 g) and for corn oils (909 mg/100 g).

Figure 1 shows the values found for the constituent phytosterols of the watermelon seed oil extracted by expeller and by solvent. The main interest in studying these phytosterols is due to their effectiveness in reducing the intestinal absorption of cholesterol, thus providing protection against cardiovascular diseases. Furthermore, epidemiological and experimental studies suggest that phytosterols in the diet may offer protection against some

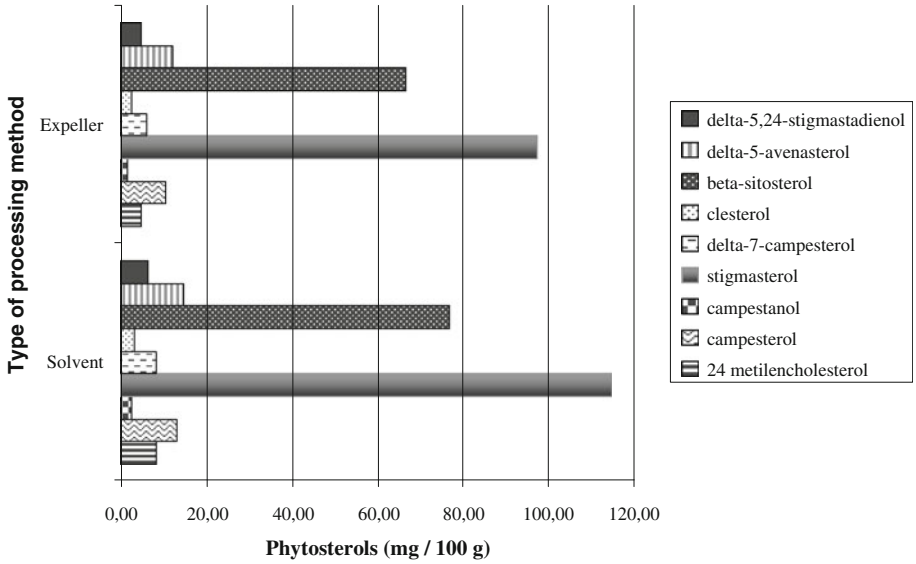

Fig. 1 Phytosterol compositions of the watermelon seed oils obtained from each type of extraction

of the more common types of cancer, such as colorectal, breast and prostate cancers [21].

The phytosterols present larger amounts in the watermelon seed oils were stigmasterol followed by β-sitosterol, corresponding to approximately 47 and 30% of the total, respectively (Fig. 1). These results indicate larger amounts of stigmasterol in watermelon seed oil (114.65 mg/100 g for S and 96.95 mg/100 g for E) as compared to oils from other sources. According to Normén et al. [20], soybean and corn oils present about 56 and 66 mg/100 g of stigmasterol, respectively.

Stigmasterol is used in the synthesis of steroid hormones which are used in the treatment of humans, especially cortisone. However, its presence in soybean oil steroid mixtures makes stigmasterol one of the most abundant sources for the synthesis of steroid hormones [22].

Conclusion

Watermelon seeds contains lipids of nutritional interest, with high concentrations of unsaturated fatty acids, including a high concentration of phytosterols, particularly stigmasterol and β-sitosterol. Watermelon oil could be suitable for culinary purposes, for manufacturing margarines and especially for use in salads, since it presents a large amount of linoleic and oleic acids.

Comparing the two extraction methods, the oil extracted by expeller presented lower extraction yields, but better values for oxidative stability, peroxide value and acidity, as well as higher pigment contents. However, the extraction method did not affect the conservation of the functional compounds, such as the phytosterols.

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