

***Cucumis melo* L. seeds as a promising source of oil naturally rich in biologically active substances: compositional characteristics, phenolic compounds and thermal properties**

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SUMMARY: This study aimed to evaluate the characteristic features, phenolic compounds and thermal analysis of melon seed oil (Maazoun variety), in order to determine its potential applications in food or pharmaceutical industries. The physicochemical properties of the seed oil revealed a high degree of unsaturation. The average contents of carotenoid and chlorophyll were 2.43 mg/kg and 5.70 mg/kg, respectively. The main fatty acids of melon seed oil were linoleic acid (68.98%) and oleic acid (15.84%), which makes this oil nutritionally valuable. Furthermore, trilinolein (LLL), accounted for 28.99% and constitutes the most abundant triacylglycerol. A chromatographic analysis showed that amentoflavone and luteolin-7-glycoside were the major phenolic compounds. A thermal analysis of melon seed oil was performed by differential scanning calorimetry (DSC). The results of sensorial properties indicated that melon seed oil is appreciated by tasters. The findings suggested that because of its composition, melon seed oil could be used successfully as an alternative source in the food and nutraceutical industries as a functional ingredient.

KEYWORDS: *Fatty acid composition; Melon (Cucumis melo L.); Phenolic compounds; Phytosterols; Seed oil; Sensory analysis; Thermal profile*

RESUMEN: *Semillas de Cucumis melo L. como prometedora fuente natural de aceite rico en sustancias biológicamente activas: composición, compuestos fenólicos y propiedades térmicas.* El objetivo de este estudio fue la caracterización, evaluación de compuestos fenólicos y el análisis térmico del aceite de semillas de melón (variedad Maazoun), con el fin de conocer sus potenciales aplicaciones en la industria alimentaria o farmacéutica. Las propiedades fisicoquímicas del aceite de semilla mostraron un alto grado de insaturación. El contenido promedio de carotenoides y clorofila fue de 2,43 mg/kg y 5,70 mg/kg, respectivamente. Los principales ácidos grasos del aceite de semilla de melón fueron el linoleico (68,98%) y oleico (15,84%), lo que hace que este aceite sea nutricionalmente valioso. Además, la trilinoleína (LLL) que representó el 28,99% constituye el triacilglicerol más abundante. El análisis cromatográfico mostró que amentoflavona y luteolin-7-glucósido eran los principales compuestos fenólicos. El análisis térmico del aceite de semilla de melón se realizó mediante calorimetría diferencial de barrido (DSC). Los resultados de las propiedades sensoriales indicaron que los catadores aprecian el aceite de semilla de melón. Los hallazgos sugieren que, debido a su composición, el aceite de semilla de melón podría utilizarse con éxito como una fuente alternativa en la industria alimentaria y nutracéutica como ingrediente funcional.

PALABRAS CLAVE: *Aceite de semilla; Análisis sensorial; Composición de ácidos grasos; Compuestos fenólicos; Fitoesteroles; Melón (Cucumis melo L.); Perfil térmico*

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1. INTRODUCTION

In the food industry, the manufacturing of vegetables and fruits generates the production of high amounts of agricultural materials such as peels, seeds and oilseed meals which are usually wasted and represent a serious environmental concern (da Silva and Jorge, 2014). These materials could be considered valuable by-products if the value of the subsequent products were to exceed the cost of further processing. In recent years, innovative features concerning the use of these by-products for further exploitation in the production of food additives or supplements with high nutritional value have gained interest since these are high-value products and their recovery may be economically attractive. Therefore, utilization of food processing by-products has increased the profitability of the food processing industry by reducing the cost of disposal of these by-products.

Seeds are regarded as the main by-products resulting from the processing of juices and wine, and they may be exploited as a source of oil and biologically active substances. There is Global trade interest in the retrieval and utilization of oils from natural plant sources (Carvalho *et al.*, 2011). Indeed, seeds and seedoils obtained from fruit by-products are rich in bioactive compounds such as fatty acids, phytosterols and carotenoids (Górnaś *et al.*, 2014).

Most kinds of *Cucurbitaceae* are promising sources of oil and their seeds have good nutritional value with important amounts of protein and oil (Nyam *et al.*, 2009). This oil has been utilized for cooking and for pharmaceutical purposes (Azhari *et al.*, 2014). Melon (*Cucumis melo* L.), which belongs to the *Cucurbitaceae* family, is a delicious fruit cultivated in warm regions of the world. The exceptional flavor of melon and its application in traditional medicine explain the economic importance of these fruits. The amount of melon seeds recovered during industrial fruit treatment was 738,000 tons based on the data of global fruit production and the percentage of seeds compared to the mass of the fruits (FAOSTAT, 2015). Despite the generation of enormous quantities, melon seeds are often discarded. Melon seeds have been reported to reduce cardiovascular disorders, help in regulating blood-fat levels (Yanty *et al.*, 2008) and aid in controlling type-2 diabetes (Chen and Kang, 2013). Melon seeds are also a good source of natural antioxidants. They have an important phenolic composition (Zeb, 2016) and they are considered as a valuable source of nutrients due to their protein, lipid, fiber and ash contents (Mallek-Ayadi *et al.*, 2018). Furthermore, these seeds are used directly for human consumption as snacks after roasting and salting in Arabian countries (Nyam *et al.*, 2009) and to add flavor to Indian dishes and desserts (Maran and Priya, 2015).

To our knowledge, scarce data has been reported on the physicochemical parameters and fatty acid

composition of the oil extracted from melon (*Cucumis melo* L.) seeds. The fatty acids of melon seed oil of the Kalahari and *tibish* varieties were determined by Nyam *et al.*, (2009) and Azhazi *et al.*, (2014). These researchers confirmed that C18:2 (linoleic acid) was predominant in melon seed oil. The work of Yanty *et al.*, (2008) aimed to investigate the physicochemical properties of *Cucumis melo* seeds *inodorus* variety. However, the literature is lacking scientific papers regarding polyphenols in melon seed oil. In a recent research, da Silva and Jorge (2014) studied the chemical composition of the oil obtained from the extraction of melon (*Cucumis melo* L.) seeds. The authors determined the total phenolic content of melon seed oil of the *inodorus* Naudin variety. Nevertheless, they did not identify the phenolic compounds present in this oil. Although some phenolic compounds of melon seed oil were quantified by HPLC, Mallek-Ayadi *et al.*, (2018) have found unknown compounds. This means that the phenolic composition of this oil may be more complex and much more variable. In addition, no previous reports are available on the sensory evaluation of melon seed oil.

An investigation of melon seed oil characteristics would be helpful to the industry to design machinery and quality control. Hence, the objectives of the present study were to determine the physicochemical parameters (acid value, peroxide value, saponification index, iodine value, refractive index, specific gravity, extinction coefficients (K_{232} and K_{270}) and color), triacylglycerols and fatty acids of the melon seed oil from the Maazoun variety. The bioactive substances, thermal analyses and sensorial properties were also studied in order to determine its nutritive value and potential applications.

2. MATERIALS AND METHODS

2.1. Seed material

Melons (*Cucumis melo* L. Maazoun cultivar) were harvested in Skhira, 80 Km from the governorate of Sfax in Tunisia (geographic position 38°94'N; 9°21'E). The rinds were removed immediately and the seeds were carefully separated by hand from the pulp, cleaned and rinsed of any residual materials. The seeds were then dried in the oven at 40 °C for 24 hours. Dried seeds were finely milled in a grinding type moulinex to a fine powder. The particle-size of the powder has a maximum of 500 µm. The seed powder was stored and preserved in hermetic bags at -20 °C until further analysis and oil extraction.

2.2. Oil content

The crude oil content of the sample was determined with a Soxhlet apparatus using hexane (99% purity, Merck, Darmstadt, Germany) for extraction (method 920.39c, AOAC, 2000).

2.3. Oil extraction

50 g of melon seed powder were macerated for 4h and then mixed with a solvent of *n*-hexane and placed in 1 L dark glass bottles at a ratio of seed powder weight to solvent volume of 1:5. The mixture was centrifuged for 15 min at 1000 g. Then, the extract was filtered through Whatman filter paper. This procedure of maceration was done with two replications. The organic phase was eliminated in a rotary evaporator at 40 °C. A stream of gas was used to dry the seed oil, which was stored at -20 °C in amber flasks until analysis.

2.4. Physicochemical analysis of oil

The acid value (method Cd 3d-63), peroxide value (Cd 8-53), saponification index (Cd 3-25), iodine index (Cd 1-25), refractive index (Cc 7-25) and specific gravity (Cc 10 a-25) of the melon seed oil were determined according to AOCS Official methods (AOCS, 2012). The extinction coefficients from absorbances at 232 and 270 nm were obtained with a UV spectrophotometer using oil in cyclohexane (1%) and a path length of one centimeter. Viscosity was calculated with a Rheologica Rheometer (Rheologica Instruments AB, Lund, Sweden) at 25 °C with a steel cone-plate (C40/4) at a constant shear rate of 100/s.

2.5. Carotenoid and chlorophyll determinations

The pigments (chlorophylls and carotenoids) were performed by measuring the absorbance at 670 and 470 nm, in cyclohexane using UV spectrophotometer (Shimadzu Co., Kyoto, Japan). The chlorophyll and carotenoid contents were calculated as follows (Chtourou *et al.*, 2013):

$$\text{Chlorophyll (mg/Kg)} = \frac{(A_{670} \times 106)}{(613 \times 100 \times d)}$$

$$\text{Carotenoid (mg/Kg)} = \frac{(A_{470} \times 106)}{(2000 \times 100 \times d)}$$

Where A is the absorbance of the oil at the respective wavelength and d is the spectrophotometer cell thickness (1 cm). The chlorophyll and carotenoid concentrations are expressed as mg per kg of oil, respectively.

2.6. Color measurements

The CIELab coordinates of melon seed oil were generated directly by a spectrophotometer Konica Minolta colorimeter previously calibrated with a standard white calibration plate. In this coordinate system, L* is an estimation of the relative luminosity of the seed oil; this parameter varies from 0 to

100 (black-white). A negative a* number designates green color; whereas an a* positive value indicates red-purple. The value of b* gives the yellow-blue character with positive values for yellow shades and negative values for blues.

2.7. Fatty acid composition

The Regulation EEC/2568/91 (EEC, 1991) was adopted to determine fatty acid methyl esters (FAMES) which were prepared by the vigorous shaking of the seed oil solution in heptane with 0.2 mL of 2N methanolic potassium hydroxide. Gas chromatography of FAMES was performed on a Gas chromatograph (HP 6890 N, Agilent Technologies) fitted with a split/splitless injector at 230 °C and a flame ionization detector at 250 °C. The column used was a capillary Agilent CP-Sil88 (length 50 m; internal diameter 0.32 mm; film thickness 0.20 µm). The temperature of the column was held at 180 °C; the carrier gas was helium at a flow rate of 1 mL/min; the split ratio was 1:50, with an injection volume of 1 µL. FAME peak identification was determined by the comparison of retention times of the authentic samples in the same conditions. FAMES were quantified according to their percentage area, obtained by integration of the peaks. The results were expressed as a percentage of individual fatty acids in the lipid fraction.

2.8. Triacylglycerol composition

The triacylglycerol profile was determined using a high-performance liquid chromatography (HPLC) apparatus, with an HP1100 Series instrument chromatographic system (Agilent Technologies, Waldbronn, Germany) fitted with a differential refractometer detector (model RID-10Avp; Shimadzu). A 0.25 g of oil sample was transferred to a graduated flask (5 mL) and dissolved with acetone (9:91, v/v). After that, separation was made with an analytical spherisorb column (250×4.6 mm) with a particle size of 5 µm from Supelco (Bellefonte, PA, USA). The mobile phase was acetone/acetonitrile (60:40, v/v) at a 1.50 mL/min flow rate. All solvents were of high performance liquid chromatography grade. The column temperature was 30 °C; the injection volume was 20 µL of sample solution prepared as indicated above. The total run time was one hour. The triacylglycerol peaks were identified based on the retention time of triacylglycerol standards: LLLn, OLL, OOL, POL, PLL, PLLn, PLP, OOO, POO, POP, PPO, POS, SOO, SLO and SLL (S, L, Ln, O and P are stearic, linoleic, linolenic, oleic and palmitic acids, respectively) purchased from Sigma (St Louis, MO, USA). Peak areas produced by the data integrator were used for quantification.

2.9. Extraction of phenolic compounds

For the extraction of the phenolic fraction, an aliquot of 2 mL of *n*-hexane and 4 mL of a solution of 60:40 methanol/water were homogenized with 4 g of sample. After vigorous mixing, they were centrifuged at 1490 g for 3 min. The extraction was performed twice and the hydroalcoholic phase was collected. Hydroalcoholic fractions were then united, washed with 4 mL of *n*-hexane to eliminate the residue of oil, concentrated and dried using a rotary evaporator at 35 °C.

2.10. Determination of total phenolic content

The total phenolic content was determined according to the the Folin Ciocalteu reagent method (Singleton and Rossi, 1965). An aliquot of 0.5 mL of the Folin reagent was added to 0.5 mL of extract. The mixture was stirred and incubated for 3 min in the dark. Then, 10 mL of a solution of Na₂CO₃ (75 g/L) were added. The mixture was shaken and incubated for 1h in the dark. Finally, the absorbance was measured at 750 nm. The results were expressed as mg of gallic acid equivalents per 100 g extract (mg GAE/100 g extract). The value presented the average of three measurements.

2.11. HPLC analysis of phenolic composition

For the identification and quantification of melon seed oil phenolic compounds, thirty-four commercial standards were used. Standards were acquired from Sigma-Aldrich (St. Louis, MO, USA) and Extrasynthèse (Genay, France).

A stock solution of each phenolic compound standard was prepared at a concentration of 1 mg/mL and stored in methanol. An intermediate solution containing all standard compounds was prepared in methanol for the calibration curve. A stock solution of cinnamic acid, used as an internal standard (1 mg/mL), was also prepared and stored in methanol. Standard stock solutions were filtered through a 0.45 µm membrane and stored at -20 °C.

For the HPLC analysis, an aliquot of 100 µL of cinnamic acid (1 mg/mL) (internal standard) was added to 100 µL of melon seed oil extract. Samples were passed through a 0.45 µm membrane filter before injection.

The qualitative evaluation of phenolic compounds was carried out using a high performance liquid chromatography technique, Hewlett-Packard system (Waldbronn, Germany) comprising a HP-1100 pump, a Rheodyne model 7725 injector (Cotati, CA), a UV detector (280 nm) and a C18 analytical column (250 mm × 8 mm). The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was

as follows: 15% A/85% B, 0–12 min; 40% A/60% B, 12–14 min; 60% A/40% B, 14–18 min; 80%A/20% B, 18–20 min; 90% A/10% B, 20–24 min; 100% A, 24–28 min. The injection volume was 20 µL. The data were stored and processed by a HPLC Chemstation (Dos Series) (Hewlette Packard). Peaks were identified by comparison of their retention times with the corresponding standards. Analyses were performed in triplicate. The amounts of phenolic compounds were expressed as mg of phenols per 100 g of dry weight (DW).

2.12. Oxidative stability by Rancimat

The oxidative-stability was measured using a Rancimat apparatus (MetrohmSeries 743). The stage of stabilization was deliberated by the oxidative induction time using 3 g of sample. The temperature was set at 100 °C and the air flow rate was 20 L/h. For the duration of the oxidation procedure, the formation of volatile acids in a receiving flask filled with distilled water (60 mL) was measured by conductometry. The values obtained were defined as induction time.

2.13. Thermal analysis by a differential scanning calorimeter (DSC)

Thermal analysis of the oil was carried out using a differential scanning calorimeter (Thermal analysis DSC-204, Germany). Seed oil samples were weighed in a differential scanning calorimeter aluminium pan. An unfilled DSC-pan was used as an inert reference. The reference and sample pans were then positioned within the calorimeter and cooled to -50 °C at 10 °C/min and held for 10 min. Then the temperature was increased to 100 °C at 10 °C/min. This process was repeated and the DSC thermographs were traced in melting transition.

2.14. Sensory evaluation

The sensorial properties of melon seed oil were compared with those of sunflower and virgin olive oil. Oil samples were presented in a perfectly homogeneous and anonymous way (identical conditions of preparation and presentation) with a simple coding of three numbers. The oil samples were served to untrained panel consisting of sixty panelists of both sexes (30 males and 30 females) from the staff members and students of the National Engineering School of Sfax in Tunisia. Their ages ranged from 23 to 48. The samples prepared for tasting were reserved in tasting glasses at 28 °C with 15 mL of oil.

Detailed directions on how to evaluate the samples were presented verbally before the panelists entered the cubicle area and then also in written form on the score paper. The panelists were given napkins and enough water to rinse their mouths between

each sample. Small containers into which samples may be spit were also provided. The prepared oils were evaluated based on a nine-point hedonic scale (1: dislike extremely, 5: neither like nor dislike, 9: like extremely). Each consumer was provided three samples tagged with arbitrary three-digit codes and asked to assess the oils for color, odor, taste, appearance and overall appreciation.

2.15. Statistical analysis

All experiments were performed in triplicate and the results were expressed as the mean values and standard deviation. One-way analysis of variance (ANOVA) was used to determine significant differences among means and Tukey's test was used to perform multiple comparisons among means using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). The significance level was defined as $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Yield oil of seeds

The lipid fraction obtained from the extraction of melon seeds (Maazoun variety) presented a yield of 30.65%, on dry weight. Such a yield may be considered economically important when compared to conventional oilseeds such as soy and corn, which contain 18–20% and 3.1–5.7% of oil, respectively (O'Brien, 2004). Belonging to the same botanical family (*Cucurbitaceae*), the oil content of Maazoun melon seed was higher than *agrestis* melon (23.33%), bitter melon (19.30%) and Kalahari melon (30.50%) seeds (Mariod *et al.*, 2009; Nyam *et al.*, 2009). Our findings were in agreement with the *inodorus* Naudin melon seeds with 30.60% of oil (da Silva and Jorge, 2014). According to Nyam *et al.*, (2009), variations in oil yield may be assigned to differences in plant variety, cultivation climate, ripening stage and the extraction method exerted. The notable percentage of oil makes Maazoun melon seeds convenient for application in the oil industry. For this reason, the profile characteristics and thermal properties of this oil should be investigated.

3.2. Physicochemical characteristics of oil

The quality parameters used frequently to evaluate the chemical and physical characteristics of edible oils are contents in free fatty acids, peroxide value, saponification value, iodine value and refractive index. Table 1 summarizes the physical and chemical properties of melon seed oil (Maazoun variety). Free fatty acid (FFAs) content is an important quality parameter and it gives information about the shelf- life of oil, since FFA can change

TABLE 1. Physicochemical characteristics of *Cucumis melo* L. seed oil (Maazoun variety).

Parameter	Value
Free fatty acids (%)	0.31 ± 0.06
Peroxide value (meq O ₂ /kgoil)	0.50 ± 0.20
Saponification value (mg KOH/goil)	179.80 ± 0.11
Iodine value (g I ₂ /100 g oil)	139.50 ± 0.53
Refractive index (25°C)	1.47 ± 0.00
Specific gravity (25°C)	0.91 ± 0.00
Extinction coefficient K ₂₃₂	1.21 ± 0.13
Extinction coefficient K ₂₇₀	0.42 ± 0.07
Viscosity (mPa.s)	90.75 ± 0.25
Carotenoid (mg/kg)	2.43 ± 0.03
Chlorophyll (mg/kg)	5.70 ± 0.08
Total phenolic compounds (mg/100g)	22.63 ± 0.85
Color L*	48.42 ± 0.84
a*	-5.81 ± 0.18
b*	15.29 ± 0.73

All determinations were carried out in triplicate and mean value ± standard deviation.

the physicochemical or organoleptic characteristics of fat. FFA content in melon seed oil was found to be 0.31% (Table 1). This low value revealed that melon seed oil was edible oil with nutritive value. Moreover, this meant that Maazoun seed oil was nearly free from the hydrolytic rancidity brought about by enzymes.

The peroxide value measures the amount of hydro peroxide in the fat and allows for determining the auto-oxidation of oils. The peroxide value of melon seed oil was equal to 0.5 meq O₂/kg of oil. This value was lower than that reported by Azhari *et al.*, (2014) for melon seed oil (2.5 meq O₂/kg oil) from Sudan. The saponification index indicates the presence or absence of triacylglycerols with high molecular weight in vegetable oils. The saponification index of Maazoun melon seed oil (179.8 mg/g oil) was higher than that described in the literature by Nyam *et al.*, (2009) for Kalahari melon seed oil (173.2 mg KOH/g oil) although lower than that of bitter melon seed oil (190.7 mg KOH/g oil), as observed by the same authors.

Iodine value is used to measure the average number of double bonds or unsaturation in fats. The iodine value of melon seed oil was determined as 139.5 g I₂/100 g oil. Compared to melon seed oils from other geographical origins, this value was higher than the iodine values for melon seed oil from Malaysia (125 g I₂/100g oil) and Sudan (110.99 g I₂/100g oil) (Nyam *et al.*, 2009; Azhari *et al.*, 2014). Such a high value might indicate that melon seed oil from Tunisia possesses higher contents in unsaturated fatty acids than most melon seed oils. Thus, it possessed adequate qualities of drying oil purposes

and edible oil. The iodine value was situated within the interval of values for other edible vegetable oils and varied from 95 to 145 g I₂/100 g (Tan and Che Man, 2002).

The refraction index increases with the unsaturation level of the oil. The refractive index of melon seed oil (1.47) was found to be higher than the previously reported value (1.44) by Azhari *et al.*, (2014). The obtained value for specific gravity (0.91) was in agreement with the value found for melon seed oil of the *tibish* variety (Azhari *et al.*, 2014). The oxidative state of every plant oil may be assessed from the specific absorption at 232 nm (K_{232}) and 270 nm (K_{270}) which designates, respectively, the presence of primary and secondary oxidation products. The extinction coefficients (K_{232} and K_{270}) were found to be 1.21 and 0.42, respectively. These results suggested that Maazoun melon seed oil's stability to oxidation was relatively good.

The viscosity of melon seed oil (90.75 mPa.s) was lower than that reported by Gohari Ardabili *et al.*, (2011) for pumpkin seed oil (93.66 mPa.s), yet, it was higher than those of most vegetable oils such as sunflower (59.7 mPa.s), soybean (63.23 mPa.s) and groundnut (69.04 mPa.s) oils due to the presence of long chain fatty acids such as linoleic and oleic acids (Chouaibi *et al.*, 2012). Similarly, Hellier *et al.*, (2015) reported an obvious trend in the relationship between fatty acids' chain length and viscosity. The authors confirmed the influence of fatty acid double bonds on viscosity.

3.3. Carotenoid and chlorophyll

The chlorophyll and carotenoid contents of melon seed oil of the Maazoun variety are illustrated in Table 1. Melon seed oil accounted for 2.43 mg/kg of carotenoids which explained the yellow color of seed oil from Maazoun variety. This amount was lower than that of virgin olive oil with 3.70 mg/kg (Issaoui *et al.*, 2010). With regards to chlorophyll, melon seed oil was found to contain 5.70 mg/kg. This value was higher than that of fig achenes oil (1.05 mg/kg) (Soltana *et al.*, 2016). However, it was lower than that of virgin olive oil (9.32–12.67 mg/kg) (Aparicio *et al.*, 1999). These pigments are beneficial because they simulate the appearance of annatos and carotenes frequently used in the fat industry (Oomah *et al.*, 2000). From a nutritional point of view, carotenoids are sources of vitamin A and they play an important role in the oxidative stability of vegetable oils.

3.4. Color

The CIE-Lab values (L^* , a^* , b^*) of melon seed oil were 48.42, -5.81 and 15.29, respectively. For comparison purposes, *Cucumis melo* seed oil of the Maazoun variety showed a higher L^* value and

lower a^* and b^* values than pumpkin (*Cucurbita maxima*) seed oil (Rezic *et al.*, 2012). This indicates that melon seed oil was lighter in color. The CIE-Lab coordinates (L^* , a^* , b^*) values for other vegetable oils, such as olive, sunflower, palm, corn and soybean ranged from 63.4 to 69.50, 3.80 to 4.40 and 9.20 to 10.40, respectively (Hsu and Yu, 2002). Hence, melon seed oil was differentiated by a negative value of the a^* coordinate which was clearly lower than that of other vegetable oils. Moreover, the melon seed oil's b^* value was higher than those of common vegetable oils. Thereby, *Cucumis melo* seed oil was more golden than the vegetable oils examined by Hsu and Hu (2002). This means the attendance of yellow pigments like carotenoids.

3.5. Total phenolic content

The phenolic compounds have a significant importance due to their nutritional and technological properties. As shown in table 1, the total phenolic content of melon seed oil was 22.63 mg/100g oil, which was higher than that of melon *inodorus* of the Naudin variety (13.07 mg/100g oil) (da Silva and Jorge, 2014). However, melon seed oil of the Maazoun variety had a relatively lower phenol content compared to olive oil, which is considered as a rich source of phenolic compounds in the Mediterranean diet. In fact, Ouni *et al.*, (2011) reported that the total phenolic content of olive oil of the *Oueslati* cultivar ranged from 33.48 to 85.98 mg/100g oil.

Phenolic compounds have a positive effect in the prevention of cancer and coronary heart disease. Furthermore, the high amount of polyunsaturated fatty acids could make melon seed oil prone to oxidation. However, this could be avoided by the natural phenolic compounds that protect the melon seed oil. Phenolics exert a beneficial effect on the oxidative stability of vegetable oils. According to Aparicio *et al.*, (1999), the contribution of polyphenols in the oxidative stability of olive oil was about 51%, fatty acids, 24% and in lower percentages in carotenoids and chlorophylls.

3.6. Fatty acids

The fatty acid composition is an essential indicator of the nutritional value of oils. The data about the fatty acid profile of melon seed oil from the Maazoun variety is presented in Table 2. This composition was dominated by C18:2 (68.98%), C18:1 (15.84%), C16:0 (8.76%) and C18:0 (5.64%). Melon seed oil belongs to the linoleic-oleic group of oil. These results are in agreement with previously published studies on other varieties such as Honeydew and *inodorus* Naudin, in which the main fatty acids were linoleic (59-59.04%), oleic (24.71-26.4%), palmitic (8.7-9.57%) and stearic acid (5.3-5.74%) (da Silva and Jorge, 2014; Gónas and Rudzińska, 2016). According to Oomah *et al.*,

TABLE 2. Fatty acid composition of *Cucumis melo* L. seed oil (Maazoun variety).

Fatty acid	Composition (%)
ΣSFA	14.76
Myristic acid (C14:0)	0.04 ± 0.01
Pentadecanoic acid (C15:0)	0.03 ± 0.01
Palmitic acid (C16:0)	8.76 ± 0.07
Margaric acid (C17:0)	0.07 ± 0.00
Stearic acid (C18:0)	5.64 ± 0.06
Arachidic acid (C20:0)	0.16 ± 0.01
Lignoceric acid (C24:0)	0.06 ± 0.01
ΣUFA	85.41
Palmitoleic acid (C16:1)	0.08 ± 0.02
Margaroleic acid (C17:1)	0.03 ± 0.01
Oleic acid (C18:1)	15.84 ± 0.03
Linoleic acid (C18:2)	68.98 ± 0.05
Linolenic acid (C18:3)	0.20 ± 0.00
Gondoic acid (C20:1)	0.26 ± 0.01
Erucic acid (C22:1)	0.02 ± 0.01
ΣMUFA	16.23
ΣPUFA	69.18
UFA/SFA	5.79

All determinations were carried out in triplicate and mean value ± standard deviation.

SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

(2000), linoleic acid has favorable nutritional implications and beneficial physiological effects in the prevention of cancer. Hence, melon seed oil has important nutritional value due to the adequate levels of linoleic and oleic acids which constituted more than 84% of total fatty acids. Additionally, melon seed oil contains arachidic, linolenic and gondoic acids, but in much smaller amounts (0.16–0.25%). Trace amounts (<0.1%) of myristic, pentadecanoic, margaric, lignoceric, palmitoleic, heptadecenoic and erucic acids were also detected.

In the current study, the percentage of the different types of fatty acids in melon seed oil recovered from the Maazoun cultivar were as follows: saturated (14.76%), monounsaturated (16.23) and polyunsaturated (69.18%). A similar observation was made by Azhari *et al.*, (2014) in *tibish* melon seed oil (saturated 19.81%; monounsaturated 18.92%; polyunsaturated 61.26%). The potential reason for the differences in the composition of fatty acids of melon seed oils could be accredited to varietal differences and abiotic factors such as climate conditions, horticultural measures and water availability.

The Maazoun melon seed oil showed an unsaturated/saturated fatty acid ratio of 5.79 (Table 2). A similar ratio of fatty acid content was found in the

TABLE 3. Triacylglycerol composition of *Cucumis melo* L. seed oil (Maazoun variety).

Triacylglycerol	ECN	Relative composition (%)
LLL	42	28.99 ± 0.01
OLL	44	23.94 ± 0.01
PLL	44	20.75 ± 0.02
OOL	46	4.87 ± 0.08
POL	46	16.13 ± 0.02
PLP	46	0.60 ± 0.005
SLL	46	0.69 ± 0.02
OOO	48	3.60 ± 0.01
POP	48	0.25 ± 0.00
SLO	48	0.17 ± 0.01

Values are mean of three replicate samples (n = 3), mean ± standard deviation.

L, linoleic; O, oleic; P, palmitic; S, stearic; ECN, equivalent carbon number.

melon seed oil from the Honeydew variety (UFA/SFA = 5.9) (da Silva and Jorge, 2014). It is well-known that the digestibility and quality of edible vegetable oils are determined by the quantity and composition in unsaturated fatty acids. According to El-Adawy and Taha (2001), the presence of unsaturated fatty acids in sufficient amounts raises great interest. Polyunsaturated fatty acids lower cardiovascular risk and their consumption should be encouraged. Consequently, melon seed oil may be recommended as a dietetic oil and suggested as a dietary supplement for diverse food purposes.

3.7. Triacylglycerols composition

The determination of the triacylglycerol profile has been considered an evaluation of the quality and purity of plant oils. The analysis of triacylglycerol molecular species in Maazoun melon seed oil revealed that trilinolein (LLL), oleo dilinolein (OLL) and palmitoyl dilinolein (PLL) were the most abundant triacylglycerols (Table 3). Therefore, linoleic acid was a major component in the three main types of triacylglycerols (LLL, OLL, and PLL). This can be explained by the fatty acid composition in which linoleic acid presents the highest concentration (68.98%; Table 2). It was remarkable that a close correspondence existed between the fatty acid composition and triacylglycerol profile of the oil. Maazoun melon seed oil also contained POL (Palmitoyl Oleoyl Linoleoylglycerol) in a lower quantity (16.13%). In comparison, these results were similar to the previous study by Yanty *et al.*, (2008) for Honeydew melon seed oil which had the following amounts of major triacylglycerols: LLL (24.90%), OLL (21.50%), PLL (15.90%) and POL (12.40%). Likewise, watermelon seed oil showed a triacylglycerol composition with dominance of

LLL (29.5%) and OLL (21.7%) (Conto *et al.*, 2011). However, da Silva and Jorge (2014) reported that OLL (33.10%) was the main triacylglycerol followed by LLL (24.70%) for melon seed oil from the variety *inodorus* Naudin. The differences in triacylglycerol amounts and types were accredited to the oil source and product processing and were mainly governed by percentages of the individual fatty acids. Furthermore, Maazoun melon seed oil contained relatively small quantities of OOL (4.87%) and OOO (3.60%).

In Table 3, all the triacylglycerols were grouped according to their equivalent carbon number (ECN). Melon seed oil contained four triacylglycerol species (from ECN42 to ECN48) in which triacylglycerols with ECN44 were dominant (44.69%), followed by TAGs ECN42 (28.99%), TAGs ECN46 (22.29%) and TAGs ECN48 (4.02%).

3.8. Identification of phenolic compounds

The quantitative analysis showed that melon seed oil from the Maazoun variety contains fourteen phenolic compounds (Table 4). Among these compounds, several flavonoids and phenolic acids were identified. The HPLC characterization of melon seed oil revealed that the amentoflavone, luteolin-7-O-glycoside and gallic acid were the major phenolic compounds accounting for 3.88 mg/100g, 1.26 mg/100g and 0.93 mg/100g, respectively. Defined as plant flavonoids, the presence of amentoflavone has been associated with an antidiabetic function; whereas the luteolin-7-O-glycoside possesses antioxidant and antibacterial

properties. Gallic acid seems to have antifungal and antiviral properties. It acts as an antioxidant and helps to protect cells against oxidative damage (Soong and Barlow, 2006). Melon seed oil was also found to contain naringenin, vanillic acid, *p*-coumaric acid, flavones and syringic acid, although in smaller amounts ranging from 0.44 mg/100 g to 0.67 mg/100g (Table 4). However, compared to the same variety, vanillic, *p*-coumaric and syringic acids were not detected in the melon seed oil of the Maazoun variety (Mallek-Ayadi *et al.*, 2018). Interestingly, vanillic acid as well as *p*-coumaric acid are components with important anticancer activity (Rocha *et al.*, 2012); whereas syringic acid possesses antibacterial ability against several microorganisms (Shi *et al.*, 2016). In comparison with other melon varieties, some of the phenolic compounds identified in this study had not been previously reported for Kalahari melon seed oil (Nyam *et al.*, 2009), in particular rosmarinic acid, which exhibits important biological activities including antioxidant properties.

The health benefits associated with the intake of polyphenols of plant origin have been scientifically established. Thereby, melon seeds are potentially a source of natural antioxidants which can be used for the prevention of oxidative stress in the human body. It is worth noting that during storage, phenolic compounds protect the polyunsaturated fatty acids of vegetable oils against oxidation by exerting their antioxidant role. Additionally, edible oils rich in natural antioxidants may play a role in reducing the risk of chronic diseases. Thus, the examined oil could be utilized in diverse food preparations to provide nutrition and health benefits.

3.9. Oxidative stability

Oxidative stability is the determination of oxidation induction time of oil and hence its resistance to oxidative phenomena. The oxidative stability index is a significant parameter to evaluate the quality of edible oils. The stability of melon seed oil, evaluated by the Rancimat method, was about 7.20 hours at 100 °C. At the same temperature, the Rancimat induction time of pumpkin seed oil was 18.61 h (Rezig *et al.*, 2012). The melon seed oil was thus less resistant to oxidation than pumpkin seed oil. With regard to the literature, it is observed that *tibish* seed oil presented lower stability (4.28 h) against oxidation (Azhari *et al.*, 2014). It seems that an arrangement of some composites is probably responsible for the good oxidative stability of unsaturated fatty acids. Decker (1998) reported that the oxidative stability could be improved by controlling the composition and the activity of reaction substrates and minimizing thus the use of food additives.

TABLE 4. Phenolic compounds (mg/100g) in *Cucumis melo* L. seed oil from the Maazoun variety.

Phenolic compounds	Composition
Protocatechuic acid	0.30 ± 0.00
Caffeic acid	0.41 ± 0.01
Gallic acid	0.93 ± 0.03
Vanillic acid	0.61 ± 0.01
Luteolin -7-O-glycoside	1.26 ± 0.02
Naringenin	0.67 ± 0.02
Syringic acid	0.44 ± 0.00
<i>p</i> -coumaric acid	0.60 ± 0.01
Oleuropein	0.17 ± 0.05
Rosmarinic acid	0.28 ± 0.02
Pinoresinol	0.40 ± 0.01
Apigenin	0.39 ± 0.01
Flavone	0.49 ± 0.02
Amentoflavone	3.88 ± 0.11

All determinations were carried out in triplicate and mean value ± standard deviation.

3.10. Thermal analysis

Differential scanning calorimetry (DSC) is an efficient method to evaluate the physical and thermal characteristics of oils. The DSC heating parameters assist in discovering the character of the phase evolution which take place when the oils are melted. As shown in figure 1, melon seed oil (Maazoun variety) presented a simple thermogram with two different peaks: a primary temperature melting peak possessing a peak temperature of $-46.02\text{ }^{\circ}\text{C}$, a melting enthalpy 88.41 J/g and an onset temperature $-50.76\text{ }^{\circ}\text{C}$ followed by a melting peak with a temperature of $-19.5\text{ }^{\circ}\text{C}$, a melting enthalpy of 138.15 J/g and an onset temperature of $-25.81\text{ }^{\circ}\text{C}$. The melting of the polymorphic forms of triacylglycerols which possess weaker stability could explain the record of the initial endothermic transition observed in the heating thermogram of melon seed oil. The second endothermic peak was clearly discernible in the melon seed oil and may be due to the more steady polymorphic form or to the presence of higher-melting triacylglycerols. The same trend was found by Cheikh-Rouhou *et al.*, (2007) for the Tunisian variety of *Nigella sativa* seed oil. The thermal properties of fats were found to correspond with their

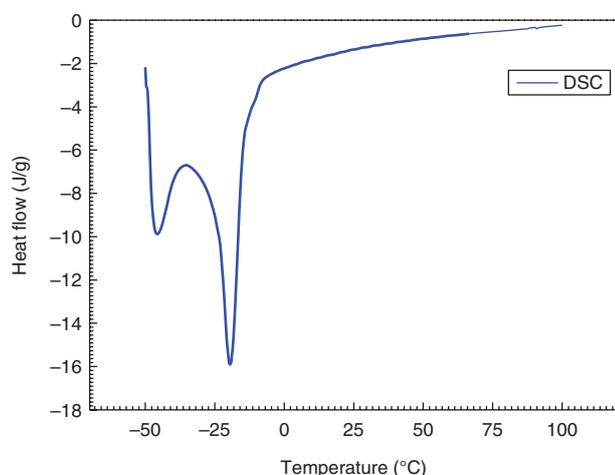


FIGURE 1. Melting thermogram of *Cucumis melo* L. (Maazoun variety) seed oil.

constitution especially the chain length of fatty acids, level of unsaturation and the distribution of fatty acids in triacylglycerols (Bahloul *et al.*, 2014).

3.11. Sensory analysis

The means of different sensory attributes (color, odor, taste, appearance and overall appreciation) of sunflower oil, melon seed oil and virgin olive oil are presented in table 5. The results of the hedonic analysis show that virgin olive oil is more appreciated by tasters than the other two vegetable oils (melon seed oil and sunflower oil) since it obtained the highest scores for all organoleptic attributes evaluated. In addition, the results from the sensory evaluation indicated that melon seed oil is also appreciated by tasters. In fact, there is no significant difference (p -value > 0.05) in color, taste and appearance between virgin olive oil and melon seed oil. Therefore, this unconventional oil extracted from melon seed of the Maazoun cultivar can be used like other vegetable food oils in culinary preparations and food formulations.

4. CONCLUSIONS

The present work shows that the melon seeds of the Maazoun variety provide an appreciable oil yield. The physicochemical characteristics asserted the added-value of the extracted oil as a dietary supplement for food applications. Moreover, melon seed oil seems to be a new promising source of biologically active substances and could be used as a functional ingredient. In fact, phenolic compounds and carotenoids are of nutritional importance for use in industrial applications. Therefore, the appropriate utilization of melon seeds may constitute an example for the valorization of fruit by-products to produce a promising new source of oil which is not widespread in the food industry yet it is not inferior in quality from the common vegetable oils.

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TABLE 5. Sensory evaluation of melon (*Cucumis melo* L.) seed oil of the Maazoun variety.

Oil	Color	Odor	Taste	Appearance	Overall appreciation
<i>Sunflower oil</i>	1.86 ± 0.71^a	2.00 ± 0.38^a	1.83 ± 0.74^a	1.86 ± 0.62^a	1.76 ± 0.32^a
<i>Melon seed oil</i>	3.01 ± 0.59^b	2.53 ± 0.97^a	$2.40 \pm 0.25^{a,b}$	2.63 ± 0.76^b	2.86 ± 0.53^b
<i>Virgin olive oil</i>	3.03 ± 0.29^b	3.50 ± 0.51^b	3.06 ± 0.34^b	2.86 ± 0.33^b	3.23 ± 0.41^c

All values given are means of three determinations, mean \pm standard deviation.

No significant difference ($p > 0.05$) among mean values in the same column and followed by the same letters (Tukey's test), significance at ($p < 0.05$).

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