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

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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) µg/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 ± 20,44 µg/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

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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 Δ7-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 ($n = 3^{e} \pm$ SEM). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

carrier gas. The temperature of the column was thermostatized 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 IC_{50} values and calculated as follows:

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

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, K_{232}, K_{270}, 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 ($n = 3^e \pm$ 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 I_2/100 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).

K_{232} and K_{270} are spectrophotometric measurements for quality assessments. The indicator of autoxidation of oil is measured by K_{232}, while K_{270} measures the presence of conjugated dienes and trienes. As shown in Table 1, K_{232} and K_{270} 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 K_{232} and K_{270} 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}	$0.12 \pm 0.01^{a,b}$	0.09 ± 0.00^{b}
24-methylen-cholesterol	$0.89 \pm 0.03^{a,b}$	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 ($n = 3^{e} \pm$ 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 ($n = 3^{e} \pm$ 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 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).

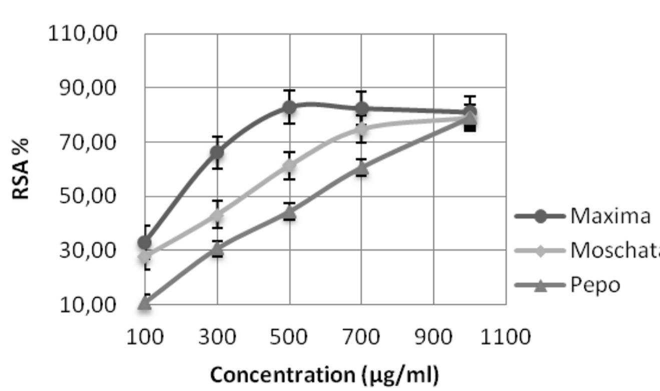

Fig. 1. The radical scavenging activity of pumpkin seed extracts.

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. IC_{50} (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 ($n = 3^e \pm$ 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 IC_{50} 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.

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

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