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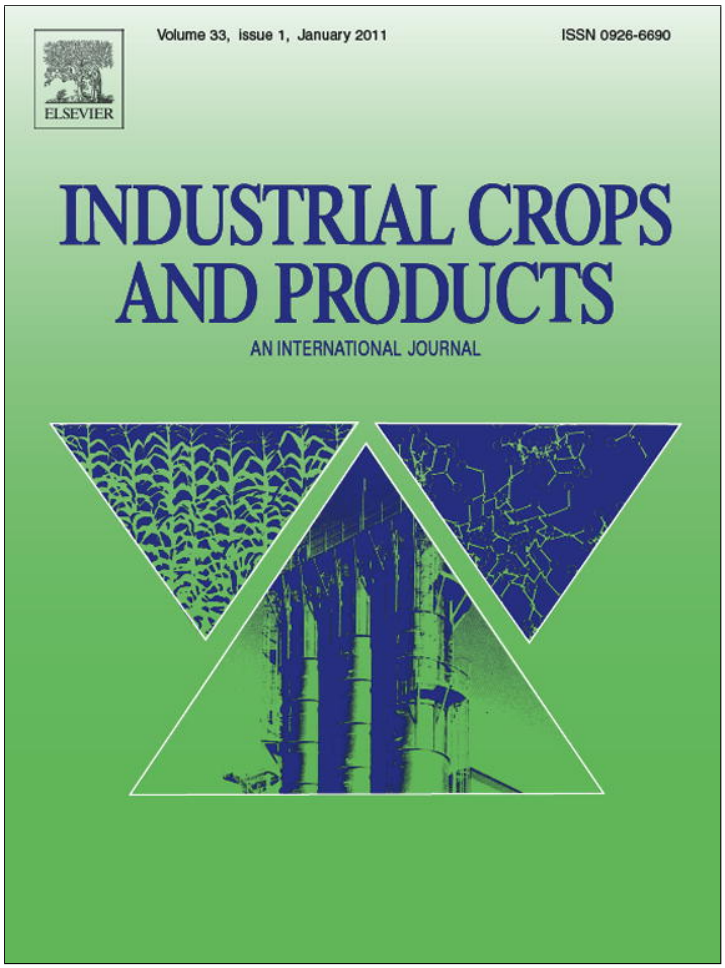

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Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil

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ABSTRACT

Seeds of a Tunisian variety (Béjaoui) of pumpkin (*Cucurbita maxima*) were analysed for their main chemical composition and for their oil properties. Expressed on dry weight basis, seed moisture was 8.46%, whereas contents of proteins, fibre, ash, fat, and total sugars established at 33.92%, 3.97%, 21.97%, 31.57%, and 0.11% respectively. Gas chromatography revealed that the major fatty acids were oleic, linoleic, and palmitic acids (44.11%, 34.77%, and 15.97% respectively). Seed oil was also found to be rich in tocopherols with a predominance of δ-tocopherol (42.27%). The sterol marker β-sisosterol accounted for 39.6% of total sterols contained in seed oil of this variety. Six phenolic acids (protocatechuic, caffeic, syringic, vanillic, p-coumaric and ferulic) were detected, the syringic acid being predominant (7.96 mg/100 g). As a whole, based on its seed oil features, pumpkin may be considered as a valuable source for new multi-purpose products for industrial, cosmetic, and pharmaceutical utilisation.

1. Introduction

Pumpkin seed refers to the edible seed of a pumpkin (genus *Cucurbita*). The seeds are specifically flat, oval, and light green inside a white hull and are used for both comestible and medicinal purposes. Pumpkin seeds are also used in culinary practices mainly in the southern parts of Austria, Slovenia, and Hungary (Murković et al., 1996). In addition, roasted pumpkin seeds are a popular snack in many African countries, especially in Tunisia.

Pumpkin seed oil is a strongly dichromatic viscous oil that has been documented for its strong antioxidant activity (Stevenson et al., 2007), and has been identified as an exceptional preventive against hypertension and carcinogenic diseases (Zuhair et al., 2000; Jian et al., 2005). In recent years, several studies have been conducted to better highlight the medicinal benefits of pumpkin seed oil (Stevenson et al., 2007). However, to the best of our knowledge, the composition and the physico-chemical properties of pumpkin seed oil remain fairly unexplored, particularly for North African varieties. The determination of both the physico-chemical properties and the oxidative stability would significantly contribute to the valorisation of pumpkin seed oil potential in cosmetic, pharmaceutical and food industries.

The present study aims at identifying the physico-chemical properties as well as the free radical scavenging activity of a pumpkin seed oil of a Tunisian variety (Béjaoui) of pumpkin seed (*Cucurbita maxima*). This may lead to the innovative utilisation of pumpkin seed oil as an alternative for industrial uses.

2. Materials and methods

2.1. Seed material

Pumpkin seeds *(C. maxima)* were brought from a local market in Chebika region (latitude 35°37′38″; longitude 10°2.15′38″; elevation 86 m), located in the south-east of Tunisia. The seeds were directly isolated, washed to remove impurities, and eventually air-dried.

2.2. Oil extraction

Pumpkin seed oil extraction was carried out according to the mechanical cold expeller method (El-Adawy and Taha, 2001) but with a slight difference. Seed samples (50 g) were milled in a heavy-duty grinder with 250 ml of petroleum ether and mixed using a shaker (GLF, Germany) at a rate of 160 rpm for 4 h. The mixture was then centrifuged for 15 min at a temperature of (4 °C). The supernatant was then filtered through a Whattman filter paper (0.45 μm). The extraction procedure was repeated twice and the solvent was removed using a rotary evaporator at 40 °C. The seed

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oil obtained was drained under a nitrogen stream (N_2) and was then stored in a freezer at (−20 °C) for subsequent analysis.

2.3. The analytical methods

2.3.1. Moisture and protein content

Moisture was determined according to (AOAC, 1990) whereas protein content was calculated from nitrogen content as follows: N (%) × 6.25 using Kjeldahl's method.

2.3.2. Ash and mineral content

Ash was obtained after mineralisation of sample seeds at 600 °C for 8 h. Mineral content was determined by atomic absorption spectrophotometer (Varian 220 FS, Belgium). Potassium and sodium were both assayed by flame emission spectrophotometer (IL 151), whereas phosphorous content was determined using the molybdenum-blue method (Hwang, 1989).

2.3.3. Fat, fibre, and total sugar contents

The fat content was measured by using a Soxhelt extraction apparatus with petroleum ether as a solvent for 8 h. Crude fibre was determined by the acid detergent fibre method (Van Soest et al., 1991). Total sugars (TS) were calculated as follows: TS = 100 − (%moisture + %protein + %ash + %fibre + %fat).

2.3.4. Chemical analysis

Official methods (American Oil Chemist's Society, AOCS, 1997) were used for the determination of the acid value (method Cd 3d-63), peroxide value (method Cd 8-53), iodone value (method Cd 1-25), saponification value (method Cd 3-25), unsaponifiable matter (method Ca 6a-40), specific gravity (using a 10 ml pycnometer at 25 °C), and the refractive index (using Abbé refractometer at 40 °C) of the pumpkin seed oil.

Specific absorptivity values K_{232} and K_{270} were determined using a UV spectrophotometer (Shimadzu Co., Kyoto, Japan) by measuring the absorbance of a 1% solution of pumpkin seed oil in cyclohexane and a path of 1 cm at 232 and 270 nm.

2.3.5. Fatty acid composition

Fatty acid composition was determined by the analytical methods described in European Parliament and of the European Council in EEC regulation 2568/91 (1991). Fatty acids were converted to fatty acid methyl esters (FAMEs) before being analysed by shaking off a solution of 0.2 g of oil and 3 ml of hexane with 0.4 ml of 2 N methanolic potassium hydroxide. The FAMEs were then analysed in a Hewlett-Packard model 4890D Gas Chromatograph furnished with an HP-INNOwax fused silica capillary columns (Cross-Linked PEG), 30 m × 0.25 mm × 0.25 μm and a flame ionisation detector (FID). Inlet and detector temperatures were held at 230 °C and 250 °C, respectively. The initial oven temperature was held at 120 °C for 1 min and then it was raised to 240 °C at a rate of 4.0 °C/min for 4 min. The FAMEs injected volume was 1 μl and nitrogen (N_2) was used as the carrier gas at 1 ml/min with a split inlet flow system at a 1:100 split ratio. Next, heptadecanoic acid C17:0 was added as an internal standard before methylation in order to measure the amount of fatty acids. Eventually, fatty acid contents were calculated using a 4890A Hewlett-Packard integrator.

2.3.6. The free radical scavenging activity of the pumpkin seed oil

A pumpkin oil sample was examined for its capacity to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl compound (DPPH) (Kalantzakis et al., 2006). One milliliter of a solution containing 0.5 g of oil in a 5 ml of ethyl acetate was added to 4 ml of a freshly prepared DPPH solution (10^{-4} M in ethyl acetate) in a screw-capped 10 ml test tube. The reaction mixture was then shaken vigorously for 10 s in a Vortex apparatus. Next, the tube was kept in complete darkness for 30 min so as to reach steady-state stability. Subsequently, the mixture absorption was measured at 515 nm against a blank solution. Meanwhile, a control sample containing 1 ml of ethyl acetate and 4 ml of the DPPH solution was prepared and equally measured.

The radical scavenging activity (RSA) towards DPPH was expressed in terms of the percentage of DPPH concentration reduction of the pumpkin seed oil components. It was calculated as follows:

$\%[DPPH]_{red} = 100 \times (1 - [DPPH]_{30}/[DPPH]_0)$, where $[DPPH]_0$ and $[DPPH]_{30}$, using the concentrations of DPPH in the control sample ($t = 0$) and in the test mixture after the 30 min reaction, respectively.

2.3.7. Oxidative stability

The oxidative stability was determined with the 743 Rancimat apparatus (Metrohm Co., Basel, Switzerland), an instrument for automatic determination of the oxidation stability of oils and fats. The level of stabilisation was measured by the oxidative-induction time (OIT) using 3.5 g of oil. The temperature was set at 100 °C, and the purified air flow passing through at a rate of 10 l/h. During the oxidation process, volatile acids were formed in the distilled water and were measured conductimetrically. The induction period was defined as the necessary time to reach the inflection point of the conductivity curve (Halbault et al., 1997).

2.3.8. Identification and quantification of phenolic acids by HPLC–MS/MS

The HPLC–MS experiments were carried out with an Agilent 1100 LC system consisting of a degasser, a binary pump, an auto sampler, and a column heater.

The column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out on a personal computer using CHEMCAD 6.3, a chemical process simulation software designed by Chemstation, Inc. For the chromatographic separation, a Knauer C-18 column (250 mm × 4.6 mm, 5 μm) was used. The column maintained at 40 °C was first held at 90% Solvent A (1% acetic acid in water) and 10% solvent B (1% acetic acid in methanol), followed by a step gradient from 10% B to 20% B in 4 min, and a second step gradient from 20% B to 100% B in 20 min. Then, it was kept for 6 min with 100% B. Finally, the elution was obtained from 100% B to 20% B for 6 min. The flow rate was 400 μl/min and the injected volume was 10 μl of the mixture that contains 100 μl of pumpkin seed oil and 900 μl of ethyl acetate.

The following parameters were used throughout the MS experiment, that is, for electrospray ionisation with negative ion polarity. The capillary voltage was set to 1.6 kV, the drying temperature to 350 °C, the nebuliser pressure to 40 psi, and the drying gas flow to 10 l/min. The maximum accumulation time was 50 ms, the scan speed was 26,000 m Z^{-1} s^{-1} (Ultra Scan Mode) whilst the fragmentation time was 30 ms. Phenolic compounds were identified using a combination of high performance liquid chromatography (HPLC) with an Agilent 1100 diode array detection and liquid chromatography with electrospary ionisation mass spectrometry (ESI–LC–MS) on the basis of their ultraviolet (UV) spectra, mass spectra and by comparing the spectra with those of available authentic standards.

2.3.9. Sterols analysis (ST)

Sterol separation was performed according to the NF EN ISO 12228 method (1999). Pumpkin seed oil (250 mg) was refluxed for 15 min with 5 ml of ethanolic KOH solution (3%, w/v) after adding 1 mg of FLUKA cholesterol as an internal standard and a few antibumping granules. The mixture was immediately diluted with 5 ml of ethanol. The unsaponifiable part was eluted over a glass column packed with a slurry of aluminium oxide (Scharlau) in ethanol (1:2, w/v) with 5 ml of ethanol and 30 ml of diethyl ether at a flow

rate of 2 ml/min. The extract was evaporated in a rotary evaporator at 40 °C under reduced pressure, and then ether was completely evaporated under a nitrogen stream. For the characterisation of sterols, a FLUKA silica gel F254 plate was developed in the solvent system n-hexane/diethyl ether (1:1, v/v). Similarly, for the detection of sterols, the thin-layer plate was sprayed with methanol; the sterol bands were scraped from the plate and recovered by extraction with diethyl ether. The trimethylsilyl ether sterols (TMS) derivatives were prepared by adding 100 μl of a silylant reagent N-methyl-N-(trimethylsilyl) trifluoroacetamide/pyridine (1:10,v/v) in a capped glass vial and heated to 105 °C for 15 min.

A mixture of standard solutions of sterols was prepared by derivatisation (cholesterol, sitosterol, stigmasterol, ergosterol and campesterol). The trimethylsilyl ether sterols derivatives were analysed using the GC system (Agilent 6890 N, CA, USA) equipped with a FID and the GC Chemstation software. An HP-5 (5% pheynyl methyl polysiloxane column) was used (0.32 mm i.d. × 30 m in length; 0.25 μm film thickness, CA, USA). The carrier gas (helium) flow was 1.99 ml/min (split-splitness) injection with a split ratio of 1:200. Both the detector and the injector were set at 320 °C, and the injected volume was of 1 μl. The total analyses were set at 71 min to ensure the elution of all ST. The operational conditions were as follows: injector temperature at 320 °C, column temperature: a gradient of 4 °C/min from 240 °C to 255 °C. Sterols peak identification was carried out according to the NF EN ISO 12228 method (1999) and confirmed by GC-MS (NIST database, 2002) database whilst operating under similar conditions as to that of the GC-FID.

2.3.10. Tocopherol composition

Prior to the HPLC analysis, 0.5 g of the seed oil was diluted with 5 ml of hexane and a 5 μl sample was injected. The tocopherol composition of pumpkin seed oil was determined using HPLC according to the NF EN ISO 9936 (2006). The sample was then analysed by an HPLC (Agilent 1100, CA, USA) consisting of a G1354 quaternary pump, a G1313A standard autosampler, a G 1321A fluorescence detector set at λ excitation = 295 nm, and λ emission = 330 nm and a Chemstation software. A normal phase column (hypersil silica, Hewlett Packard) (250 mm × 4.6 mm × 5 μm) was used with hexane/isopropanol (99.5:0.5, v:v) as a mobile phase. The system was operated isocratically at a flow rate of 0.5 ml/min.

The separations were carried out at 30 °C. The quantification was based on an external standard method and the tocopherol standards mixed in hexane solution (2 mg/ml) were prepared on the basis of the standard compounds: α-, β-, γ-, and the δ-tocopherols.

2.3.11. Differential scanning calorimetry (DSC)

Thermal characteristics of pumpkin seed oil were performed using a modulated differential scanning calorimeter (DSC 2920 Modulated DSC-TA Instruments, Newcastle, DE, USA). The oil sample (2 ± 0.10 mg) was weighed directly into a DSC-pan (SFI-Aluminium, TA Instrument T11024). The seed oil was quickly cooled to −50 °C with a speed of 15 °C/min, maintained for 15 min and heated to 90 °C with a heating speed of 15 C/min. The heating operation was repeated twice and the DSC thermographs were recorded during the second melting. The instrument was calibrated for temperature and heat flow using eicosane (T_p = 36.8 °C, H = 247.70 J/g) and dodecane (T_p = −9.65 °C, H = 216.73 J/g). An empty DSC-pan was used as a reference.

2.3.12. CIE L^* a^* b^* coordinates

CieLab coordinates (L^*, a^* and b^*) were directly read with a spectrophotometer colorimeter (Trintometer Lovibond PFX 195, UK). In this coordinate system, the L^* value is a measure of lightness, ranging from 0 (black) to 100 (white), the a^* value represents the red/green axis and varies from −100 (greenness) to +100 (redness) and the b^* value represents the yellow/blue axis and ranges from −100 (blueness) to +100 (yellowness).

2.3.13. Analytical methods

Analysis was carried out in triplicate. The values of different parameters were expressed as the mean ± standard deviation ($\bar{x} \pm SD$).

3. Results and discussion

3.1. Chemical composition of pumpkin seeds

As illustrated in Table 1, total sugars, protein, fibre, fat and moisture levels were 0.11%, 33.92%, 21.97%, 31.57% and 8.46%, respectively. The oil yield did not exceed that described by Nyam et al. (2009) for *Cucurbita pepo* seed oil, which belongs to the same botanical family (Cucurbiatceae). The high percentages of oil make these seeds suitable for the oil industry application.

Variation in the oil yield could be attributed to differences in plant variety, cultivation climate, ripening stage and the extraction method used (Nyam et al., 2009). Since pumpkin (*C. maxima*) var. "Béjaoui" seeds also contain a high amount of crude fibre, they may constitute a valuable source of dietary fibre in animal feed.

Interestingly, variety Béjaoui seeds were found to contain significant amounts of minerals (Table 1). Potassium was the most prevalent element, followed, in decreasing order by phosphorus, sodium, calcium, magnesium, copper, zinc, iron, and manganese. Such a chemical composition reveals the valuable potencies of such a pumpkin seeds.

3.2. Chemical analysis

Table 2 shows the physicochemical characteristics of pumpkin seed oil var. "Béjaoui". The specific gravitational value (0.91) comparable to that found by El-Adawy and Taha (2001). However, the iodine value (153.66 g I_2/100 g oil) was higher than that reported in the literature (Mitra et al., 2009; El-Adawy and Taha, 2001). High iodine is due to its high content of unsaturated fatty acids (Table 3) and indicates that the seed oil has the good qualities of edible oil and drying oil purposes (Eromosele et al., 1997). The acid and peroxide values were very low, (7.54% and 2.33 meq O_2/kg oil, respectively). The peroxide values suggest that the oil has begun to degrade since it was identified as a highly unsaturated oil. Saponification value of pumpkin seed oil was determined as 175 mg of KOH/g of oil. This value was lower than that reported by El-Adawy and Taha (2001)

Table 1
Chemical characteristics (dry basis) of pumpkin seeds.

Component	
Moisture content[a]	8.46 ± 0.62
Crude oil[a]	31.57 ± 3.71
Crude protein[a]	33.92 ± 3.16
Crude fibre[a]	21.97 ± 4.32
Total ash[a]	3.97 ± 0.02
Total sugars[a]	0.11 ± 0.08
Copper[b]	36.66 ± 4.93
Zinc[b]	25.19 ± 0.63
Iron[b]	15.37 ± 0.87
Manganese[b]	3.42 ± 0.76
Magnesium[b]	146.13 ± 9.91
Sodium[b]	356.75 ± 18.43
Calcium[b]	271.89 ± 24.81
Potassium[b]	886.56 ± 16.34
Phosphorus[b]	824.53 ± 21.38

Values are means ± SD of three determinations.
[a] % (w/w).
[b] mg/100 g of dry weight flour.

Table 2
Physicochemical characterisation of pumpkin seed oil.

Parameter	
Refractive index (40 °C)	1.46
Specific gravity (25 °C)	0.91
Acid value (mg KOH/g oil)	7.54 ± 0.02
Saponification value (mg KOH/g oil)	175 ± 1.30
Iodine value (g I_2/100 g oil)	153.66 ± 0.65
Peroxide value (meq O_2/kg oil)	2.33 ± 0.65
Unsaponifiable matter (%)	1.25 ± 0.15
k_{232}	3.10 ± 0.10
k_{270}	1.66 ± 0.04
$\%[DPPH]_{red}$	36.22 ± 0.60
Oil stability index (h)	18.61 ± 0.42

Values are means ± SD of three determinations.

in their research on pumpkin (*C. pepo*) seed kernel oil and that of Mitra et al. (2009) on pumpkin (*C. maxima*) seed oil. Besides, the oil showed an average of unsaponifiable matter of 1.25%.

Specific extinction coefficients calculated from absorbances at 232 and 270 nm were 3.10 and 1.66, respectively. They indicated that pumpkin seed oil contains primary (hydroperoxides) and secondary oxidation products.

3.3. Fatty acid composition

The fatty acid composition of pumpkin seed oil is illustrated in Table 3. Amongst the current seven fatty acids, four were unsaturated. The most abundant fatty acids were oleic acid (44.11%), linoleic acid (34.77%), palmitic acid (15.97%). These values are close to those obtained by Mitra et al. (2009), Nyam et al. (2009) and El-Adawy and Taha (2001). It is worth mentioning that the high amount of linoleic acid makes pumpkin seed oil specifically prone to oxidation. Yet, this fatty acid may have favourable nutritional implications and beneficial physiological effects in the prevention of both coronary heart disease and cancer (Oomah et al., 2000).

Table 3
Fatty acid (%) and phenolic acids (mg/100 g) composition of pumpkin (*Cucurbita maxima* var. "Béjaoui") seed oil.

Fatty acids	Composition
Palmitic (C16:0)	15.97 ± 0.39
Palmitoleic (C16:1)	tr.
Stearic (C18:0)	4.68 ± 0.56
Oleic (C18:1)	44.11 ± 0.63
Linoleic (C18:2)	34.77 ± 0.95
Linolenic (C18:3)	tr.
Arachidic (C20:0)	0.41 ± 0.40
SAFA	21.07 ± 1.19
MUFA	44.12 ± 0.57
PUFA	34.78 ± 0.85
Phenolic acids	**Composition**
Protocatechuic acid	1.81 ± 0.26
Caffeic acid	3.88 ± 0.03
Syringic acid	7.96 ± 0.13
Vanillic acid	2.46 ± 0.37
p-coumaric acid	2.50 ± 0.95
Ferulic acid	4.99 ± 0.29
ni	*

SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; tr.: trace amounts (less than 0.2%).
ni: not identified. *: Compound detected but concentration in sample not determined.
Values are means ± SD of three determinations.

3.4. Free radical scavenging activity

After 30 min of incubation, 36.22% of DPPH radicals were quenched by pumpkin seed oil (Table 2). Kalantzakis et al. (2006) found that the antioxidant activity of virgin olive oil and other vegetable oil samples like refined olive, cottonseed, sunflower, soybean and commercial oils (expressed as the % of DPPH· concentration reduction by the components of the oils under the same conditions) ranged from 28.4% for refined olive oil to 78.9% for cottonseed oil. This variation was attributed to the tocopherol and phenol contents of each oil sample and greatly contributes to stability towards oxidation.

3.5. Oxidative stability

The result of the Rancimat test is shown in Table 2. The stability of pumpkin seed oil expressed as the oxidation induction time was about 18.61 h. This value may be justified by the high contents of MUFA and PUFA (Besbes et al., 2004). A linear regression based on the oleic/linoleic ratio and the contents of phenols and tocopherols, in virgin olive oil, showed a good correlation with the oxidative stability measured by Rancimat (Aparicio et al., 1999).

Similarly, date seed oil presented a high oxidation stability (33–45 h.), as it was measured by Rancimat under the same conditions. This high stability was justified by the relatively low content of PUFA and the high content of natural antioxidants, such as phenolic compounds (Besbes et al., 2004). The contribution of phenolic and orthophenolic compounds in the oxidation stability of olive oil was about 51%, 24% for fatty acids and, to fewer percentages α-tocopherols, carotenoids and chlorophylls (Aparicio et al., 1999).

3.6. Phenolic acids

Phenolic compounds are part of the unsaponifiable matter and are known as minor oils constituents. These compounds play a determinant role due to their attributes such as flavour, shelf life and resistance against oxidation. A list of the detected phenolic acids with their HPLC retention times and concentration is illustrated in Table 3.

An unidentified peak at a retention time of 29.2 min and a M/Z $(M-H)^-$ of 295.2 was observed. The M/Z 295.2 ion was subjected to MS^2 analysis to produce a signal at M/Z 277.2. This should be attributed to the loss of an hydroxyl group and an hydrogen atom ($\Delta m = 18$). The peak is believed to correspond to simple hydrolysable phenolic acids or to two typical phenolic acids of pumpkin seed oil. These may contribute to their oxidative stability. We believe further study is needed to identify this compound.

In pumpkin seed oil, six phenolic acids were identified, namely syringic acid (having the highest relative content), ferulic acid, caffeic acid, p-coumaric acid, vanillic acid and protocatechuic acid. All these phenolic acids were identified in pumpkin (*Cucurbita pep* L.) seed oil according to Nyam et al. (2009), but at a lower level. Nevertheless, gallic and p-hydroxybenzoic acids were not detected in seed oil of Béjaoui variety. Their concentrations were 0.26 mg/100 g and 0.20 mg/100 g, respectively.

3.7. Phytosterols

The Δ7-sterols are specific to pumpkin seed oil and supposed to confer to this oil a beneficial effect in the treatment and prophylaxis of the prostate gland and the bladder disorders (Nederal Nakić et al., 2006; Schilcher et al., 1987).

The Sterol composition of pumpkin seed oil is presented in Table 4. Sitosterol (39.6%) was the major sterol in the seed oil, followed by Δ5,24-stigmastadienol (21.3%). Sitosterol was also the sterol marker in pumpkin (*C. pepo*) seed oil, in bitter melon seed oil

Table 4
Sterol and tocopherol composition of pumpkin (*Cucurbita maxima* var. "Béjaoui") seed oil (mg/100 g).

Sterol	Composition
Cholesterol	25.35 ± 0.56
Cholestanol	3.03 ± 0.07
24-Methylenecholesterol	3.62 ± 0.11
Stigmasterol	3.17 ± 0.13
Sitosterol	50.64 ± 1.96
Δ5,24-Stigmastadienol	27.25 ± 0.45
(24R)-24-Ethyl cholesta-7,25 (27)-dien-3β-ol	8.52 ± 0.57
Δ7-Avenasterol	6.3 ± 0.30
Total	127.88 ± 2.97
Tocopherol	**Composition**
α-Tocopherol	128 ± 14.42
γ-Tocopherol	113.66 ± 1.52
δ-Tocopherol	177 ± 14.17
Total	418.66 ± 33.36

Values are means ± SD of three determinations.

and in Kalahari melon seed oil. It ranges from 75.7% to 87.3% (Nyam et al., 2009). As found by Nyam et al. (2009), Δ7-sterols were not the main sterols of the seed oil (11.58% of the total sterols). Differences between the contents of Δ5- and Δ7-sterols could be attributed to the maturity stage of seeds or to the solvent used in the extraction procedure.

3.8. Tocopherols

Owing to their role in the protection against oxidative deterioration of polyunsaturated fatty acids in plant material, tocopherols in seed oil are extremely important. They are natural lipophilic antioxidants mainly found in vegetable oils. In seed oil of Béjaoui variety, only α-, γ- and δ-tocopherols were present (Table 4). δ-tocopherol was the main component and represented about 42.27% of total tocopherols, then followed by α-tocopherol and γ-tocopherol. α-tocopherol is recommended for human and animal consumption because it has a higher biological activity than other tocopherols, but γ-tocopherol shows a higher antioxidant capacity as compared to α-tocopherol (Fatnassi et al., 2009). Tocopherol content in seed oil of *C. maxima* var. Béjaoui (418 mg/100 g oil) is higher than that of *C. pepo* L. seed oil (80.65 mg/100 g oil) but much lower than those of other pumpkin seed oils (Stevenson et al., 2007).

3.9. Thermal behaviour

The melting curve of Pumpkin seed oil is displayed in Fig. 1. The oil sample exhibited four transitions when heated from −50 °C to 90 °C. The first and the last transitions, occurring respectively at −35 °C and 0 °C, illustrated approximately the same temperature range (the range of the transitions can be calculated as temperature difference between T_{on} and T_{off}). Theses transitions were higher than the second and the third ones. The first endothermic transition observed in heating thermogram of pumpkin seed oil could be attributed to the melting of the lowest stability polymorphic forms of triacylglycerols (TAG) (e.g. α) (Ferrari et al., 2007). A distinct high endothermic peak occurred at −23.42 °C with a shoulder merging one at about −18 °C. It was typical and clearly distinguishable in the seed oil. The last transition occurring respectively at 0 °C could be associated with the melting of the highest stability polymorphic forms of TAG, mainly monosaturatedtriacylglycerols (MSTAG). Yet, the disaturatedtriacylglycerols (DSTAG) could not be excluded. Nyam et al. (2009) reported that vegetable oils with high content of saturated fatty acids (SFA) experienced DSC melting profiles at higher regions as compared to oils with a high content of unsaturated fatty acids (UFA). The complex endothermic events occurring at higher temperatures were attributed to the melting of crystallised lipids and were characterised by multiple overlapping contributions as previously observed in vegetable (Tan and Che Man, 2000, 2002) and olive oils (Tan and Che Man, 2002; Jiménez Márquez and Beltrán Maza, 2003; Chiavaro et al., 2008). No endothermic phenomenon was illustrated beyond 0 °C. Such a feature may confirm the liquid state of the pumpkin seed oil at room temperature (25 °C) and consequently the absence of crystals.

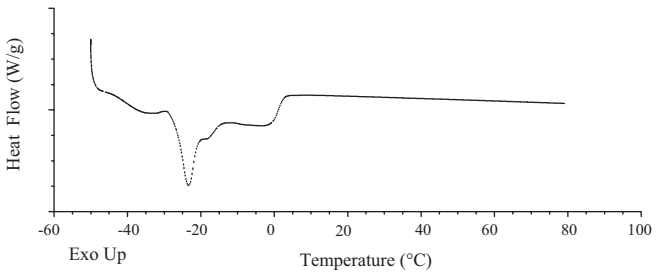

Fig. 1. Melting thermogram of pumpkin (var. Béjaoui) seed oil.

It is noteworthy that the asymmetry of the obtained peak at −23.42 °C could be regulated by two components of different weights. The fact that thermogram seemed to correspond to a number of components higher than the visible ones suggested the presence of triglyceride fractions with melting points too close to be differentiated under the used conditions (Herrera and Añón, 1991). In fact, after total solidification, mixed glyceride crystals could be formed by intersolubility and could be associated into different crystalline groups with different melting points (Besbes et al., 2004). The obtained data are useful to control fractionation of oil during production and may assist in the identification of unknown seed oil samples.

3.10. Colour

CieLab coordinates values (L^*, a^*, b^*) of pumpkin seed oil were 44.8 ± 0.32, −0.18 ± 0.13 and 28.88 ± 0.21. Pumpkin (*C. maxima*) seed oil showed a higher L^* value and lower a^* and b^* values than pumpkin (*C. pepo*) seed oil studied by Nyam et al. (2009). This means that *C. maxima* seed oil was lighter in colour than *C. pepo* seed oil.

The CieLab (L^*, a^*, b^*) values of other vegetable oils, such as palm, soybean, sunflower, olive, and corn ranged from 63.4 to 69.5, 3.8 to 4.4 and 9.2 to 10.4, respectively (Hsu and Yu, 2002). Hence, the pumpkin (*C. maxima*) seed oil's b^* value was higher than those of other vegetable oils. Pumpkin seed oil was also yellower than the vegetable oils studied by Hsu and Yu (2002). This suggests the presence of yellow pigments such as carotenoids. Pumpkin seed oil was also characterised by another colour specification: a^* negative value which was markedly lower than the a^* value of common vegetable oils.

4. Conclusion

This study has revealed that pumpkin seeds are a rich source of many important nutrients that appear to have a very positive effect on human health. In the same way, phenolics, tocopherols and sterols, could provide high protection against oxidative stress. Finally, a good shelf life as well as other desirable characteristics makes pumpkin seed oil an ideal ingredient in pharmaceutics and cosmetics.

The use of pumpkin seed oil for industrial applications could necessitate its exposure to high thermal treatments that could lead to changes in quality characteristics of the oil. So, a study of thermo-oxidation effects on physicochemical parameters of pumpkin seed

must be undertaken. The production of oil from pumpkin seeds provides the use of renewable resource, and at the same time adding value to agricultural products.

Conflict of interest

The authors hereby declare that there are no conflicts of interest.

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