

## Chemical Composition and Physicochemical Properties of Pumpkin Seeds (*Cucurbita pepo* Subsp. *pepo* Var. *Styriaca*) Grown in Iran

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### ABSTRACT

Chemical composition and physicochemical properties of pumpkin seeds and fatty acids of their oil were determined. It was found that the seeds contained 41.59% oil and 25.4% protein. Moisture, crude fiber, total ash, and carbohydrate contents were 5.2%, 5.34%, 2.49%, and 25.19%, respectively. The specific gravity, dynamic viscosity, and refractive index of the extracted pumpkin seed oil were 0.915, 93.659 cP, and 1.4662, respectively. Acid value (mg KOH/g oil), peroxide value (meq O<sub>2</sub>/kg oil), iodine value (g I<sub>2</sub>/100 g oil), saponification number (mg KOH/ g oil), and unsaponifiable matter content (%) of the extracted oil from pumpkin seeds were 0.78, 0.39, 10.85, 104.36, 190.69, and 5.73, respectively. Total phenolics compounds (mg gallic acid/kg oil), total tocopherols (mg  $\alpha$ -tocopherol/kg oil), total sterols (%), and waxes (%) were 66.27, 882.65, 1.86, and 1.58, respectively. Specific extinctions at two wavelengths of 232 nm (K<sub>232</sub>) and 270 nm (K<sub>270</sub>) and R-value (K<sub>232</sub>/K<sub>270</sub>) were 3.80, 3.52 and 0.74, respectively. Gas chromatographic analysis of the pumpkin seed oil showed that the linoleic (39.84%), oleic (38.42%), palmitic (10.68%) and stearic (8.67%) acids were the major fatty acids. Compared with other vegetable oils, the present study revealed that pumpkin seed oil can be a valuable source of edible oil.

**Keywords:** *Cucurbita pepo* subsp. *pepo* var. *Styriaca*, Fatty acid composition, Physicochemical properties, Pumpkin seed oil.

### INTRODUCTION

Vegetable oils are essential in meeting global nutritional demands and are utilized for many food and other industrial purposes (Idouraine *et al.* 1996). Despite the broad range of sources for vegetable oils, the world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively (Stevenson *et al.*, 2007). These conventional sources of vegetable oil no longer meet the ever increasing demands of domestic and industrial sectors (Idouraine *et al.*, 1996). Therefore, the need exists to look for other

sources to supplement the supplies. From this view point, non-conventional oilseeds are of much concern to cope this challenge. More recently, research activities have focused on examining and characterizing new sources of edible oils. Esuoso *et al.* (1998) reported that seeds of some species of Cucurbitaceae can be the edible oil sources to meet the increasing demands for vegetable oil.

Pumpkins belong to the family Cucurbitaceae. The majority of the species in this family are used as food and are found in five genera: *Citrullus* (water melons and wild colocynths), *Cucumis* (cucumbers, gherkins and melons), *Lagenaria* (gourds), *Sechium* (chayotte) and *Cucurbita*. The

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genus *Cucurbita*, which is economically the most important one, includes five species: *C. maxima*, *C. pepo*, *C. moschata*, *C. ficifolia*, and *C. turbaniformis* in which *C. pepo* exhibits the widest variation, especially with respect to fruit characteristics (Gemrot *et al.*, 2006). *C. pepo* is a native species of North America and has been cultivated there for several thousand years (Paris, 1989). It is claimed that *C. pepo* is more persistent and less liable to deterioration, which certainly is reflected in the quality of the extracted oil (Markovic and Bastic, 1975). Hull-less or naked pumpkin seed are widely grown in the southern regions of Austria (Styria province) and the adjacent regions in Slovenia and Hungary (Idouraine *et al.*, 1996). The pumpkin seed is valued in regard to nutritional points. Several studies have reported the chemical composition and oil characteristics of the pumpkin seed from different origins and varieties (Lazos 1986; Stevenson *et al.*, 2007). The four fatty acids presented in significant quantities are palmitic, stearic, oleic, and linoleic acids (Stevenson *et al.*, 2007). The pumpkin seed is a good source of potassium, phosphorus and magnesium, and also contains moderately high amounts of other trace minerals (calcium, sodium, manganese, iron, zinc, and copper) and these elements make pumpkin seed valuable for food supplements (Lazos, 1986).

Raw or roasted pumpkin seeds are used as a snack food for human consumption in many cultures all over the world. The kernels of pumpkin seeds have been utilized as flavor enhancers in gravies and soups, and used in cooking, baking and ground meat formulations as a nutrient supplement and a functional agent (Tsaknis *et al.*, 1997; El-Adawy and Taha, 2001). The oil of pumpkin seeds are being used as a cooking oil in some countries in Africa and the Middle East, and as a salad oil in the south of Austria and the adjacent regions in Slovenia and Hungary (Wenzl *et al.*, 2002). The pumpkin seeds possess valuable dietary and medicinal qualities besides being the source of good-quality edible oils. Pumpkin

seed oil has been used traditionally as medicine in many countries such as China, Yugoslavia, Argentina, India, Mexico, Brazil, and America. It is applied in therapy of small disorders of the prostate gland and urinary bladder caused by hyperplasia (BHP). Pumpkin seed extract has been reported to have antidiabetic, antitumor, antibacterial, anticancer, antimutagenic, and antioxidant activities. It has also been found to have strong hypotriglyceridemic and serum cholesterol-lowering effects (Fu *et al.*, 2006). The health benefits of pumpkin seeds are attributed to their macro- and micro-constituent compositions. They are a rich natural source of proteins, triterpenes, lignans, phytosterols, polyunsaturated fatty acids, antioxidative phenolic compounds, carotenoids, tocopherol, and minerals (Fu *et al.*, 2006).

Due to the differences among the species and/or varieties of *Cucurbita* grown in different areas of the world, the present study was undertaken to determine the composition of whole seed, and physicochemical properties of the crude oil of *Cucurbita pepo* subsp. *pepo* var. *Styriaca* grown in Iran.

## MATERIALS AND METHODS

### Materials

The dried pumpkin seeds (*C. pepo* subsp. *pepo* var. *Styriaca*) were obtained from Tabriz, Iran. They were stored in a sealed vessel wrapped with a polyethylene bag at 4 °C until analysis and oil extraction. All chemicals and solvents, and fatty acid methyl ester (FAME) standards used in this study were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO).

### Compositional Analysis

The recommended methods of the Association of Official Analytical Chemists

(AOAC, 2005) were used to determine the chemical composition of the pumpkin seeds including the contents of moisture, ash, crude protein, crude fat, and crude fiber. The moisture content was determined by drying the seeds in an oven at  $105 \pm 1^\circ\text{C}$  to a constant weight. Total lipids were determined by continuous extraction in a Soxhlet apparatus for 12 h using hexane as solvent. After evaporation of the solvent, the oil content was determined gravimetrically. Ash was determined by incinerating the sample at  $550^\circ\text{C}$  in a muffle furnace. Crude protein was calculated from the nitrogen content measured by Kjeldahl method with Gerhardt model Vat 20 instrument using a factor 6.25. Crude fiber was determined according to the gravimetric procedure. Total carbohydrate was obtained by subtracting (crude protein + crude fat + ash + crude fibre) from 100. The moisture content was expressed in g/100 g sample and the other values were reported on dry basis. All the analyses were performed in triplicate.

### Oil Extraction Procedure

After cleaning and removal of the sand and foreign materials, the dried pumpkin seeds were ground to a fine powder using a grinder (Toos Shekan, Iran). The oil was extracted with n-hexane (1:4 w/v) by agitation in a shaker at room temperature in the dark for 36 h. The solvent was evaporated *in vacuo* at  $40^\circ\text{C}$  to dryness. The extracted oil was stored in sealed and dark bottles under nitrogen gas until analysis.

#### Physical and Chemical Analysis of the Extracted Oil

Specific gravity was determined at  $30^\circ\text{C}$  using a 25 ml capacity pycnometer. Refractive index was measured with an Abbe refractometer (Atago Co. Ltd, Tokyo, Japan) equipped with a thermostated circulator. A glass capillary viscometer Model A200 (Duran, Mainz, Germany) calibrated with distilled water was used to determine dynamic viscosity. Specific

extinctions ( $E_{1\text{cm}}^{1\%}$ ) at 232 nm ( $K_{232}$ ) and 270 nm ( $K_{270}$ ) were determined according to the AOCS official method Ch5-91 (AOCS 1993) using a UV-Vis Spectrophotometer (Model 160 A Shimadzu).

Free fatty acid content and acid value were measured by a titration method defined in American Oil Chemists' Society (AOCS 1993) Official Methods Ca 5a-40 and Cd 3d-63, respectively. Peroxide value was determined with the spectrophotometric method of the International Dairy Federation (Shantha and Decker, 1994; Farhoosh and Moosavi, 2009) (thiocyanate method).

Iodine value and saponification number were determined according to the AOAC (2005) Official Methods 920.158 (Hanus method) and 920.160, respectively. Determination of the unsaponifiable matters was carried out by the procedure of Lozano *et al.* (1993). Determination of the total phenolics content was done spectrophotometrically using Folin-Ciocalteu's reagent as described by Capannesi *et al.* (2000). A calibration curve of gallic acid in methanol was performed in a concentration range of 0.04–0.40 mg/ml. Total tocopherols content was determined according to the colorimetric method described by Wong *et al.* (1988). Total sterols content was quantified according to the Lieberman–Burchard color reaction (Sabir *et al.*, 2003). Lieberman–Burchard reagent (sulfuric acid and acetic anhydride) reacts with sterols to produce a characteristic green color whose absorbance is determined by spectrophotometry at 640 nm.

Determination of the wax content was carried out by the procedure of Mezouari *et al.* (2006). Briefly, an accurately weighed quantity of oil (5 g) was put in an Erlenmeyer flask and five times its volume of acetone was added. The solution (oil/acetone) was cooled and kept at  $4^\circ\text{C}$  for 24 h to crystallize the waxes. The solid fraction was filtered on a previously weighed Whatman No. 1 filter paper, then dried at  $45^\circ\text{C}$  in a vacuum oven, and



weighed to obtain the acetone-insoluble matter.

Oxidative stability index (OSI) was measured following the procedure described by Farhoosh (2007). A Metrohm Rancimat model 743 (Herisau, Switzerland) was used for the determination of the OSI of the extracted oil. Briefly, 3g oil was carefully weighed into reaction vessels and analysis was performed at 120 °C at an airflow rate of 15 l/h. The OSI were automatically recorded and corresponded to the break point of the plotted curve.

### Fatty Acid Composition

Fatty acid composition of the pumpkin seed oil was determined by injecting the fatty acid methylesters into a gas-liquid chromatograph (Hewlett-Packard, Santa Clarita, USA) equipped with a flame ionization detector and a BPX 70 capillary column (60 m × 0.22 mm i.d., 0.2 mm film thickness), using helium as the carrier gas at a flow rate of 0.7 ml/min. The FAMES were prepared by shaking a solution of oil in hexane (0.3 g in 7 mL) with 7 ml of 2 N methanolic potassium hydroxide. The solution was kept at 50-55 °C for 15 min. After shaking, the solution was allowed to settle for 5 min. The upper layer was collected for GC analysis after mixing with some anhydrous sodium sulfate and filtering. The oven temperature was maintained at 198 °C and those of the injector and detector at 280 and 250 °C, respectively. Analysis was done in duplicate and the data was reported as relative area percentages.

## RESULTS AND DISCUSSION

### Chemical Composition of Dried Pumpkin Seeds

The results of chemical composition of the dried pumpkin seeds are presented in Table 1. The dried seeds contained  $5.2 \pm 0.3\%$  of

**Table 1.** Proximate analysis of the whole pumpkin seed (*Cucurbita pepo* subsp. *pepo* var. *Styriaca*)<sup>a</sup>

Parameter	Content (%)
Moisture	$5.20 \pm 0.28$
Oil	$41.59 \pm 2.71$
Protein	$25.40 \pm 0.61$
Ash	$5.34 \pm 0.04$
Fiber	$2.49 \pm 0.11$
Carbohydrate	$25.19 \pm 3.3$

<sup>a</sup> Means  $\pm$  standard deviation of three determinations.

moisture and they were safe for long period storage without spoilage, because, generally, dried pumpkin seeds having this low moisture content are not highly susceptible to microorganism attack (Ajayi *et al.*, 2006).

The oil content was found to be  $41.6 \pm 2.7\%$  (Table 1). This value fell in the range reported for different species of *cucurbita* (9.8-52.1%) and different varieties of *C. pepo* (31.2-51.0%) (Stevenson *et al.*, 2007). This oil content was much lower than that reported for the European varieties i.e.54.9%, (Murkovic *et al.*, 1999) and Egyptian varieties i.e.51.0% (El-Adawy and Taha 2001), and higher than that reported for African ones i.e.21.9-35.0% (Younis *et al.*, 2000). It has been claimed that such differences in the oil content can be attributed to genetic diversity and climate conditions (Stevenson *et al.*, 2007). Also, the oil content of the pumpkin seed in the present study was found to exceed, or be comparable to, that of some common edible oils such as cottonseed (22-24%), safflower (30-35%), soybean (18-22%), rapeseed (40-48%), and olive (12-50%) (Nichols and Sanderson, 2003). Therefore, the pumpkin seed can be considered as a potential source of vegetable oil for domestic and industrial purposes.

The protein content ( $25.4 \pm 0.6\%$ ) found in this study (Table 1) was in good agreement with those indicated by Al-Khalifa (1996) for *C. pepo* (26.5%) and *C. moschata* (24.0%). The protein content of cucurbit seeds was shown to be 13.0% for *Citrullus lanatus*, 16.9% for *Lagenaria siceraria* (Achu *et al.*,

2005), and 16.0% for *Telfairia occidentalis* (Esuoso *et al.*, 1998), which are much lower than that of our study for *C. pepo*. However, some other researchers reported higher amounts (29-44%) of oil content in *C. pepo* and other species (Idouraine *et al.*, 1996; Achu *et al.*, 2005). These differences may be caused by the species variations and environmental conditions. In addition, the protein content of the pumpkin seed from our study was higher than those of other oilseeds, e.g. cashew nuts (22.8%), cottonseed (21.9%), and sesame (18.7%), and that of animal proteins (16.0-18.0%) such as lamb, fish, and beef (Ajayi *et al.*, 2006). Overall, the pumpkin seeds are considered to be rich in protein. The protein content of the pumpkin seed suggests that it can contribute to the daily protein need of 23.6 g/100 g for adults as recommended by some authorities (Ajayi *et al.*, 2006).

Total carbohydrate content was calculated to be  $25.2 \pm 3.3$  % of the dry matter (Table 1). This value was much higher than 5.6% reported by Lazos (1986) for pumpkin. In addition, it was the same as the total carbohydrate content of cashew nuts

(26.2%) and sesame (26.0%) (Achu *et al.*, 2005). Total ash content ( $5.3 \pm 0.0\%$ ) was close to that obtained by some researchers (Idouraine *et al.*, 1996; Alfawaz 2004) for *C. maxima* and *C. pepo* but higher than the others (Al-Khalifa 1996; Younis *et al.*, 2000). Ash content determination is important because it is an index of the quality of feeding materials used by animal feed producers for poultry and cattle feeding (Esuoso *et al.*, 1998). Crude fiber content ( $2.5 \pm 0.1\%$ ) was low compared to 12.1% for *C. pepo* and *C. maxima* (Lazos 1986) and 9.3% for *T. occidentalis* (Esuoso *et al.*, 1998). The low level of crude fiber can probably be due to the use of dehulled seed samples.

#### Physicochemical Characterization of Pumpkin Seed Oil

Physicochemical properties of the pumpkin seed oil are shown in Table 2. Physical properties of lipids derive directly from their chemical structures and functional groups and greatly influence the functions of lipids in foods and the methods required for

**Table 2.** Physicochemical characteristics of the pumpkin seed (*Cucurbita pepo* subsp. *pepo* var. *Styriaca*) oil. <sup>a</sup>

Parameter	Value
Acid value (mg KOH/g oil)	$0.78 \pm 0.02$
Free fatty acid content (% as oleic acid)	$0.39 \pm 0.01$
Peroxide value (meq O <sub>2</sub> /kg oil)	$10.85 \pm 0.62$
Iodine value (g of I <sub>2</sub> /100 g oil)	$104.36 \pm 0.04$
Saponification number (mg KOH/g oil)	$190.69 \pm 1.40$
Unsaponifiable matters content (% of oil)	$5.73 \pm 0.82$
Total sterol content (% of oil)	$1.86 \pm 0.10$
Total phenolics content (mg gallic acid/kg oil)	$66.27 \pm 3.69$
Total tocopherols content (mg $\alpha$ -tocopherol/kg oil)	$882.65 \pm 18.32$
Wax content (% of oil)	$1.58 \pm 0.13$
Oxidative stability index (OSI) (h)	$6.57 \pm 0.09$
Specific extinctions at 232 nm ( $K_{232}$ )	$4.80 \pm 0.22$
Specific extinctions at 270 nm ( $K_{270}$ )	$3.52 \pm 0.05$
R-value ( $K_{232}/K_{270}$ )	$0.74 \pm 0.033$
Dynamic viscosity (cP, 30 °C)	$93.66 \pm 0.48$
Specific gravity (30 °C)	$0.9151 \pm 0.0002$
Refractive index (30 °C)	$1.4662 \pm 0.0001$
State at room temperature	Liquid
Color	Greenish brown

<sup>a</sup> Means  $\pm$  standard deviation of three determinations.



their manipulation and processing. They can also be used to assess the purity or quality of lipid material in reference to known standards or preferred characteristics (Nichols and Sanderson, 2003). The pumpkin seed oil was greenish brown in color with nut-like taste. It was liquid at room temperature and even in a refrigerator. Specific gravity ( $0.915 \pm 0.0002$ ) of the oil fell in the reported range of 0.903-0.926 (Nichols and Sanderson, 2003) and was well comparable with the value of 0.9159 (Markovic and Bastic, 1975) for *C. pepo*. This value also fell in the range reported for olive (0.910-0.920), coconut (0.908-0.921), rapeseed (0.910-0.920), and canola (0.914-0.920) oils (Nichols and Sanderson, 2003).

Refractive index is used by most processors to measure the change in unsaturation as the fat or oil is hydrogenated. The refractive index of oils depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation (Nichols and Sanderson, 2003). The pumpkin seed oil showed a refractive index of  $1.4662 \pm 0.0001$ , which was similar to those reported by Lazos (1986) for pumpkin (1.4616) and melon (1.4662) seed oils (Table 2). This value that fell in the range reported for the pumpkin seed oils (1.466-1.474) was lower than the range reported for sunflower and olive oils; higher than that for palm, palm kernel and coconut oils; and within the range reported for canola, rapeseed and corn oils (Nichols and Sanderson, 2003). Pure oils have marked ranges of refractive index and density; thus, the degree of variation of a typical oil from its true values may indicate its relative purity.

The viscosity measured in the present work ( $93.659 \pm 0.48$  cP, Table 2) was higher than those reported by Tsaknis *et al.* (1997) for *C. maxima* and *C. pepo* (72 cP) and that reported by Alfawaz (2004) for *C. maxima* (48.09 cP). Oomaha *et al.* (2000) reported a value of 26.0, 47.3 and 49.4 cP for raspberry, safflower, and grape seed oils, respectively. Viscosity is an important parameter for the design of industrial

processes. It can also be used to evaluate the quality of fats and oils used in frying (Nichols and Sanderson, 2003).

Spectrophotometric measurements are widely used in quality assessments. The  $K_{232}$  is usually considered as an indicator of the oil autoxidation and has been well correlated with peroxide value, but the  $K_{270}$  is a more useful quantity that measures the presence of conjugated dienes and trienes. Furthermore, both measurements have been used to determine the addition of an oil to pure ones (Ogutcu *et al.*, 2008). As can be seen in Table 2, the  $K_{232}$ ,  $K_{270}$ , and R-value ( $K_{232}/K_{270}$ ) of pumpkin seed oil were  $4.80 \pm 0.22$ ,  $3.52 \pm 0.05$ , and  $0.74 \pm 0.033$ , respectively. There are few published data on the  $K_{232}$  and  $K_{270}$  for *Cucurbita* species and *C. pepo*. Markovic and Bastic (1975) found that oils with the same peroxide values show different specific extinctions and the pumpkin seed oil had specific extinctions considerably higher than those of other vegetable oils at both wavelengths. The  $K_{232}$ ,  $K_{270}$ , and R-value for the pumpkin seed oil were lower than those reported by Markovic and Bastic (1975) i.e. 6.17-9.00, 1.73-4.42, 1.34-3.7, respectively, and Tsaknis *et al.* (1997) i.e. 6.32, 3.93, 1.61, respectively. At the same peroxide value, the  $K_{232}$  and  $K_{270}$  for sunflower, olive, and the pumpkin seed oils were reported to be 4.93 and 0.51, 3.32 and 0.65, and 8.88 and 1.99, respectively (Markovic and Bastic, 1975).

Considering the content of free fatty acids ( $0.39 \pm 0.01$  % as oleic acid), acid value ( $0.78 \pm 0.02$  mg KOH/g oil) and peroxide value ( $10.85 \pm 0.62$  meq  $O_2$ /kg oil) (Table 2), the extracted pumpkin seed oil had an acceptable initial quality. The Codex Alimentarius Commission expressed the permitted maximum acid values of 10 and 4 mg KOH/g oil for virgin palm and coconut oils, respectively (Alfawaz, 2004). It has been shown that oils become rancid when the peroxide value ranges from 20.0 to 40.0 meq  $O_2$ /kg oil (Ajayi *et al.*, 2006). On the other hand, according to the Codex Alimentarius Commission, the peroxide value for unrefined olive oil may be

maximum 20 meq/kg oil (Markovic and Bastic, 1975). Therefore, considering that the oil studied was unrefined and its initial quality indicators were within the reported limits, the pumpkin seed oil can be regarded as an edible oil with good quality.

The pumpkin seed oil had an iodine value of  $104.4 \pm 0.0$  (Table 2), indicating a high degree of unsaturation. This value was close to 103.2, 107.0, and 105.1 reported by, respectively, Lazos (1986), Tsaknis *et al.* (1997), and Alfawaz (2004), but higher than 80.0 that was indicated by Esuoso *et al.* (1998), and lower than 123.0 of Younis *et al.* (2000) and 116.0-133.4 of Markovic and Bastic (1975) for *Cucurbita* species. It also lied within the range reported for cottonseed, canola, rapeseed, and corn oils (Nichols and Sanderson, 2003).

Saponification number (SN) is an indicator of the average molecular weight and, hence, chain length. It is inversely proportional to the molecular weight of the lipid. The SN of the examined oil was  $190.7 \pm 1.4$  mg KOH/g oil (Table 2) and fell in the 174-197 range reported for the pumpkin seed oils (Nichols and Sanderson, 2003). This value indicated that the pumpkin seed oil had fatty acids with higher number of carbon atoms in comparison with coconut (248-265) and palm kernel (230-254) oils (Nichols and Sanderson, 2003). This result was in good agreement with the 185.5-195.3 range of Markovic and Bastic (1975), however, it was lower than 200-218 range reported by Al-Khalifa (1996), 206 of El-Adawy and Taha (2001) and 201 of Tsaknis *et al.* (1997) and was higher than 132.3 reported by Younis *et al.* (2000) for *Cucurbita* species. Furthermore, it fell in the range reported for olive, canola, corn, and sunflower oils (Nichols and Sanderson, 2003).

Unsaponifiable matters in the vegetable oils are a variety of nonglyceridic bioactive substances containing variable mixture of hydrocarbons, aldehydes, ketones, alcohols, sterols, pigments, and fat-soluble vitamins that may occur naturally or may be formed during processing or degradation of oils (Badifu, 1991). The content of

unsaponifiable matters ( $5.7 \pm 0.8\%$ ) in the oil experimented (Table 2) was much higher than the values reported in the literature (Al-Khalifa, 1996; Esuoso *et al.*, 1998) for the pumpkin seed oil, but it was in a close agreement with the 3-7% range reported by Anwar *et al.* (2005) for rice bran oil. The sterol content was found to be  $1.9 \pm 0.1\%$  of the oil. In contrast to the other vegetable oils with  $\Delta^5$ -sterols ( $\beta$ -sitosterol, campesterol and stigmasterol) as the major components, Wenzl *et al.* (2002) showed that the pumpkin seed oil contains specific  $\Delta^7$ -phytosterols that provide fingerprint for detection of adulteration. These  $\Delta^7$ -sterols are supposed to give the pumpkin seed oil a beneficial effect in the treatment and prophylaxis of disorders of the prostate gland and the urinary bladder (Nakic *et al.*, 2006). Hence, more detailed examinations of the composition of the sterol fraction of this oil will be of special interest.

Recently, there has been an increasing interest in studying phenolic compounds from oilseeds, because they represent potentially health-promoting substances and have industrial applications (Pericin *et al.*, 2009). These naturally occurring compounds have proven to possess important role in the stability and sensory and nutritional characteristics of the product and may prevent deterioration through quenching of radical reactions responsible for lipid oxidation (Siger *et al.*, 2008). Total phenolics content of the extracted oil was found to be  $66.7 \pm 3.7$  mg gallic acid/kg oil (Table 2). Total phenolics content differs from one oil to another. Wide ranges have been reported (50-1000 mg/kg), but the values are usually between 100 and 300 mg/kg. Cultivar, extraction system, and the conditions of processing and storage are critical factors for the content of phenolic compounds (Boskou, 2006). Tocopherol homologues are phenolic antioxidants that occur naturally in vegetable oils and provide some protection against oxidation by terminating free radicals. The determination of tocopherol homologues in the kernel oils is important owing to their antioxidative



effects and their positive nutritional influences in human metabolism as biological antioxidants (Yoshida *et al.*, 2006). As shown in Table 2, the pumpkin seed oil had a high level of total tocopherols ( $882.7 \pm 18.3$  mg  $\alpha$ -tocopherol/kg oil), which would be expected to contribute good oxidative stability of the oil during storage and processing. The total tocopherols content in the pumpkin seed oil we studied was considerably higher than that reported in the literature (Tsaknis *et al.*, 1997, Nakic *et al.*, 2006).

The pumpkin seed oil had a wax content of  $1.58 \pm 0.13\%$  (Table 2) that was less than the reported range (3-4%) for rice bran oil. Waxes are high-melting-point esters of long-chain carboxylic acids and long-chain alcohols. They are removed from crude oils by a dewaxing step during the refining process to clarify the oil. They have potential applications in cosmetics, pharmaceuticals, foodstuffs, polymers, lubricants, and leather industries (Vali *et al.*, 2005).

The oil oxidative stability index (OSI) is a criterion of oxidative stability of the oils and fats defined as the hours for an oil sample to develop a measurable rancidity. The OSI of the pumpkin seed oil examined in this study was  $6.57 \pm 0.09$  h. Tsaknis *et al.* (1997) reported the OSIs of 5.55, 7.22, and 3.40 h (120 °C, 20 l/h) for the pumpkin seed, olive, and sunflower oils, respectively. In a previous study on rice bran oil, the OSI (120 °C, 20 l/h) ranged from 5.99 to 7.40 h (Anwar *et al.*, 2005).

#### Analysis of Fatty Acids of Pumpkin Seed Oil

Data of fatty acid composition of the pumpkin seed oil, which can be used to evaluate its stability and nutritional quality, is shown in Table 3. A higher degree of oil unsaturation makes it more susceptible to oxidative deterioration. On the other hand, there are considerable data to recommend a reduction in saturated and a moderate increase in monounsaturated and *n*-3 and *n*-6 polyunsaturated fatty acids in human nutrition in order to prevent coronary heart disease and other diseases (Nakic *et al.*, 2006). The composition of fatty acids varies depending on several factors including variety, growing area, climate and ripeness (Murkovic *et al.*, 1999). As can be seen in Table 3, four major fatty acids, namely, linoleic, oleic, palmitic, and stearic, were found in the pumpkin seed oil and they constituted more than 97% of the total amount. This fatty acid profile is confirmed by several authors (Markovic and Bastic, 1975; Murkovic *et al.*, 1999; Nakic *et al.*, 2006). The pumpkin seed oil contained 19.4% saturated fatty acids, with the major one being palmitic acid (10.7%) followed by stearic acid (7.7%), while it was high in unsaturated fatty acids with a total content of 80.7%. This total content of the unsaturated fatty acids was similar to that of the other studies on *C. pepo* and all pumpkin species in Cucurbitaceae (Stevenson *et al.*, 2007). The main unsaturated fatty acids were linoleic acid followed by oleic acid with concentrations of 39.8 and 38.4 %, respectively.

**Table 3.** Fatty acid composition of the pumpkin seed (*Cucurbita pepo* subsp. *pepo* var. *Styriaca*) oil. <sup>a</sup>

Fatty acid	Content (%)
Palmitic (C16:0)	$10.68 \pm 0.42$
Palmitoleic (C16:1)	$0.58 \pm 0.14$
Stearic (C18:0)	$8.67 \pm 0.27$
Oleic (C18:1)	$38.42 \pm 0.37$
Linoleic (C18:2)	$39.84 \pm 0.08$
Linolenic (C18:3)	$0.68 \pm 0.14$
Gadoleic (C20:1)	$1.14 \pm 0.00$
Total saturated fatty acids	$19.35 \pm 0.16$
Total unsaturated fatty acids	$80.65 \pm 0.16$

<sup>a</sup> Means of duplicate determinations.



respectively. In most other investigations on the fatty acid composition of *C. pepo* (Lazos, 1986; Al-Adawy and Taha, 2001), the percentage of linoleic acid was higher (43.1-55.6%) than that of oleic acid (20.4-37.8%), while, in the present study, the percentages of linoleic and oleic acids were almost the same (39.84 and 38.42%, respectively). Despite the high content of total unsaturated fatty acids in the pumpkin seed oil, linolenic acid was very low (0.7%), which was in good agreement with all other similar studies. Also, the level of other fatty acids in the pumpkin seed oil was very low, similar to the results reported in the literature (Stevenson *et al.* 2007).

### CONCLUSION

Our results in this study showed that pumpkin seed was rich in oil and protein and, considering its fatty acid profile, it lies in linoleic-oleic group such as cottonseed, corn, sesame, sunflower, and soybean oils. With a high yield of oil and physicochemical characteristics similar to those of the other commercial edible oils, the pumpkin seed oil can be considered as a new and valuable source of edible oil.

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## ساختار شیمیایی و ویژگیهای فیزیکوشیمیایی دانه کدوی تخم کاغذی کشت شده در ایران

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### چکیده

ساختار شیمیایی و ویژگیهای فیزیکوشیمیایی دانه کدو و ترکیب اسید چربی روغن استخراج شده تعیین شد. نتایج نشان داد دانه‌ها غنی از روغن (۴۱/۵۹ درصد) و پروتئین (۲۵/۴ درصد) هستند. مقدار رطوبت، فیبر خام، خاکستر کل و کربوهیدرات به ترتیب ۵/۲، ۵/۳۴، ۲/۴۹ و ۲۵/۱۹ درصد تعیین گردید. وزن مخصوص روغن استخراج شده ۰/۹۱۵، گرانیروی ۹۳/۶۵ سانتی پواز، و ضریب شکست آن ۱/۴۶۶۲ بود. عدد اسیدی، عدد پراکسید، عدد یدی، عدد صابونی و مقدار ترکیبات صابونی ناشونده به ترتیب ۰/۷۸، ۱۰/۸۵، ۱۰۴/۳۶، ۱۹۰/۶۹ و ۵/۷۳ اندازه‌گیری شد. مقدار کل ترکیبات فنلی ۶۶/۲۷ میلی گرم بر کیلوگرم روغن، توکوفرول کل ۸۸۲/۶۵ میلی گرم بر کیلوگرم روغن، مقدار کل استرول ۱/۸۶ درصد و مقدار موم ۱/۵۸ درصد به دست آمد. مقادیر  $k_{270}$ ،  $k_{272}$  و عدد  $R (k_{272}/k_{270})$  به ترتیب ۳/۸۰، ۳/۵۲ و ۰/۷۴ بود. بررسی ساختار اسید چربی روغن دانه کدو به روش کروماتوگرافی گازی نشان داد اسید لینولئیک (۳۹/۸۴ درصد)، اسید اولئیک (۳۸/۴۲ درصد)، اسید پالمیتیک (۱۰/۶۸ درصد) و اسید استئاریک (۸/۶۷ درصد)، اسیدهای چرب عمده روغن مذکور هستند. مقایسه روغن دانه کدو با دیگر روغنهای گیاهی نشان داد روغن حاضر را می‌توان به عنوان منبعی ارزشمند از روغنهای خوراکی محسوب نمود.