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Physicochemical properties and stability of pumpkin seed oil as affected by different extraction methods and species

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Abstract

The present work evaluated the influence of extraction method and species on the physicochemical properties, oxidative stability, fatty acid profiles, and rheological behaviour of pumpkin seed oils. The seeds of two pumpkin species (*Cucurbita argyrosperma* Huber [CA] and *Cucurbita moschata* Duchesne [CM]) were obtained from small-scale pumpkin processors in Yucatán, Mexico. The oils were extracted by two methods: mechanical pressing (MP) and organic solvent (OS). It was found that the oil extraction method, species, and their interaction significantly influenced the physicochemical properties and oxidative stability of the seed oils. The composition and fatty acid content of the oils were comparable to those of other pumpkin species. The oil yield from the MP method was lower than that from the OS method. Also, CA oil extracted by MP had an olive-green colour as compared to the reddish-yellow colour of CM oil, and also had a higher oxidative stability. The viscosity of CA oil extracted by MP was superior to that extracted by OS. Also, CA oil had a higher content of iron, monounsaturated fatty acids (MUFA), stearic acid, and oleic acid as well as viscosity in comparison to CM oil, although CM oil had a higher content of linoleic acid. This information can be used to obtain more stable pumpkin oils with enhanced properties that would benefit both producers and processors.

Introduction

Edible vegetable oils can be characterised as refined, partially refined, virgin, or cold-pressed based on their level and type of processing (Redondo-Cuevas *et al.,* 2018). At present, consumers show a preference for vegetable oils that have not been refined, with distinctive taste and odour characteristics that make them suitable for use in salads or cold dishes (Matthäus, 2008). Notably, the refining process reduces the content of antioxidant compounds (phenolic compounds, tocopherols, phytosterols, and carotenoids), which renders the oil more susceptible to oxidative deterioration (Redondo-Cuevas *et al.,* 2018).

Pumpkins (Cucurbita spp.) are well-known gourds that are both consumed and used for decoration. The oil extracted from their seeds has high nutritional and functional values. The oil extracted from certain species has a high content of fatty acids such as oleic and linoleic acids. At present, pumpkin oil is commercialised in the United States, Austria, Slovenia, Croatia, and Hungary (Fruhwirth and Hermetter, 2007; Xiang *et al.,* 2017). The processing of oil influences its quality and nutritional properties, especially because oils are susceptible to oxidative deterioration, which reduces their shelf life (Rezig *et al.,* 2012). Also, the physicochemical properties (fatty acids, density, iodine value, saponification value, colour, viscosity, etc.) determine the nutritional value, and influence the physical quality of oil. These aspects are important to take into consideration for the commercialisation of oils and the preservation of their quality.

In south-eastern Mexico, particularly in the state of Yucatán, two species of pumpkins which are important for Mayan gastronomy; *Cucurbita moschata* (CM) and *C. argyrosperma* (CA) are cultivated.

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However, the quality and stability of oil from their seeds have been poorly studied. The quality of oil can be affected by climate, soil, and growing conditions. Therefore, the oil obtained from pumpkin species cultivated in south-eastern Mexico can have distinct characteristics from that obtained from other species cultivated in other regions of the world. So far, only one partial report exists on the chemical composition of oil extracted from pumpkin seeds cultivated in Yucatán using solvent (hexane) (Moo-Huchin *et al.,* 2013).

In addition, the yield, quality, and oxidative stability of oil can be affected by the extraction method. Conventionally, vegetable oil is extracted by mechanical pressing (MP) or organic solvent (OS). Recently, the use of compressed propane, pressurised carbon dioxide, and ultrasonic-assisted extraction using ethanol have been verified as methods for sustainable extraction of oil from *C. maxima* (Cuco *et al.,* 2019a; 2019b; Massa *et al.,* 2019).

The quality of oil and its oxidative stability are important for the desired uses of consumers. For this reason, it is necessary to study how the quality varies according to the extraction method, species, and other factors; in order to improve the production, commercialisation, and final physicochemical characteristics and oxidative stability of oils destined for consumers. The aim of the present work was therefore to compare the effects of two extraction methods (MP and OS) and two species (CM and CA), on the physicochemical properties and oxidative stability of pumpkin seed oils. The results may be of interest to the pumpkin oil extraction industry.

Materials and methods

Chemicals

All solvents and chemicals were of analytical grade. A standard of 37 fatty acid methyl ester (FAME) mix was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Pumpkin seeds

Seeds (CM and CA) were acquired from small-scale pumpkin processors in the state of Yucatán, Mexico. The maturation characteristics (moisture content and electric conductivity) of the pumpkin seeds were previously reported by Can-Cauich *et al.* (2019). Selected seeds (without physical damage) were dried in an oven (Felisa FE-293D) at 35.00 ± 1.00°C for 48 h. Dried seeds were then stored in polyethylene bags at 4.00 ± 1.00°C in darkness until oil extraction.

Partial characterisation of CM and CA seeds

Physical and chemical parameters

Length (mm) and width (mm) of whole seeds, length-to-width ratio, weight of the peel of kernel (g), weight of the kernel (g), weight of the kernel and peel, and weight of 100 whole seeds (g) were reported as mean ± standard deviations following the method described by Jafari *et al.* (2012).

Humidity, ash, and crude protein contents were determined following the methods of the Association of Official Analytical Chemists (AOAC, 1997). Crude fibre content was determined by alkali-acid digestion (Tejeda, 1992). Carbohydrate content was calculated using Eq. 1 (Rezig *et al.,* 2012):

Carbohydrate (%) = 100 - (%moisture + %protein + %ash + %fibre + %lipids) (Eq. 1)

Minerals

Two hundred mg of seed ashes which was obtained by calcination (550°C for 7 h) were dissolved in 25 mL of HNO_3 (20%, v/v, H_2O), and subsequently filtered through a 0.22-µm Millipore membrane (Millex-HV). Potassium (λ = 766.5 nm), calcium (λ = 422 nm), zinc (λ = 213.9 nm), sodium (λ = 589 nm), and iron (λ = 248.3 nm) were directly measured in the resulting solution by atomic absorption spectroscopy (55 AA, Agilent Technologies), using an oxidising flame of acetylene and hollow cathode lamps. The quantification (mg/100 g of seed dry weight, DW) of the different elements was carried out using the calibration curves (at an interval between 0.001 - 0.03 mg/mL) of the respective standard solution of each mineral.

Pumpkin seed oil extraction

Organic solvent (OS) and mechanical pressing (MP)

First, a Soxhlet apparatus was used to extract seed oil into an OS. Seed particle sizes, solvent, and extraction conditions were previously reported in Can-Cauich *et al.* (2019).

Second, a screw press was used to extract seed oil by MP (Komet CA59G, IBG Monforts Oekotec GmbH and Co. KG, Mönchengladbach, Germany) following the procedure described in Can-Cauich *et al.* (2019).

Oil yield was expressed in both cases as a percentage based on the weight of the utilised pumpkin seeds. Recovered oil was immediately stored in amber-coloured jars under nitrogen at -20°C to avoid possible changes in the chemical composition until further analysis.

Analysis of extracted pumpkin oil

Physical and chemical parameters

The following physicochemical properties of the extracted oils were determined follwoing the official methods of the American Oil Chemists' Society (AOCS, 1998): acidity (method Cd 3d-63), peroxide (method Cd 8-53), iodine (method Cd 1-25), saponification (method Cd 3-25), and the refractive index (using an Abbe refractometer at 40°C). Relative density was determined with a 25-mL pycnometer at 40°C, and content of substances reactive to 2-thiobarbituric acid (TBARS) (Cd 19-90) was also determined. K232 and K270 extinction coefficients were determined using a UV-visible spectrophotometer (Agilent Technologies Cary 60); specifically, absorbance of 1% solution of oil in cyclohexane and a quartz cuvette was measured at a path length of 1 cm at 232 and 270 nm.

Iron

Iron (Fe) content was determined following the procedure described by Ixtaina *et al.* (2011) with slight modifications. Ten grams of oil was calcinated at 600°C over a period of 7 h, then the resulting ashes were dissolved in 25 mL of HNO_3 (20%, v/v, H_2O), and filtered through a 0.22-µm Millipore membrane (Millex-HV). Solutions of the samples were analysed using the same equipment previously described in section "Minerals". The quantification of iron (mg/kg of oil) was carried out using the calibration curve of a standard at an interval of 0.001 to 0.03 mg/mL.

Colour

Oil colour was measured with a colorimeter (X-rite, SP 62). Twenty grams of oil was deposited in a 50-mL vial (exterior diameter of 41.67 mm, height of 55.19 mm, and glass thickness of 2.51 mm), and analysed in the colorimeter. The colour was expressed in the CIELAB space as L* (lightness, from 0 = black to 100 = white), a* (+a = redness, -a = greenness), and b* (+b = yellowness, -b = blueness). The obtained parameters were used to calculate the hue angle (*h**) and chromaticity (C*) (Eq. 2 and 3, respectively):

$$hue\ angle\ (h*) = tan^{-1}\frac{b*}{a*} \quad \text{(Eq. 2)}$$

$$Chromaticity\ (C*) = \sqrt{(a*)^2 + (b*)^2} \quad \text{(Eq. 3)}$$

Carotenoid and chlorophyll determinations

Carotenoid extraction was performed following the method described by Moo-Huchin *et al.* (2017) with some adaptations. Three grams of oil was stirred for 2 h with 25 mL of extraction solvent (hexane:acetone:ethanol, 70:15:15, v/v/v). Then, 2.5 mL of a methanolic solution of KOH (40%, w/v) was added to the mixture for saponification at 25°C with continuous stirring for 2 h. Following saponification, 15 mL of *n*-hexane was added to the sample and centrifuged at 4°C for 30 min at 8,000 rpm to recover the supernatant. The residue was subjected to another extraction under the same conditions. After two extractions, the supernatants (carotenoid extracts) were pooled, and the absorbance was immediately measured at 450 nm against an extraction solvent blank (Moo-Huchin *et al.,* 2017) using a UV-visible spectrophotometer (Agilent Technologies Cary 60). Total carotenoid concentration was expressed as mg of β-carotene/kg of oil using a calibration curve of β-carotene between 0.001 and 0.01 mg/mL.

To determine chlorophyll content, the methodology published by Minguez-Mosquera *et al.* (1991) was followed. Oil (7.5 g) was dissolved in 25 mL of cyclohexane. The absorbance of the solution was measured at 670 nm, and the total chlorophyll content was expressed as mg/kg of oil using a molar extinction coefficient of 613 L/mol*cm.

Fatty acid composition

Fatty acid composition was determined using a gas chromatograph (Autosystem, Perkin-Elmer, Norwalk, CT, USA) equipped with a flame ionisation detector (FID). Fatty acid methyl esters (FAMEs) were prepared following the methodology described by Moo-Huchin *et al.* (2013), and were injected (0.5 µL) in a SP-2380 Supelco capillary column (L × I.D., 100 m × 0.25 mm, df 0.20 µm). The temperatures of the detector and injector were 260 and 240°C, respectively. The oven temperature was programmed to increase from 140 to 240°C at a rate of 4°C per min, using nitrogen as the carrier gas (54.1 psi). Fatty acids were identified by comparing their retention times to the those of the standard FAMEs under the same conditions; for the quantification, methyl heptadecanoate was used as an internal standard (50 mg/g). The results were expressed as g of fatty acid identified in 100 g of oil.

Oxidative stability index

The oxidative stability of the oils was evaluated by means of the Rancimat method (Metrohm AG Series 679, Herison, Switzerland). Five grams of oil was heated to 120°C with an air flow speed of 20 L/h. The stability was expressed as the time of induction, IT (h), following the method of Halbaut *et al.* (1997).

Rheological measurements

Oil flow behaviour was analysed using a rheometer (AR 2000, TA Instruments, New Castle, DE) with parallel plates. Flow curves were plotted at 25°C over a shear rate range of 10 - 1200 s^{-1}. Additionally, to investigate the effect of temperature on oil viscosity, a temperature sweep test was conducted in the range of 20 - 65°C, and the data were fitted to the Arrhenius equation (Eq. 4):

$$\mu = Ae^{(-\frac{Ea}{RT})} \quad \text{(Eq. 4)}$$

where, μ = viscosity (Pa.s), A = equation constant, T = temperature (K), R = gas constant (8.314 J/mol/K), and Ea = energy of activation (kJ/mol).

Statistical analysis

Data were reported as mean ± standard deviations of three repetitions; and within each repetition, the analyses were performed in triplicate. Two-way analyses of variance (ANOVA) were performed using the Statgraphics Plus software, version 5.1 (Manugistic, Inc., Rockville, MD, USA) followed by Tukey's mean comparison test to identify any significant differences. Pearson's correlation coefficients (r) were calculated to evaluate the relationship between the time of induction and total carotenoid content.

The chemical composition of the oils was subjected to a principal component analysis (PCA) to classify the oils based on species and extraction method using the Statgraphics Plus software, version 5.1.

Results and discussion

Physicochemical characterisation of pumpkin seeds

The present work is the first to compare the chemical compositions of CA and CM seeds cultivated in south-eastern Mexico. The chemical compositions of seeds are important since they provide information on the quality of seeds and influence the nutritional and energetic levels of the final product. Table 1 presents the average values of the physical and chemical characteristics of the evaluated CA and CM seeds.

The physical characteristics of seeds differed between the pumpkin species: The CA seeds were larger than the CM seeds.

Table 1. Physical and chemical characteristics of seeds from two pumpkin species, *C. argyrosperma* and *C. moschata*.

Seed characteristic	Species	
	C. argyrosperma	***C. moschata***
Length (L) (mm)	22.32 ± 2.72[b]	11.98 ± 0.74[a]
Width (W) (mm)	11.23 ± 1.50[b]	8.47 ± 1.02[a]
Length/width (L/W)	2.04 ± 0.42[b]	1.43 ± 0.09[a]
Weight of the peel of kernel (g)	0.07 ± 0.01[b]	0.03 ± 0.00[a]
Weight of the kernel (g)	0.18 ± 0.02[b]	0.09 ± 0.00[a]
Weight of kernel + peel (g)	0.25 ± 0.03[b]	0.12 ± 0.00[a]
Weight of 100 seeds whole (g)	25.01 ± 2.92[b]	12.00 ± 0.40[a]
Water content (%)	9.46 ± 0.01[b]	7.16 ± 0.18[a]
Crude oil (%)	47.53 ± 0.86[a]	46.18 ± 0.83[a]
Crude fibre (%)	32.72 ± 0.67[b]	30.66 ± 0.25[a]
Crude Protein (%)	6.72 ± 0.12[a]	8.51 ± 0.44[b]
Ash (%)	4.87 ± 0.60[a]	4.93 ± 0.38[a]
Carbohydrate content (%)	3.51 ± 0.09[a]	4.43 ± 017[b]
Minerals		
Potassium (mg/100 g)	109.45 ± 1.97[a]	125.09 ± 2.03[b]
Calcium (mg/100 g)	52.50 ± 2.50[a]	50.83 ± 3.81[a]
Zinc (mg/100 g)	5.00 ± 2.04[a]	5.01 ± 2.04[a]
Sodium (mg/100 g)	9.68 ± 2.03[b]	4.41 ± 0.01[a]
Iron (mg/100 g)	4.58 ± 0.16[a]	6.52 ± 0.16[b]

Values are mean ± standard deviations (n = 9). Different lowercase letters in the same row indicate significant difference ($p \leq 0.05$).

The chemical composition also varied between the pumpkin species: The CM seeds had the highest content of crude protein, carbohydrate, potassium, and iron in comparison to the CA seeds. This difference can be attributed to species-specific characteristics and the environmental conditions of the region (Ardabili *et al.,* 2011).

The seed oil content of both species was greater than that reported by Rezig *et al.* (2012) and Montesano et al. (2018) for the seeds of *C. pepo* and *C. maxima*, respectively, supporting the use of CA and CM seeds from south-eastern Mexico to produce oil.

Physicochemical properties of pumpkin seed oil

The physicochemical properties of edible oils play an important role in their organoleptic characteristics and quality. Therefore, it is of interest to learn how different factors influence these properties. Table 2 shows how the extraction method (MP and OS), species (CA and CM), and their interaction affected ($p \leq 0.05$) the physicochemical properties of the pumpkin oils. The studied factors and their interaction had a significant influence on almost all of the properties.

The pumpkin oil of both species extracted by MP had a higher density, refraction index, and total carotenoid and chlorophyll content. High density indicates more molecules per volume. Differences in fatty acid composition, molar mass, and oxidation state as well as possible impurities present in the oils can explain the higher refraction index of the oil extracted by MP. Notably, CM oil presented a higher refraction index as compared to CA oil. Also, extraction by MP was carried out at a lower temperature (45°C) and in a shorter time (45 min) in comparison to the Soxhlet extraction (76°C and 4 h), which may partially explain why the oil extracted by MP had a higher content of total carotenoids, as these are thermosensitive molecules (Durante *et al.,* 2014). In particular, CA oil had a higher content of total carotenoids as compared to CM oil in addition to a higher chlorophyll content. Meanwhile, considering the oils extracted using OS, CM oil presented a higher refraction index and a higher total carotenoid content in comparison to CA oil.

Independent of the extraction method, CA oil had a higher level of iron. This can be explained by the seed characteristics of each pumpkin species, harvesting conditions, and factors relating to the climate and environmental characteristics of the cultivation zone (Aktaş *et al.,* 2018).

The physicochemical values reported herein are similar to those reported by Rezig *et al.* (2012), Moo-Huchin *et al.* (2013), and Aktaş *et al.* (2018), and are also similar to those recommended by Codex Alimentarius (1999) for oils that have not been subjected to a refining process. The total carotenoid content of both oils was higher as compared to *C. maxima* seed oil extracted by ultrasound with ethanol (Massa *et al.,* 2019).

Based on the colorimetry results, CA oil exhibited a green colour, whereas CM oil had a reddish yellow colour. CA oil extracted by MP obtained higher values for L*, a*, b*, *C**, and *h**, which imparted a greener colour (olive green) in comparison to that extracted by OS (Table 2). This difference in the colour can be attributed to the degree of oxidation and content of oil pigments. Turek and Stintzing (2011) found that the oxidative deterioration of oil is generally accompanied by a loss in quality and colour changes. However, these parameters do not concur with the studies of Nyam *et al.* (2009) and Rezig *et al.* (2012) on oils extracted from *C. pepo* and *C. maxima*, respectively. These differences can be attributed to the treatments, conditions, and methods used to extract oil as well as species differences.

Oxidative state

The oxidation of oil is often related to the deterioration of lipids, leading to the development of rancidity, and formation of compounds that cause undesirable flavours, polymerisation, and other types of reactions that reduce the shelf life and nutritional value. It can also be influenced by the species and the utilised extraction method. The oxidative state of oils is determined using the peroxide value (PV), specific extinction at 232 and 270 nm, TBARS value, and Rancimat test. PV measures the quantity of peroxides in the oil; these are important intermediates of oxidative reactions that decompose via transition metal irradiation or at elevated temperatures to form free radicals. The specific extinction coefficients at 232 and 270 nm are related to the degree of primary and secondary oxidation, respectively, and thus directly correlate with the amount of peroxide. TBARS are indicators of secondary products of lipid oxidation.

In the present work, the extraction method, species, and their interaction had a significant effect ($p \leq 0.05$) on the PV, TBARS, and IT of the pumpkin oil. The method and species had a significant effect on the K270 value, and the interaction of the factors had a significant effect on the K232 value (Table 2).

For both species, the oil extracted by MP had the lowest values for PV, TBARS, and K270 in comparison to the oil extracted by OS. This result

Table 2. Physicochemical properties and oxidative state of seed oils from two pumpkin species, *C. argyrosperma* and *C. moschata*, as extracted by solvent and pressing.

	C. moschata		***C. argyrosperma***		***F*-ratio**		
	Solvent extraction	**Pressing**	**Solvent extraction**	**Pressing**	**Species**	**Method**	**Species × Method**
Density (40°C)	0.911 ± 0.00[a]	0.918 ± 0.00[b]	0.910 ± 0.00[a]	0.918 ± 0.00[b]	0.01	1065.20*	0.73
Acid value (mg KOH/g oil)	1.60 ± 0.01[a]	1.69 ± 0.00[a]	1.69 ± 0.00[a]	1.69 ± 0.00[a]	0.01	0.49	0.29
Iodine value (g I_2/100 g oil)	145.09 ± 5.86[a]	149.32 ± 1.47[a]	155.24 ± 11.72[a]	148.47 ± 4.40[a]	1.35	0.10	1.88
Saponified value (mg KOH/g oil)	210.57 ± 0.20[a]	219.08 ± 5.46[a]	210.27 ± 0.94[a]	221.22 ± 2.01[b]	0.29	32.56*	0.51
Refractive index (40°C)	1.4676 ± 0.00[a]	1.4702 ± 0.00[b]	1.4673 ± 0.00[a]	1.4693 ± 0.00[b]	584.53*	10552.51*	133.63*
Metal content (mg of Fe/kg oil)	2.27 ± 0.53[a]	6.52 ± 0.53[b]	11.67 ± 1.63[a]	13.62 ± 0.31[a]	247.19*	34.98*	4.82
Total carotenoids (mg of β-carotene/kg oil)	18.38 ± 0.50[a]	26.46 ± 0.68[b]	14.66 ± 0.38[a]	37.21 ± 0.06[b]	175.47*	3322.30*	741.63*
Chlorophyll (mg/kg oil)	2.89 ± 0.72[a]	7.01 ± 0.33[b]	2.50 ± 0.48[a]	8.54 ± 0.15[b]	0.39	0.57*	0.21*
Colour (CIELAB)							
L* value	31.47 ± 0.57[a]	30.44 ± 0.43[a]	28.69 ± 0.22[a]	30.61 ± 0.59[b]	29.99*	3.28	26.05*
a* value	-0.16 ± 0.48[a]	1.44 ± 0.29[b]	-0.32 ± 0.22[a]	-4.44 ± 0.68[b]	148.55*	25.98*	123.31*
b* value	8.03 ± 2.11[b]	6.16 ± 0.55[a]	1.84 ± 0.41[a]	9.97 ± 1.19[b]	15.26*	53.20*	153.83*
Chromaticity	8.05 ± 2.13[a]	6.33 ± 0.54[a]	1.87 ± 0.44[a]	10.92 ± 1.36[b]	5.62*	13.98*	71.09*
Hue angle	90.42 ± 2.94[b]	76.29 ± 0.05[a]	98.63 ± 4.53[a]	113.50 ± 1.00[b]	204.16*	1.53	91.46*
Peroxide value (meq O_2/kg of oil)	3.33 ± 0.14[b]	1.33 ± 0.29[a]	7.16 ± 0.14[b]	5.41 ± 0.28[a]	902.50*	202.50*	0.90*
TBARS (μM/kg of oil)	5.74 ± 0.12[b]	5.04 ± 0.10[a]	8.40 ± 0.28[b]	4.01 ± 0.24[a]	48.38*	475.45*	249.04*
K232	3.70 ± 0.04[a]	3.59 ± 0.06[a]	3.57 ± 0.05[a]	3.69 ± 0.00[b]	0.25	0.00	17.38*
K270	1.66 ± 0.01[b]	1.45 ± 0.04[a]	1.57 ± 0.00[b]	1.36 ± 0.00[a]	45.28*	0.00*	244.02
Induction time (h)	7.03 ± 0.10[a]	8.20 ± 0.05[b]	5.25 ± 0.05[a]	14.09 ± 0.08[b]	2550.03*	11544.01*	6594.50*

*Significant at the 0.05 probability level. Values are mean ± standard deviations ($n = 9$). Different lowercase letters in the same row within each species indicate significant difference ($p \leq 0.05$).

confirms that the pumpkin oil extracted by MP experiences less oxidation. The use of high temperatures during the extraction of vegetable oils promotes the formation of primary oxidation products (peroxides and hydroperoxides) and secondary oxidation products (aldehydes and carbonyls, among others), which might explain why MP extraction at 45°C, in contrast with OS extraction at 76°C, results in less oxidation.

Of the two extraction methods, the CM and CA oils extracted by MP had greater oxidative stability (longer induction time of oxidation), with values between 8.20 ± 0.05 and 14.09 ± 0.08 h for CM and CA, respectively. Also, the total carotenoid content of the oils showed a linear relationship (r = 0.9676) with the IT values, indicating that carotenoids can increase the oxidative stability of edible oils due to their antioxidant properties (Nour *et al.,* 2018).

In addition, CA oil presented the highest PV value in comparison with CM oil. The PV of both oils does not exceed the maximum value for the category of cold pressed virgin oils (15 meq O_2/kg) according to Codex Alimentarius (1999). Moreover, the PV values were inferior to those reported by Bardaa *et al.* (2016) for oil from *C. pepo* seeds.

Between the two species, CM oil had a longer induction time of oxidation when extracted by OS, while CA oil had a longer induction time of oxidation when extracted by MP (Table 2). The stability of oil can be attributed to variability in the phenolic acid and flavonoid contents, and antioxidant activity, as previously reported by Can-Cauich *et al.* (2019).

The values for PV, IT, TBARS, K232, and K270 of the oils studied herein concur with those reported for the seed oil of *C. maxima* and *C. pepo* (Rezig *et al.,* 2012; Bardaa *et al.,* 2016). Also, the IT of the oils is higher as compared to the oxidative stability of *C. maxima* oil extracted using pressurised carbon dioxide (Cuco *et al.,* 2019b).

Fatty acid composition

Essential fatty acids are a fundamental part of vegetable oils, and exhibit excellent nutritional and physiological properties that help to prevent cancer and coronary diseases (Akin *et al.,* 2018). Therefore, it is important to study how different factors influence the content of essential fatty acids in edible oils.

In the present work, the content of most of the fatty acids in the analysed pumpkin oils was mainly affected by the method and species; interaction between the factors was only found to be significant for the content of linoleic acid, behenic acid, and polyunsaturated fatty acid (PUFA) (Table 3).

For both species, the oil yield was greater when the OS extraction method was used (46.18 ± 0.83 and 47.53 ± 0.86% for CM and CA, respectively) as compared to the MP extraction method (32.37 ± 0.65 and 34.73 ± 0.84% for CM and CA, respectively). The highest yield was generated by the OS method; the use of a thermal treatment allows the breakage of the cohesive and adhesive interactions between the oil molecules and molecules of the oil matrix (Tuberoso *et al.,* 2007). These results concur with those obtained by Ixtaina *et al.* (2011) and Aguirre *et al.* (2014), who reported a greater yield of chia and sunflower oil, respectively, using the OS method as compared to the MP method.

Between the extraction methods, CM oil extracted by OS had a lower content of linoleic acid and higher content of oleic acid. Given that CM oil also had a greater content of total carotenoids and was extracted under high temperatures, it could also contain degradation products that act as pro-oxidants of polyunsaturated oils, as reported by Steenson and Min (2000). Moreover, the formation of fatty acid radicals increases with increasing unsaturation; thus, oleic acid is 10 to 40 times less susceptible to oxidation as compared to linoleic acid (Yun and Surh, 2012). This supports the findings reported herein, and has also been reported for sunflower oil in which the high concentration of β-carotene acts as a pro-oxidant of unsaturated fatty acids (Zeb, 2011).

As compared to CA oil, CM oil extracted by MP had a higher value of PUFA (43.56 ± 1.37 g/100 g of oil), which was also higher than the oil extracted by OS (40.22 ± 0.89 g/100 g of oil). This finding supports the potential commercialisation of CM oil extracted by MP as a dietary supplement with certain health benefits, such as lowering the risk of cerebrovascular disease (Billingsley and Carbone, 2018).

Also, CM oil extracted by both methods had the highest values of palmitic acid, palmitoleic acid, linoleic acid, and arachidic acid; while CA oil had the highest values of stearic acid and oleic acid. These significant differences in the fatty acid content of the pumpkin seed oils could be attributed to the species characteristics and their growing conditions (Petropoulos *et al.,* 2015).

Considering both extraction methods, CA oil was richer in MUFA and had a lower content of saturated fatty acid (SFA) as compared to CM oil. Because of its high MUFA content, CA oil could also have beneficial health properties. In the literature, it

Table 3. Oil yield (%), fatty acid composition (g/100 g), viscosity, and Arrhenius model parameters of seed oils from two pumpkin species, *C. argyrosperma* and *C. moschata*, as extracted by solvent and pressing.

	C. moschata		***C. argyrosperma***		***F*-ratio**		
	Solvent extraction	**Pressing**	**Solvent extraction**	**Pressing**	**Species**	**Method**	**Species × Method**
Oil yield	46.18 ± 0.83^{b}	32.37 ± 0.65^{a}	47.53 ± 0.86^{b}	34.73 ± 0.84^{a}	25.77*	885.59*	0.00
Fatty acids							
Myristic acid ($_{C14:0}$)	0.15 ± 0.01^{a}	0.14 ± 0.00^{a}	0.14 ± 0.02^{a}	0.15 ± 0.01^{a}	0.06	0.06	0.88
Palmitic acid ($_{C16:0}$)	22.25 ± 0.70^{a}	21.51 ± 0.34^{a}	18.36 ± 0.28^{a}	17.54 ± 0.67^{a}	161.38*	6.41*	0.02
Palmitoleic acid ($_{C16:1}$)	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}	0.07 ± 0.01^{a}	0.09 ± 0.01^{a}	89.30*	3.08	0.33
Stearic acid ($_{C18:0}$)	8.48 ± 0.41^{a}	7.84 ± 0.41^{a}	10.05 ± 0.14^{a}	9.77 ± 0.61^{a}	48.98*	3.41	0.53
Oleic acid ($_{C18:1}$)	25.59 ± 0.34^{b}	23.28 ± 1.49^{a}	37.00 ± 0.27^{a}	35.79 ± 1.34^{a}	785.68*	12.58*	0.42
Linoleic acid ($_{C18:2}$)	40.22 ± 0.89^{a}	43.56 ± 1.37^{b}	32.46 ± 0.09^{a}	33.02 ± 1.17^{a}	247.07*	11.23*	5.70*
Arachidic acid ($_{C20:0}$)	1.55 ± 0.83^{a}	1.78 ± 0.09^{a}	0.59 ± 0.27^{a}	0.61 ± 0.10^{a}	17.23*	0.23	0.16
Behenic acid ($_{C22:0}$)	0.31 ± 0.10^{a}	0.42 ± 0.07^{a}	0.15 ± 0.01^{a}	0.69 ± 0.03^{b}	1.73	74.93*	34.18*
PUFA	40.22 ± 0.89^{a}	43.56 ± 1.37^{b}	32.46 ± 0.09^{a}	33.02 ± 1.17^{a}	247.07*	11.23*	5.70*
MUFA	25.15 ± 0.91^{a}	23.03 ± 1.74^{a}	37.17 ± 0.25^{a}	36.55 ± 0.48^{a}	466.14*	5.34*	1.62
SFA	32.78 ± 0.55^{b}	31.48 ± 0.64^{a}	29.30 ± 0.43^{a}	29.22 ± 0.20^{a}	103.91*	6.67*	5.19
Viscosity (Pa.s)	0.030 ± 0.00^{b}	0.023 ± 0.00^{a}	0.031 ± 0.00^{a}	0.043 ± 0.00^{b}	1116.74*	71.16*	990.42*
Ea (KJ/mol)	19.42 ± 0.30^{a}	20.63 ± 0.34^{b}	19.38 ± 0.60^{a}	20.98 ± 0.62^{b}	0.10	8.00*	0.16
A (Pa.s)	1.41E-05 ± 0.03^{b}	1.35E-05 ± 0.03^{a}	1.64E-05 ± 0.05^{b}	1.21-05 ± 0.04^{a}	2.22	61.05*	35.36*

*Significant at the 0.05 probability level. Values are mean ± standard deviations (n = 9). Different lowercase letters in the same row within each species indicate significant difference ($p \leq 0.05$). SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, and PUFA: polyunsaturated fatty acid.

has been demonstrated that the consumption of oils rich in MUFA maintains HDL (high-density lipoprotein) cholesterol levels while reducing LDL (low-density lipoprotein) cholesterol levels (Lopes *et al.,* 2016).

The fatty acid composition of the pumpkin oils evaluated herein is similar to that reported by Rodríguez-Miranda *et al.* (2012) and Montesano *et al.* (2018) for *C. maxima* and *C. pepo* oils, respectively, which have been considered as alternative substitutes for cotton, sesame seed, sunflower, and soybean oils in the Mexican diet. The oleic acid content of the evaluated oils is also comparable to that of an oil extracted from a mixture of *C. maxima* seeds and peel using compressed propane (Cuco *et al.,* 2019a). However, the linoleic acid content of the evaluated oils (both extraction methods) was lower as compared to that of *C. maxima* seed oil extracted by ultrasound and compressed propane (Massa *et al.,* 2019; Cuco *et al.,* 2019a).

Rheological behaviour

A similar flow pattern was found for the CA and CM oil extracted by MP and OS (steady shear measurements at 25°C). It was characterised by a straight line, as shown in Figure 1a, indicating that the shear stress is directly proportional to the shear rate. Therefore, the oil of the pumpkin seeds has a Newtonian flow. Fresh vegetable oils have previously been shown to exhibit Newtonian flow behaviour because of their long-chain molecules (Santos *et al.,* 2004).

The viscosity is calculated from the slope of the curve of shear stress *versus* shear rate. It was constant for all oil samples regardless of the shear rates tested. However, it was significantly affected by the species, method, and their interaction (Table 3). Between the methods, CA oil extracted by MP presented the highest viscosity. Delfan-Hosseini *et al.* (2017) also reported that purslane oil extracted by cold press showed greater viscosity in comparison to oil obtained using OS.

Considering both extraction methods, CA oil had higher viscosity as compared to CM oil. The differences in fatty acid composition might explain the differences in oil viscosity. Between species, CM oil (less viscous) extracted by both methods was distinguished by a greater proportion of PUFA, while CA oil (more viscous) was characterised by its high MUFA content. Kim *et al.* (2010) observed a reduction in the viscosity of seven edible oils, with an increase in the content of fatty acids with two or more double bonds in the chain. The presence of double bonds does not allow the fatty acid molecules to pile together, interfering with the packaging in the crystalline stage. Thus, oils with higher PUFA content do not have a rigid structure and are more fluid (Delfan-Hosseini *et al.,* 2017), which could explain the lower viscosity of CM oil.

The flow behaviours of the extracted oils were also studied at a temperature interval of 25 to 70°C (Figure 1b). The oils exhibited the same viscosity pattern, showing a reduction in viscosity when the temperature increased. The effect of temperature on oil viscosity was evaluated by the Arrhenius model, which describes an exponential reduction in viscosity with increasing temperature. The curve of Arrhenius versus 1/T (R^2 = 0.999) was used to determine the thermodynamic parameters of the pumpkin seed oils.

The activation energy (Ea) was only significantly affected by the method. The value of constant A was influenced by the method, interaction of method, and species. Between the extraction

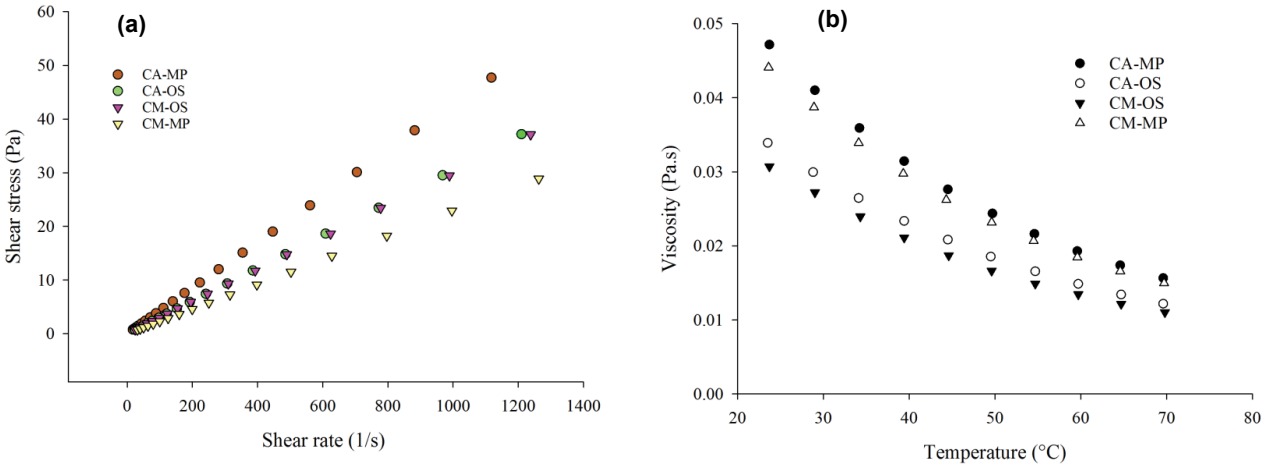

Figure 1. Rheological behaviour of pumpkin seed oils from two pumpkin species, *C. argyrosperma* (CA) and *C. moschata* (CM) as extracted by OS and MP: (a) curves of shear stress vs. shear rate at 25°C, and (b) effect of temperature on the flow behaviours.

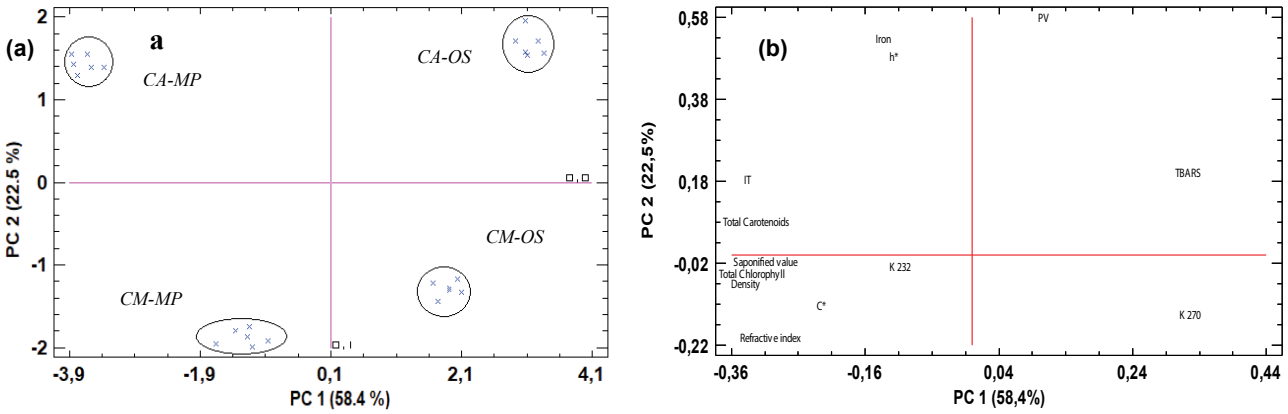

Figure 2. PCA of the physicochemical characteristics of the pumpkin seed oils from two pumpkin species, *C. argyrosperma* (CA) and *C. moschata* (CM) as extracted by OS and MP: (a) scores graph of PC1-PC2, and (b) loadings graph of PC1-PC2.

methods, the oil extracted by OS exhibited a lower Ea value and higher A value, indicating that oil extracted by OS is less sensitive to changes in temperature as compared to oil extracted by MP. Similar findings were observed by Delfan-Hosseini *et al.* (2017), who reported that the oil of purslane seeds extracted by OS had a lower Ea value as compared to that of oil extracted by cold press.

The effect of extraction method on the colour (b* and hue angle) and viscosity of the oils extracted from the two species was completely opposite. Similar findings were observed by Can-Cauich *et al.* (2019), who established that the effect of the extraction method on the chrysin and lutein content of *C. argyrosperma* Huber and *C. moschata* Duchesne oils was opposite. The content of secondary metabolites in pumpkin oil could explain the different effects of the extraction method on each species. Another possible explanation is perhaps the different content of gums or impurities in the oils, since they were not exposed to a refining process.

Principal component analysis (PCA)

A PCA was used to group and separate the oils of the two species, extracted by two methods according to their physicochemical characteristics. Four groups can be clearly distinguished (Figure 2a) in the PC1-PC2 scores graph, indicating that the classification of the oils according to species and extraction method as a function of their physicochemical characteristics could be possible.

The loadings graph (Figure 2b) was used to represent the weight of the variables in the different PCs. The loadings of the components are represented by the first three factors of the PCA, which explain 96.28% of the variability of the samples. PC1 explains 58.4% of the total variation and relates positively with the content of TBARS and K270. PC2 explains 22.5% of the total variation and correlates positively with the values of PV, iron, *h**, total carotenoids, and IT. Finally, PC3 explains 16.3% of the total variation and correlates positively with the values of K232, *C**, and chlorophyll.

CA oil obtained by the OS method exhibited a higher positive value for PC1, and was situated further from the other oils. It is characterised by its high content of TBARS and PV. CA oil obtained by the MP method presented a high positive value for PC2. It is characterised by its higher values of *h**, iron, total carotenoids, and IT. CM oil extracted by the OS method obtained a higher positive value for PC3. It is characterised by a high K270 value. Finally, CM oil obtained by the MP method presented a high negative value for PC1 and a high positive value for PC3. It is characterised by its lower TBARS values and PV (Figure 2b).

The results of the PCA confirm the importance of selecting the pumpkin species and extraction method based on the desired qualities of the final seed oil, as different methods and species can yield oils with contrasting characteristics.

Conclusion

The physical characteristics and chemical compositions of pumpkin seeds varied among the studied species. The species, extraction method, and their interaction were found to be important determinants of the physicochemical properties, oxidative stability, and rheology of the pumpkin seed oil. The difference in the composition of fatty acids of the oils can also be attributed to the species and extraction method. Extraction by MP is recommended to obtain a pumpkin oil (from both species) that is less oxidised, and has a greater content of carotenoids and chlorophyll. PUFA and MUFA are the principal

components of the pumpkin seed oils, and contribute to the flow behaviour. To produce oil with a high PUFA content, the CM species and either oil extraction method (OS or MP) can be used. To produce oil with a high MUFA content, the use of the CA seeds is recommended. A comparative study on the quality of oil extracted from CA and CM using supercritical carbon dioxide is suggested.

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