

The influence of the species on the quality, chemical composition and antioxidant activity of pumpkin seed oil[★]

Ihssan Boujemaa, Sara El Bernoussi, Hicham Harhar^{*} and Mohamed Tabyaoui

Laboratoire de matériaux, nanotechnologie et environnement (LMNE), Faculté des sciences, Université Mohammed V de Rabat, BP 1014, Rabat, Maroc

Received 30 April 2020 – Accepted 1 July 2020

Abstract – Oilseed pumpkin seeds are known to be rich in oil and nutrients. Their content in bioactive components gives them some assets that make them beneficial for human health. Although commonly consumed as a snack, pumpkin seeds are ready to claim more uses. The identification of pumpkin species is a major resource in this study. Thus, we worked with three pumpkin species: *Cucurbita maxima* (CMa), *Cucurbita moschata* (CMo) and *Cucurbita pepo* (CP). The species effect on the chemical composition, the content of bioactive compounds and the antioxidant activity was studied. As a result, the analysis of pumpkin seed oil revealed a polyunsaturated fatty acids (PUFAs) content ranging from 52.23% to 57.65%. Our study also revealed that this oil was a good source of phenolic compounds, in particular CMa with a value of 27.52 mg gallic acid equivalents per gram of methanolic extract and 633.51 mg/kg of total tocopherols, which gives it a very strong antioxidant character. In addition, it showed a high antioxidant potency (126.20 ± 20.44 $\mu\text{g/ml}$ for CMa). In this respect, it can be said that the species effect can be a very important factor influencing the nutritional quality of pumpkin seed oil.

Keywords: *Cucurbita pepo* / *Cucurbita moschata* / *Cucurbita maxima* / chemical composition / DPPH

Résumé – Influence de l'espèce sur la qualité, la composition chimique et l'activité antioxydante de l'huile de pépins de courge. Les graines de courge sont connues pour être riches en huile et en nutriments. Leur teneur en composants bioactifs leur confère certains atouts qui les rendent bénéfiques pour la santé humaine. Bien qu'elles soient couramment consommées comme en-cas, les graines de courge peuvent prétendre à d'autres utilisations. L'identification des espèces de courge est une ressource majeure dans cette étude. Ainsi, nous avons travaillé avec trois espèces de citrouilles : *Cucurbita maxima* (CMa), *Cucurbita moschata* (CMo) et *Cucurbita pepo* (CP). L'effet de l'espèce sur la composition chimique, la teneur en composés bioactifs et l'activité antioxydante ont été étudiés. En conséquence, l'analyse de l'huile de pépins de courge a révélé une teneur en acides gras polyinsaturés (AGPI) allant de 52,23 % à 57,65 %. Notre étude a également montré que cette huile était une bonne source de composés phénoliques, en particulier la CMa avec une valeur de 27,52 mg équivalent d'acide gallique par gramme d'extrait méthanolique et 633,51 mg/kg de tocophérols totaux, ce qui lui confère un très fort caractère antioxydant. En outre, a été mis en évidence un pouvoir antioxydant élevé ($126,20 \pm 20,44$ $\mu\text{g/ml}$) pour la CMa. À cet égard, on peut dire que l'effet d'espèce peut être un facteur très important influençant la qualité nutritionnelle de l'huile de pépins de courge.

Mots clés : *Cucurbita pepo* / *Cucurbita moschata* / *Cucurbita maxima* / composition chimique / DPPH

1 Introduction

Vegetable oils are important sources of nutritional value and are used in many food and industrial applications. The need

for cooking oil is becoming more and more important, not only in developed countries but also in developing countries. Fats and oils provide highly concentrated energy reserves to keep the body temperature at an optimal level.

Humans use about 40 million tons of fats and oils each year, indicating their nutritional importance and widespread daily use (Dhiman *et al.*, 2009). Recently, plants have taken a very strong position in the biomedical field. Indeed, many

[★] Topical issue on: Minor oils from atypical plant sources / Huiles mineures de sources végétales atypiques

*Correspondence: hichamoo79@yahoo.fr

antioxidants have been extracted from plants such as vegetables, fruits, and seeds (Indrianingsih *et al.*, 2019). Pumpkin is commonly cultivated in Morocco for its high nutritional value and digestive effects (Walters *et al.*, 2018). It belongs to the Cucurbitaceae family, which includes 130 genera and more than 800 species (Perez-Gutierrez, 2016). The different species offer a diversity of fruit characteristics such as shape, size, color, taste, and seeds. These are very closely related to the cultivated species, three of which, CMo, CP and CMa, are grown worldwide and used in cooking as an additional ingredient in bread, pastries, and salads. They are rich in minerals, vitamins and β -carotene (Seo *et al.*, 2005; Akwaowo *et al.*, 2000).

Pumpkin seeds account for about 30–50% of oil, which contains a high concentration of phytosterols, the majority of which are β -sitosterol and Δ^7 -sterols (Phillips *et al.*, 2005). Studies on oils obtained from raw seeds reveal a richness in mono (MUFAs) and polyunsaturated fatty acids (PUFAs). The main components of PUFAs in pumpkin oil are linoleic acid, oleic acid and palmitic acid (Sabudak, 2007). Also, it is very rich in phytosterols, pigments, antioxidant vitamins, carotenoids, tocopherols and phenolic compounds (Stevenson *et al.*, 2007; Xanthopoulou *et al.*, 2009; Kim *et al.*, 2012), which enable it to contribute to a healthy diet for humans, shrink the prostate (Tsai *et al.*, 2006; Gossell-Williams *et al.*, 2006) and reduce diabetes by increasing hypoglycemic activity. It also has antihypertensive, antitumor, immunomodulatory, antibacterial, and anti-hypercholesterolemic activity (Caili *et al.*, 2006).

Because of their positive effects on humans, several studies on pumpkin seeds have been conducted to assess the content of phenolic compounds and tocopherols. It was reported that β - and γ -tocopherol was mainly present in raw pumpkin seeds with a mean of 338 mg/kg (Vogel, 1978). However, another study found that the content of α -tocopherol ranged from 2.0 to 4.9 mg/100 g, while that of γ -tocopherol was 1.5 to 5.4 mg/100 g (Murkovic and Pfannhauser, 2000).

The present study aims to evaluate the effect of the species cultivated in Morocco (*Cucurbita maxima* [CMa], *Cucurbita moschata* [CMo] and *Cucurbita pepo* [CP]) on the physico-chemical properties (FFA index, iodine index, saponification index, K_{232} and K_{270}), the content of carotenoid, chlorophyll and bioactive compounds, as well as the content and composition of fatty acids and sterols and the antioxidant activity of pumpkin seed oil.

2 Materials and methods

2.1 Plant materials

Three species of pumpkin seeds, belonging to CMa, CP and CMo were used in this study. Harvesting was carried out in January and April 2019, in the regions of Khemissat and Guercif, Morocco. The seeds were air-dried, separated from their scales and crushed to a granular powder. This was sealed in plastic containers and stored in a refrigerator at 4 °C until extraction.

2.2 Oil extraction

Fifty grams of ground pumpkin seeds were extracted in a Soxhlet extractor for 8 hours using 250 ml of n-hexane.

This solvent was removed at 50 °C under reduced pressure using a rotary evaporator. The extracted oils were subsequently placed in brown glass bottles and stored at 4 °C. The oil yields of CMa, CMo and CP were obtained at (46.39±2.02)%, (32.82±2.30)%, (52.56±3.11)%, respectively. For each species, three extractions were needed to calculate the yield.

2.3 Physicochemical properties

The free fatty acid index (FFA), the specific extinction coefficients (K_{232} and K_{270}), the saponification index and iodine index were determined according to the practices recommended by the American Oil Chemists' Society (AOCS), respectively Ca 5a-40, Ch 5-91, Cd 3b-76, Cd 1c-85 (AOCS, 1997). The FFA was expressed in mg KOH/g oil and the specific extinction coefficients (K_{232} and K_{270}) were expressed as the specific extinction of a 1% (w/v) solution of oil in cyclohexane, using a spectrophotometer (LLG-uniSPEC 2) and the saponification index was expressed in mg KOH/g oil.

2.3.1 Carotenoids and chlorophylls

These natural pigments were determined at 470 and 670 nm, respectively. The oil was dissolved in cyclohexane and the values obtained were expressed in mg/kg (Gharby *et al.*, 2018). Thus, pigment contents were determined using the following expressions:

$$[\text{Chlorophylls}] \text{mg/kg} = A_{670} \times \frac{10^6}{613 \times 100 \times S}$$

$$[\text{Carotenoids}] \text{mg/kg} = A_{470} \times \frac{10^6}{2000 \times 100 \times S}$$

where A is the absorbance and S is the thickness of the spectrophotometer cell (1 cm). Chlorophyll and carotenoid contents were expressed as mg of pheophytin and lutein per kg of oil, respectively.

2.4 Fatty acid composition

Fatty acid methyl esters were prepared and analyzed by flame ionization coupled with Varian CP-3800 gas chromatography (GC) equipped with a CP-Wax 52CB type column (30 m × 0.25 mm diameters). The initial and final temperatures used were 170 °C and 230 °C with an increase of 4 °C/min. Helium was applied as carrier gas with a flow rate of 1 ml/min. The data were processed using a Varian Star Workstation v 6.30 and the results were expressed as a relative percentage of each fatty acid present in the sample (Harhar *et al.*, 2019; ISO, 1990).

2.5 Sterol composition

The sterol composition was determined according to the ISO 6799. The trimethylsilylation of the crude sterol fraction was prepared and analyzed using a flame ionization coupled to Varian 3800 gas chromatography equipped with a VF-1 ms GC column (30 cm and 0.25 mm) and using helium (1.6 ml/min) as

Table 1. Physicochemical properties, chlorophyll and carotenoid content of pumpkin seed oils extracted from different pumpkin species.

	CMa	CMo	CP
FFA %	0.57 ± 0.05 ^a	0.65 ± 0.05 ^a	0.86 ± 0.05 ^b
Saponification index (mg KOH/g oil)	203.53 ± 3.01 ^a	173.64 ± 3.18 ^b	178.91 ± 1.86 ^b
Iodine index (g I ₂ /100 g oil)	122.13 ± 1.78 ^a	119.57 ± 1.02 ^a	114.11 ± 1.43 ^b
K ₂₃₂	1.59 ± 0.01 ^a	1.43 ± 0.01 ^b	1.53 ± 0.01 ^c
K ₂₇₀	0.68 ± 0.01 ^a	0.37 ± 0.01 ^b	0.88 ± 0.01 ^c
Carotenoid (mg/kg)	0.25 ± 0.05 ^a	0.66 ± 0.04 ^b	0.34 ± 0.00 ^c
Chlorophyll (mg/kg)	0.46 ± 0.06 ^a	1.37 ± 0.03 ^b	0.85 ± 0.06 ^c

The data are presented in the form of the average of three individual repetitions ($n = 3^e \pm \text{SEM}$). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

carrier gas. The temperature of the column was thermostated to 270 °C, and the temperature of the injector and detector was about 300 °C. A volume of 1 µL was injected for each analysis. In addition, the data was processed using the Varian Star Workstation v 6.30 (ISO, 1991).

2.6 Tocopherols composition

The tocopherol contents were determined according to the standard method ISO 9936 using an HPLC equipped with a fluorometric detector (excitation wavelength 290 nm – emission wavelength 330 nm) on a silica column (25 cm × 4 mm). Elution was carried out using a mixture of isooctane: isopropanol (99:1, v/v) at a flow rate of 1.2 ml/min for an analysis time of 20 minutes. In addition, quantification was performed using external standard curves of α-, β-, γ- and δ-tocopherols and daily reference of quantitative and qualitative tocopherol standards (ISO, 2006).

2.7 Extraction and determination of total phenolic compounds

Phenolic compounds were extracted by a Soxhlet extractor from the residue of delipidated seeds for 6 hours using 200 ml of methanol 70%. The solvent was removed at 50 °C under reduced pressure using a rotary evaporator. The samples were stored in a refrigerator at 4 °C until analysis. The total phenolic content was determined using the Folin-Ciocalteu's reagent according to Xu *et al.* (2008). Absorbance was measured using LLG-uniSPEC2 spectrophotometer at 765 nm. The amount of total phenols was calculated using gallic acid as a standard in the range of 1–200 µg GA/100 µL in methanol. The results were expressed in mg gallic acid equivalents (GAE)/g extract.

2.8 Determination of antioxidant activity

The evaluation of the antioxidant activity of different pumpkin species was carried out by DPPH (1,1-diphenyl-2-picrylhydrazyl) according to the protocol described by Debasis *et al.* (2017) with some modifications. It is based on the free radical scavenging

power of pumpkin seed extracts. 0.5 ml of methanolic solution of DPPH (0.2 mMol) was added to extract solutions with a concentration range of 0.1 to 1 mg/ml. The reaction mixture is vigorously stirred and then stored in a dark place for 30 minutes. The absorbance of the mixture was measured at 517 nm relative to a control sample and reported to the negative control (NC). All samples were prepared in triplicate. The results were reported as IC₅₀ values and calculated as follows:

$$\frac{(\text{NC})\text{Abs} - (\text{Sample})\text{Abs}}{(\text{NC})\text{Abs}} \times 100.$$

2.9 Statistical analysis

Analysis of variance (ANOVA) was affected by the software IBM SPSS Statistics 21, for the checking of the statistical significance by Tukey's tests at a confidence level of 95.0%, as well as, and the results were presented as means ± standard error of the mean

3 Results and discussion

3.1 Physicochemical properties, chlorophyll and carotenoid content

The contents of FFA, saponification index, iodine index, K₂₃₂, K₂₇₀, carotenoid and chlorophyll in pumpkin seed oils are shown in Table 1. The results showed that the FFA index ranged from 0.57 to 0.86% of oleic acid. This parameter gives an indication of the shelf life and edibility value of the oil. Indeed, it is an inversely proportional relationship. These values indicate the good quality of the oil samples as their FFA indexes did not exceed the maximum limit of 4.0 mg KOH/g of oil according to the Codex Alimentarius Commission (2015). As reported by some authors, the FFA index ranged between 2.75 to 4.93% of oleic acid for CP (Hernández-Santos *et al.*, 2016) and 1.0% for CMo (Al-Khalifa, 1996). It can be said that these values are higher than those obtained in our work. For CMa, Habib *et al.* (2015) reported a lower value of 0.26% but still insignificant.

Table 2. Fatty acids (%) of oils from different species of pumpkin seeds.

	CMa	CMo	CP
Myristic acid (C14:0)	0.10 ± 0.01 ^a	0.09 ± 0.03 ^a	0.14 ± 0.04 ^a
Palmitic acid (C16:0)	17.41 ± 0.20 ^a	17.39 ± 0.42 ^a	15.83 ± 0.17 ^a
Palmitoleic acid (C16:1)	0.08 ± 0.01 ^a	0.08 ± 0.01 ^a	0.12 ± 0.03 ^a
Stearic acid (C18:0)	6.56 ± 0.16 ^a	7.26 ± 0.23 ^a	7.33 ± 0.15 ^a
Oleic acid (C18:1)	18.12 ± 1.80 ^a	17.03 ± 0.45 ^a	23.86 ± 0.89 ^a
Linoleic acid (C18:2)	56.98 ± 1.77 ^a	57.40 ± 0.67 ^a	52.11 ± 0.71 ^a
Linolenic acid (C18:3)	0.28 ± 0.03 ^a	0.25 ± 0.04 ^a	0.12 ± 0.01 ^a
Arachidic acid (20:0)	0.47 ± 0.05 ^a	0.50 ± 0.02 ^a	0.49 ± 0.06 ^a
ΣSFA	24.54 ± 0.42 ^a	25.24 ± 0.72 ^a	23.79 ± 0.43 ^a
ΣMUFA	18.20 ± 1.81 ^a	17.11 ± 0.46 ^a	23.98 ± 0.92 ^a
ΣPUFA	57.26 ± 1.80 ^a	57.65 ± 0.71 ^a	52.23 ± 0.072 ^a

The data are presented in the form of the average of three individual repetitions ($n = 3 \pm \text{SEM}$). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

The saponification index is considered a measure of the molecular size of free fatty acids contained in the oil. The values obtained in Table 1 range from 173.64 to 203.53 mg KOH/g. This difference is probably related to the molecular size and proportions of fatty acids contained in the three different species of pumpkin seed oils. This theory can be supported by the results reported by some authors; 214.0 for CMo (Al-Khalifa, 1996), 185.0–195.3 for CP (Nichols and Sanderson, 2003), 173.9 and 236.0 for CMa (Lyimo *et al.*, 2012; Ziaul *et al.*, 2019).

The iodine index refers to the levels of oils unsaturation. The results presented in Table 1 range from 114.11 to 122.13 g I₂/100 g. The iodine value is higher in CMa than the other two species. It can be concluded that CMa has a high level of unsaturated fatty acids. These results are higher than 114.33 for CMa (Habib *et al.*, 2015), 113.50 for CMo and 111.50 for CP (Al-Khalifa, 1996). In addition, iodine index and FFA were negatively correlated ($r = -0.999$) which is related to the level of oxidative rancidity. An increase in the iodine index is consistent with the increase in double bonds, making the oil less stable (Alireza *et al.*, 2010).

K₂₃₂ and K₂₇₀ are spectrophotometric measurements for quality assessments. The indicator of autoxidation of oil is measured by K₂₃₂, while K₂₇₀ measures the presence of conjugated dienes and trienes. As shown in Table 1, K₂₃₂ and K₂₇₀ of pumpkin seed oil range from 1.43 to 1.53 and from 0.37 to 0.88, respectively. These results are lower than those obtained by Ardabili *et al.* (2011). Indeed, the spectroscopic index K₂₃₂ and K₂₇₀ are 4.80 and 3.52, respectively. Consequently, these parameters support a satisfactory quality of the three pumpkin seed oils studied.

The chlorophyll pigment is used to determine the pro-oxidant action of the oil. In the oils studied, the chlorophyll content varies from 0.46 to 1.37 mg/kg ($P < 0.05$). These values are lower than 2 mg/kg, thus ensuring a good conservation of the oils (Boulfane *et al.*, 2015). On the other hand, the carotenoid content varies from 0.25 to 0.66 mg/kg ($P < 0.05$). These natural pigments have a considerable advantage in the prevention of prostate cancer (Stevenson *et al.*, 2007).

3.2 Fatty acid composition

As shown in Table 2, the oils contain high levels of unsaturated fatty acid. The main one is linoleic acid, which represents (56.98 ± 1.77)% for CMa, (57.40 ± 0.67)% for CMo and (52.11 ± 0.71)% for CP. It is necessary for the formation of the cell membrane and various hormones. Oleic acid is also present in pumpkin seed oil. It is very effective in reducing both the risk of cardiovascular disease and infection (Aktas *et al.*, 2018). Results show that oleic acid represents 18.12% for CMa, 17.03% for CMo and 23.86% for CP. We also found a negative correlation between linoleic and oleic acids ($r = -0.997$). These results are in agreement with those reported by Alfawaz (2004). Indeed, linoleic and oleic acids represent respectively 52.69% and 18.14%. However, there are slight differences in the composition of fatty acid compositions with those reported by Cuco *et al.* (2019). Linoleic and oleic acids represent from 49.4 to 55.4 and from 23.4 to 27.0, respectively. This may be due to climatic conditions, time of harvest, level of maturity, drying and storage conditions. As the correlation between oleic and linoleic fatty acid contents is negative ($r = -0.997$), it confirms the formation of linoleic acid by direct desaturation of oleic acid, as described by Murkovic and Pfannhauser (2000).

3.3 Sterol composition

The composition and sterol content of pumpkin seed oil are shown in Table 3. In this study, total sterols ranged from 189.48 to 310.56 mg/100 g oil. For nutritional and medicinal purposes, high sterol content is highly recommended as it has the ability to inhibit intestinal cholesterol absorption by reducing plasma total and LDL cholesterol levels (Ryan *et al.*, 2007). β-sitosterol has a value of (116.33 ± 0.15) mg/100 g of oil for CMa, (84.40 ± 1.10) mg/100 g of oil for CMo and (90.87 ± 3.62) mg/100 g of oil for CP. It is followed by Δ-5-24-stigmastadienol, with (79.90 ± 2.66) mg/100 g of oil for CMa, (56.24 ± 2.37) mg/100 g of oil for CMo and (44.82 ± 0.88) mg/100 g of oil for CP and Δ-7-avenasterol with (74.68 ± 1.83) mg/100 g of oil for CMa, (47.18 ± 2.19) mg/100 g

Table 3. Composition and sterol content of pumpkin seed oil (mg/100 g of oil).

	CMa	CMo	CP
Cholesterol	0.16 ± 0.00 ^a	0.12 ± 0.01 ^{a,b}	0.09 ± 0.00 ^b
24-methylen-cholesterol	0.89 ± 0.03 ^{a,b}	0.79 ± 0.03 ^a	0.99 ± 0.04 ^b
Campesterol	0.53 ± 0.05 ^a	0.89 ± 0.01 ^b	0.54 ± 0.02 ^a
Stigmasterol	1.80 ± 0.07 ^a	2.97 ± 0.01 ^b	0.82 ± 0.00 ^c
Δ7-campesterol	2.66 ± 0.05 ^a	4.02 ± 0.00 ^b	1.21 ± 0.04 ^c
β-sitosterol	116.33 ± 0.15 ^a	84.40 ± 1.10 ^b	90.87 ± 3.62 ^b
Sitostanol	6.24 ± 0.17 ^a	3.18 ± 0.04 ^b	1.46 ± 0.06 ^c
Δ5-avenasterol	1.58 ± 0.06 ^a	0.71 ± 0.01 ^b	3.42 ± 0.02 ^c
Δ5,24-stigmastadienol	79.90 ± 2.66 ^a	56.24 ± 2.37 ^b	44.82 ± 0.88 ^c
Δ7-stigmastenol	25.80 ± 1.78 ^a	15.72 ± 0.70 ^b	13.37 ± 0.34 ^b
Δ7-avenasterol	74.68 ± 1.83 ^a	47.18 ± 2.19 ^b	31.88 ± 0.01 ^c
Total sterols	310.56 ± 2.40 ^a	216.20 ± 4.92 ^b	189.48 ± 2.41 ^c

The data are presented in the form of the average of three individual repetitions ($n = 3 \pm \text{SEM}$). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 4. Tocopherols (mg/kg of oil) and total phenolics compound content (mg EAG/g extract) of pumpkin seed oil.

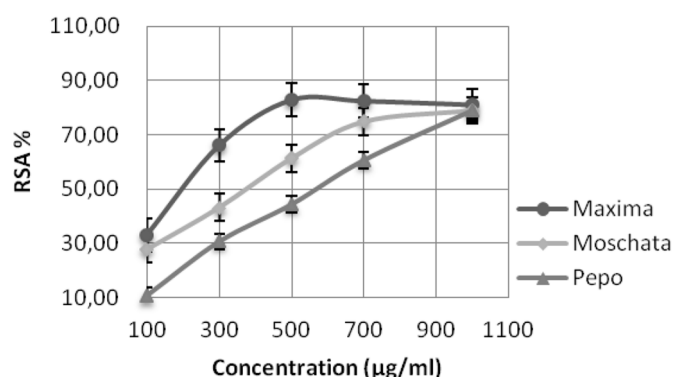
	CMa	CMo	CP
α-tocopherol	22.02 ± 1.82 ^a	18.96 ± 0.51 ^a	8.49 ± 0.34 ^b
γ-tocopherol	626.67 ± 47.45 ^a	476.86 ± 29.14 ^b	334.54 ± 13.56 ^c
δ-tocopherol	19.98 ± 0.45 ^a	11.06 ± 0.29 ^b	7.02 ± 1.14 ^c
Total tocopherols (TT)	633.51 ± 49.69 ^a	506.88 ± 28.34 ^b	350.05 ± 15.04 ^c

The data are presented in the form of the average of three individual repetitions ($n = 3 \pm \text{SEM}$). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

of oil for CMo and (31.88 ± 0.01) mg/100 g of oil for CP. A significant variation in sterol content was observed between the three species ($P < 0.05$). The Δ7 sterols are specific to pumpkin seed oil and are believed to have a beneficial effect in the prevention of prostate and bladder disorders (Nakić *et al.*, 2006). A similar sterols composition was found in Bardaa *et al.* (2016) and Rezig *et al.* (2012) studies.

3.4 Tocopherol composition

Tocopherols are found naturally in vegetable oils. They offer some protection against oxidation by blocking free radicals. As shown in Table 4, total tocopherols range from 350.05 to 633.51 mg/kg of oil. The results showed that the CMa species is very rich in γ-tocopherol. The values were 1.2–1.8 times higher than those of the other species. Indeed, γ-tocopherol ranged from 334.54 to 626.67 mg/kg of oil. On the other hand, the values of α- and δ-tocopherol ranged from 8.49 to 22.02 mg/kg and from 7.02 to 19.98 mg/kg, respectively. The results of this study were similar to those obtained by Petkova and Antova (2019). 89.9% of the tocopherol levels were γ-tocopherol, followed by α- and δ-tocopherol, 5.6 and 2.1%, respectively. In contrast to those obtained by Ziaul *et al.* (2019), δ-tocopherol was predominant with 544 mg/kg, followed by γ- and α-tocopherol, 112.0 and 54.0 mg/kg, respectively. Several factors may affect the tocopherol content such as the oil extraction process, drying maturity, storage conditions, climate and the method of

**Fig. 1.** The radical scavenging activity of pumpkin seed extracts.

determination of tocopherols (Murkovic *et al.*, 1996; Rabrenovic *et al.*, 2014). However, it is interesting to note that the γ form has much higher antioxidant properties and therefore could be important in controlling or preventing prediabetes or vascular injury (Yadav *et al.*, 2010; Lampi *et al.*, 1999).

3.5 Antioxidant activity and total phenolic compounds

DPPH is a stable free radical that can be efficiently scavenged by antioxidants and has a high absorbance at 517 nm.

Table 5. IC₅₀ (μg/ml) of three species of pumpkin seed extracts.

Species	CMa	CMo	CP
IC ₅₀ (μg/ml)	126.20 ± 20.44 ^a	396.95 ± 12.73 ^b	586.47 ± 15.73 ^c
TPC (mg EAG/g extract)	27.52 ± 0.20 ^a	16.64 ± 3.03 ^b	13.70 ± 1.39 ^b

The data are presented in the form of the average of three individual repetitions ($n = 3^e \pm \text{SEM}$). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Based on Figure 1, it can be seen that inhibition of DPPH increased by increasing the concentration. As shown in Table 5, the lowest concentration of 50% of radical scavenging activity (126.20 ± 20.44) μg/ml was determined for CMa, followed by CMo (396.95 ± 12.73) μg/ml and CP (586.47 ± 15.73) μg/ml ($P < 0.05$). Statistical analysis showed that TPC values were positively correlated with TT values ($r = 0.928$) and negatively correlated with IC₅₀ of DPPH ($r = -0.976$). According to the results obtained, the CMa had a greater antioxidant power than the other species; it showed a free radical inhibition of more than 80%.

The total phenolic compounds of the methanolic extracts were shown in Table 4. The values range from 13.70 to 27.52 mg EAG/g extract. As seen for tocopherols, the highest value belongs to the maxima species, which also has the highest TPC value. There is therefore a positive correlation between these two parameters. However, the total phenolic compounds found in this study showed lower values than those obtained by Kulaitienė *et al.* (2018). The values belong to the methanolic extracts obtained from oil extracted by cold pressing and vary between 37.0 to 60.6 mg EAG/kg. It can be concluded that the extraction method can have a considerable influence on the content.

Several studies have demonstrated that the antioxidant potential of seeds can be attributed to PUFA, tocopherols and TPC (Latif and Anwar, 2011; Zhang *et al.*, 2010). CMa showed higher antioxidant activity, which can be explained in part by the higher levels of PUFA (57.26 ± 1.80)%, TT (633.51 ± 49.69) mg/kg of oil and TPC (27.52 ± 0.20) mg EAG/g extract. Similarly, the presence of polyphenols and carotenoids prevents the harmful effects of free radicals by strengthening the antioxidant defense mechanism and thus helps to combat hypertension, atherosclerosis, type 2 diabetes and cancer (Kulczyński and Gramza-Michałowska, 2019).

4 Conclusion

The results of this study showed that pumpkin seed oil contains eight fatty acids; the most predominant being unsaturated fatty acids. In addition, the oil contains many different sterols, the majority of which are β-sitosterol, Δ⁵,24-stigmastadienol and Δ⁷-avenasterol. γ-tocopherol is very abundant in pumpkin seed oil. It also has a strong antioxidant activity. According to the comparison between the three species, it is possible to indicate that CMa has a higher free radical scavenging power than the others. Therefore, we can suggest that all three varieties of pumpkin seed oil can be used as an alternative source of high fatty acid oil.

Acknowledgments. The authors would like to kindly thank Mr. Maamri Mohamed, Mr. El Guezane Chakir and Mr. Tabyaoui Yanis for the precious scientific contribution and generous efforts during this study.

Conflicts of interests. The authors declare that they have no conflicts of interest in relation to this article.

References

- Aktas N, Gerçekaslan KE, Nevşehir TU. 2018. The effect of some pre-roasting treatments on quality characteristics of pumpkin seed oil. *OCL* 25: A301.
- Akwaowo EU, Ndon BA, Etuk EU. 2000. Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis Hook.f.*). *Food Chem* 70: 235–240.
- Alfawaz MA. 2004. Chemical composition and oil characteristics of pumpkin (*Cucurbita maxima*) seed kernels. *J King Saud Univ Agric Sci* 129: 5–18.
- Alireza S, Tan CP, Mirhosseini H, Che Man YB. 2010. Effect of frying process on fatty acid composition and iodine value of selected vegetable oils and their blends. *Int Food Res J* 17: 295–302.
- Al-Khalifa S. 1996. Physicochemical characteristics, fatty acid composition, and lipoxygenase activity of crude pumpkin and melon seed oils. *J Agric Food Chem* 44: 964–966.
- AOCS. 1997. Official methods and recommended practices of the American oil Chemists' Society, 5th ed. Champaign, USA: AOCS Press.
- Ardabili AG, Farhoosh R, Khodaparast Haddad MH. 2011. Chemical composition and physicochemical properties of pumpkin seeds (*Cucurbita pepo* subsp. *Pepo* var. *Styriaca*) grown in Iran. *J Agric Sci Tech* 13: 1053–1063.
- Bardaa S, Ben Halima N, Aloui F, *et al.* 2016. Oil from pumpkin (*Cucurbita pepo L.*) seeds: evaluation of its functional properties on wound healing in rats. *Lipids Health Dis* 15: 73.
- Boulfane S, Maata N, Anouar A, Hilali S. 2015. Caractérisation physicochimique des huiles d'olive produites dans les huileries traditionnelles de la région de la Chaouia-Maroc. *J Appl Biosci* 87: 8022–8029.
- Caili F, Huan S, Quanhong L. 2006. A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods Hum Nutr* 61: 73–80.
- Codex Alimentarius Commission. 2015. Joint FAO/WHO food standards programme codex committee on contaminants in foods. 5th Session, The Hague, the Netherlands.
- Cuco RP, Massa TB, Postae N, *et al.* 2019. Oil extraction from structured bed of pumpkin seeds and peel using compressed propane as solvent. *J Supercrit Fluids* 152: 104568.

- Debasis N, Sarbani A, Pradipta RR, Bismita N. 2017. Assessment of antioxidant, antimicrobial and anti-osteosarcoma potential of four traditionally used Indian medicinal plants. *J Appl Biomed* 15: 119–132.
- Dhiman AK, Sharma K, Attri S. 2009. Functional constituents and processing of pumpkin: a review. *J Food Sci Technol* 46: 411–417.
- Gharby S, Harhar H, Farssi M, *et al.* 2018. Influence of roasting olive fruit on the chemical composition and polycyclic aromatic hydrocarbon content of olive oil. *OCL* 25: A303.
- Gossell-Williams M, Davis A, O'Connor N. 2006. Inhibition of testosterone-induced hyperplasia of the prostate of Sprague-Dawley rats by pumpkin seed oil. *J Med Food* 9: 284–286.
- Habib A, Biswas S, Siddique AH, *et al.* 2015. Nutritional and lipid composition analysis of pumpkin seed (*Cucurbita maxima* Linn.). *J Nutr Food Sci* 5: 4.
- Hernández-Santos B, Rodríguez-Miranda J, Herman-Lara E, *et al.* 2016. Effect of oil extraction assisted by ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (*Cucurbita pepo*). *Ultrason Sonochem* 31: 429–436.
- Harhar H, Gharby S, El Idrissi Y, *et al.* 2019. Effect of maturity stage on the chemical composition of argan fruit pulp. *OCL* 26: 15.
- Indrianingsih AW, Rosyida VT, Apriyana W, *et al.* 2019. Comparisons of antioxidant activities of two varieties of pumpkin (*Cucurbita moschata* and *Cucurbita maxima*) extracts. *IOP Conf Ser Earth Environ Sci* 251: 012021.
- ISO 5508. 1990. Animal and vegetable fats and oils – analysis by gas chromatography of methyl esters of fatty acids.
- ISO 6799. 1991. Determination of the sterol fraction by gas chromatography.
- ISO 9936. 2006. Animal fats and vegetable “determination of tocopherols and tocotrienols by liquid chromatography high performance”.
- Kim MY, Kim EJ, Kim YN, *et al.* 2012. Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts. *Nutr Res Pract* 6: 21–27.
- Kulaitienė J, Černiauskienė J, Jarienė E, *et al.* 2018. Antioxidant activity and other quality parameters of cold pressing pumpkin seed oil. *Not Bot Horti Agrobo* 46: 161–166.
- Kulczyński B, Gramza-Michałowska A. 2019. The profile of carotenoids and other bioactive molecules in various pumpkin fruits (*Cucurbita maxima* Duchesne) cultivars. *Molecules* 24: 3212.
- Lampi A, Kataja L, Kamal-Eldin A, *et al.* 1999. Antioxidant activities of α - and γ -tocopherols in the oxidation of rapeseed oil triacylglycerols. *J Am Oil Chem Soc* 76: 749–755.
- Latif S, Anwar F. 2011. Aqueous enzymatic sesame oil and protein extraction. *Food Chem* 125: 679–684.
- Lyimo ME, Shayo NB, Kasanga A. 2012. Physical-chemical properties, storage stability and sensory evaluation of pumpkin seed oil. *J Open Univ Tanzan* 12: 110–117.
- Murkovic M, Hillebrand A, Winkler J, Pfannhauser W. 1996. Variability of vitamin E content in pumpkin seeds (*Cucurbita pepo* L.). *Z Lebensm Unters Forsch* 202: 275–278.
- Murkovic M, Pfannhauser W. 2000. Stability of pumpkin seed oil. *Eur J Lipid Sci Tech* 102: 607–611.
- Nakić SN, Rade D, Škevin D, *et al.* 2006. Chemical characteristics of oils from naked and husk seeds of *Cucurbita pepo* L. *Eur J Lipid Sci Technol* 108: 936–943.
- Nichols DS, Sanderson K. The nomenclature structure and properties of food lipids. In: Sikorski ZE, Kolakowska A, eds. *Chemical and functional properties of food lipids*. New York: CRC Press, 2003, pp. 1–31.
- Perez-Gutierrez RM. 2016. Review of *Cucurbita pepo* (pumpkin) its phytochemistry and pharmacology. *Med Chem* 6: 12–21.
- Petkova Z, Antova G. 2019. A comparative study on quality parameters of pumpkin, melon and sunflower oils during thermal treatment. *OCL* 26: 32.
- Phillips KM, Ruggio DM, Ashraf-Khorassani M. 2005. Phytosterol composition of nuts and seeds commonly consumed in the United States. *J Agric Food Chem* 53: 9436–9445.
- Rabrenovic BB, Dimic EB, Novakovic MM, *et al.* 2014. The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds. *LWT-Food Sci Technol* 55: 521–527.
- Rezig L, Chouaibi M, Msaada K, Hamdi S. 2012. Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. *Ind Crop Prod* 37: 82–87.
- Ryan E, Galvin K, O'Connor TP, *et al.* 2007. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Foods Hum Nutr* 62: 85–91.
- Sabudak T. 2007. Fatty acid composition of seed and leaf oils of pumpkin, walnut almond, maize, sunflower and melon. *Chem Nat Compd* 43: 465–467.
- Seo JS, Burri BJ, Quan Z, Neidlinger TR. 2005. Extraction and chromatography of carotenoids from pumpkin. *J Chromatogr A* 1073: 371–375.
- Stevenson DG, Eller FJ, Wang L, *et al.* 2007. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *J Agric Food Chem* 55: 4005–4013.
- Tsai YS, Tong YC, Cheng JT, *et al.* 2006. Pumpkin seed oil and phytosterol-F can block testosterone/prazosin-induced prostate growth in rats. *Urol Int* 77: 269–274.
- Vogel P. 1978. Untersuchungen über Kurbiskernöl. *Fette Seifen Anstr* 80: 315–317.
- Walters SA, Bouharroud R, Mimouni A, Wifaya A. 2018. The deterioration of Morocco's vegetable crop genetic diversity: an analysis of the Souss-Massa region. *Agriculture* 8: 49.
- Xanthopoulou MN, Nomikos T, Fragopoulou E, Antonopoulou S. 2009. Antioxidant and lipoxigenase inhibitory activities of pumpkin seed extracts. *Food Res Int* 42: 641–646.
- Xu G, Liu D, Chen J. 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chem* 106: 545–551.
- Yadav M, Jain S, Tomar R, *et al.* 2010. Medicinal and biological potential of pumpkin: an updated review. *Nutr Res Rev* 23: 184–190.
- Zhang S, Zu YG, Fu YJ, *et al.* 2010. Supercritical carbon dioxide extraction of seed oil from yellow horn (*Xanthoceras sorbifolia* Bunge.) and its anti-oxidant activity. *Bioresour Technol* 101: 2537–2544.
- Ziaul MA, Tehera I, Farhana M, *et al.* 2019. Comparative assessment of the physicochemical and biochemical properties of native and hybrid varieties of pumpkin seed and seed oil (*Cucurbita maxima* Linn.). *Heliyon* 5: e02994.

Cite this article as: Boujemaa I, Bernoussi SE, Harhar H, Tabyaoui M. 2020. The influence of the species on the quality, chemical composition and antioxidant activity of pumpkin seed oil. *OCL* 27: 40.