Food Research 4 (3) : 578 - 584 (June 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

MINI REVIEW

Pumpkin (*Cucurbita maxima*) seed oil: chemical composition, antioxidant activities and its authentication analysis

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Article history:
Received: 2 July 2019
Received in revised form: 3 October 2019
Accepted: 15 October 2019
Available Online: 19 October 2019

Keywords:
Pumpkin seed oil,
Physico-chemical properties,
Antioxidant activity,
Authentication analysis,
FTIR spectroscopy

DOI:
https://doi.org/10.26656/fr.2017.4(3).242

Abstract

Pumpkin seed oil (PSO) has been known as functional food oil due to some bioactive components contained such as phenolics and tocopherols with beneficial effects to human health including antioxidants, prevention of certain cancers, and retardation of hypertension progression and alleviation of diabetes mellitus. To extract PSO from corresponding fruit seed, numerous extraction techniques either conventional like Soxhlet extraction or modern extraction systems such as ultrasound-assisted extraction and supercritical extraction were optimized and developed to get maximum yields of PSO with maximum bioactive components. PSO contained tocopherols and other phenolics compounds, therefore, it has potential application in the treatment of diseases related to oxidative stress. Due to the different price of PSO with other vegetable oils, the adulteration practice involving the substitution or addition of PSO with lower price oils such as palm oil and corn oils is possible, therefore analytical method capable of detecting the adulteration practice is available. The aim of this review was to highlight the physico-chemical properties, extraction procedure, antioxidant activities and authentication analysis of PSO.

1. Introduction

Pumpkin with the scientific name of *Cucurbita maxima* belongs to Cucurbitaceae family which widely grows in tropical regions and has a relatively high economic importance around the world. The food industry has exploited pumpkin for the production of juices, purees, jams and alcoholic beverages (Jiao *et al*., 2014). Meanwhile, pumpkin seed refers to the edible seed of a pumpkin, containing some bioactive compounds frequently used as herbal medicines and functional foods. Pumpkin seeds are also commonly used in culinary practices mainly in Southeast Asian countries. In Indonesia, pumpkin is one of the popular vegetables consumed and considered as functional food due to the extraordinary rich sources of bioactive compounds having beneficial health effects (Montesano *et al*., 2018). In addition, pumpkin seed oil (PSO) has gained great attention in fats and oils industry not only as edible oil but also as a potential nutraceutical (Rezig *et al*., 2012). The world production of pumpkins was 27 million tonnes, and China led the total production accounting of 29%.

PSO has been reported to contain phytosterols, phenolic compounds, antioxidants, tocopherols, and small levels of carotenoids responsible to some biological activities which are beneficial to human health (Cuco *et al*., 2019) including prevention of gastric, breast, colorectal and lung cancers (Elfiky *et al*., 2012), retardation of hypertension progression, antihypertension (Zuhair *et al*., 2000), prevention of prostate disease, mitigation of hypercholesterolemia and arthritis, alleviation of diabetes mellitus by enhancing hypoglycemic activity, reduction of bladder and urethral pressure (Fruhwirth *et al*., 2003a; Fu *et al*., 2006), improving bladder compliance and urinary disorder in human overactive bladder (Nishimura *et al*., 2014), and offering good antioxidant sources (Nawirska-Olszańska *et al*., 2013; Naziri *et al*., 2016).

Due to the beneficial effects of PSO to human health, several studies also focused on the chemical characterization including triacylglycerol compositions, fatty acids (FA) composition, tocopherols, sterols, and phenolic acids and correlated these components with those biological activities (Romano *et al*., 2014). In the market of fats and oils industry, PSO may have high price value compared with other vegetable oils such as palm and corn oils and may have lower price value than,

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for example, olive oil (Rohman *et al*., 2015), as a consequence, PSO could be adulterated with other oils. This review article highlighted some extraction methods, physico-chemical characterization of PSO, antioxidant activities, and the authentication of PSO.

2. Methodology

To accomplish this review article, numerous reputable databases such as Web of Science, PubMed and Scopus containing review and original articles related to the covered topics were identified and downloaded. The keywords explored during literature searching consisted of physico-chemical properties + pumpkin seed oil or antioxidant activities + pumpkin seed oil + *in vitro* or *in vivo* or biological activities + pumpkin seed oil.

2.1 Extraction of pumpkin seed oil

Numerous different methods have been employed for the extraction of PSO from the pumpkin seeds in order to obtain PSO with high yield and high content of bioactive compounds. These methods include the extraction by organic solvents, cold pressing (Rezig *et al*., 2018), mechanical pressing (Rabrenović *et al*., 2014), supercritical fluid extraction employing liquid CO_2 (Durante *et al*., 2014), aqueous enzymatic extraction assisted by micro-wave (Jiao *et al*., 2014), ultrasound-assisted extraction, and microwave-assisted extraction (Kouba *et al*., 2016). However, the extraction with an organic solvent and mechanical pressing are the most common methods applied for the commercial production of vegetable oils.

Supercritical CO_2 using the experimental design of composite rotatable design has been used for extraction of PSO. Some parameters affecting extraction efficiency (maximum yield) and physicochemical properties were optimized which included pressure, temperature, and time. The maximum extraction yield obtained was 30.7% using optimized conditions of pressure, temperature, and time of 32,140 kPa, 68.1^{o}C and 94.6 mins, respectively. These three variables exhibited quadratic effects and all parameters affected the extraction yield significantly (Mitra *et al*., 2009).

Rezig *et al.* (2018) have compared two different extraction methods, namely solvent extraction with extracting solvents of pentane, hexane, and the mixture of chloroform: methanol (3:1, v/v) and cold pressing. These extraction techniques affected the oil stability and antioxidant activities. PSO extracted by mechanical extraction exhibited the best stability and highest tocopherol levels than other PSOs. PSO extracted by mechanical pressing also had the highest values in total carotenoids, total phenolic compounds (TPC), β-carotene, quercetin, squalene, fecosterol, stigmasterol and antioxidant activities DPPH using radical scavenging, ABTS radical scavenging, and reducing power. PSOs were extracted using *n*-hexane as the extracting solvent in the Soxhlet apparatus by thermal cycles at 76°C for 4 hrs (Can-Cauich *et al*., 2019). These antioxidant activities correlated with the levels of tocopherol in which PSO has α-tocopherol of 23.68±2.08 mg/kg of oil and γ-tocopherol of 12.35±1.10 mg/kg of oil.

Hernández-Santos *et al.* (2016) have evaluated the effects of amplitude and time of ultrasound-assisted extraction (UAE) during PSO extraction. Using experimental design, the amplitude of 62.50% and a time of 5 mins offered a higher extraction yield than others. The extraction yield increased with the increased amplitude because the larger ultrasonic wave amplitude could enhance the cell disruption which led thus the extraction efficiency increased (Perez-Serradilla *et al*., 2007).

Compared to organic solvent extraction, aqueous enzymatic extraction technology is considered as safe, cheap, and environmentally-friendly. Jiao *et al.* (2014) performed PSO's extraction based on microwave-assisted aqueous enzymatic comprising of cellulose, pectinase and proteinase (w/w/w). Some extraction conditions namely extraction temperature, enzyme concentration (%, wt/wt), time (min) and power (W) were optimized using response surface methodology (RSM). Using optimum condition (extraction temperature of 44^{o}C and enzyme concentration of 1.40% (wt/wt), extraction time of 66 min and irradiation power of 419 W), the highest oil yield of 64.17% was achieved. PSO extracted using microwave-assisted aqueous enzymatic extraction showed higher oxidative stability than that extracted using Soxhlet extraction with hexane as extracting solvents.

The production processes of PSO also affected the chemical properties as studied by Nederal *et al.* (2012). Three different extraction procedures namely pressing of roasted pumpkin seed paste, pressing of unroasted ground pumpkin seeds, and pressing of unroasted ground pumpkin seeds while cooling the press two seed varieties (husked and naked) were evaluated and the results showed that PSO produced higher total phenol content, higher initial peroxide value and better oxidative stability while cold-pressed oils had higher tocopherol content. But, FA composition and TAG profiles were not affected significantly by these extraction processes. The chemometrics of principal component analysis (PCA), based on the score plot of

first principle component (PC1) and second principle component (PC2), using fatty acid and triglyceride compositions as variables could classify PSOs from two varieties and three production processes.

3. Chemical characterization of pumpkin seed oil

Determination of physico-chemical properties of PSO was needed to characterize which could be used further for the authentication purposes. The chemical composition and PSOs properties depend on several factors including varieties (cultivars) of pumpkin and region of origins. The fatty acid (FA) composition of PSO was significantly different among various cultivars of PSO (Stevenson *et al*., 2007). Table 1 compiles the physicochemical characterization of pumpkin seed oil which include some constants specific for PSO. Habib *et al.* (2015) have reported that the physical properties of PSO were as follows: specific gravity of 0.9412 (at 31° C), the iodine value of 114.33 gI_2/g PSO, saponification value of 193.73 mg KOH/g PSO, acid value of 0.516 mg KOH/g PSO and percentage of free fatty acid of 0.2646%.

Table 1. Physicochemical characterization of pumpkin seed oil (Rezig *et al*., 2012)

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

Value are expressed as means ± standard deviation (SD)

PSO is known to have nutritional values because it contained some vitamins needed by the human diet (Nishimura *et al*., 2014), as shown in Table 2. Rezig *et al.* (2012) have reported that the major FAs in PSO were oleic, linoleic, and palmitic acids. In addition, PSO also contained δ-tocopherol, β-sitosterol, and syringic acid was the predominant phenolic acid present in PSO (Table 3). In addition, the chemical composition of PSO in terms of fatty acid composition exhibited that PSO contained 40.58% oleic acid, 27.06% stearic acid, 17.39% palmitic acid and 14.97% linoleic acid. Siano *et al*. (2016) have noted that the polyunsaturated FA (PUFA), monounsaturated FA (MUFA) and saturated FA (SFA) contents in PSO cultivated in southern Italy were of 48.14%, 25.54%, and 25.20%, respectively. The high degree of unsaturated FA makes PSO suitable to be used as valuable drying agent, while low value of free FA contents indicates the suitability of PSO as edible oils.

4. Antioxidant activities

Potočnik *et al.* (2018) have investigated the antioxidant activity of PSO due to roasting temperature using the capability to scavenge 2,2-diphenyl-1-picrylhydrazil (DPPH) radicals. PSO used was obtained from the extraction of pumpkin seed Gleisdorf variety and the Rustikal hybrid variety produced in Slovenia. The results showed that antiradical activity values were 31.4-70.6% and 19.3-47.7% in the Gleisdorf and Rustikal samples, respectively at the same concentrations of PSO. This activity was correlated with the levels of polyphenol and tocopherol contents. Fruhwirth *et al.* (2003b) also suggested that 59% polar phenolics and 41% tocopherols contributed to the antioxidant capacities of PSO. The antioxidant activities of PSO from 12 cultivars have been also investigated (Nawirska-Olszańska *et al*., 2013). Using Trolox equivalent antioxidant capacity (TEAC), the values of 0.443 to 1.220 μM Trolox/g, the highest value was observed in PSO cultivar Karowita (1.220 TEAC), and the lowest value was obtained in PSO cultivars of Danka and Pyza, each with TEAC value of 0.443 and 0.446 TEAC, respectively.

PSO obtained from microwave-assisted aqueous enzymatic extraction (MAAEEO) and that extracted by Soxhlet extraction (SEO) has been evaluated for its antioxidant activity using two different antioxidant methods, namely DPPH radical scavenging activity and β-carotene/linoleic acid bleaching test (Jiao *et al*., 2014). PSO obtained from MAAEEO exhibited higher antioxidant activity based on DPPH radical scavenging assay with IC_{50} of 123.93±1.85 mg/mL than that obtained from SEO with IC_{50} of 150.38 ± 1.07 mg/mL. In addition, using β-carotene/linoleic acid bleaching method, the lipid peroxidation inhibitory activity of PSO obtained by MAAEEO was 152.84±2.34 mg/mL, significantly higher ($p<0.05$) than that obtained from SEO with lipid peroxidation inhibitory activity of 183.26±3.79 mg/mL. Therefore, PSO obtained by MAAEEO showed higher antioxidant activities than that obtained using SEO either in aqueous (DPPH assay) or lipid-based system (β-carotene/linoleic acid bleaching) which can be explained by higher contents of antioxidant compounds. PSO obtained from MAAEEO exhibited higher PUFA accounting of 57.65% than that in SEO of 53.90%. Besides, MAAEEO method increased the release of tocopherols and phenolics compounds from PSO, thus contributing to the higher antioxidant activities than SEO. Several reports have exhibited that

the antioxidant activities of edible oils can be attributed to polyunsaturated fatty acids (PUFA), tocopherols and phenolics compounds (Zhang *et al*., 2010; Latif and Anwar, 2011; Rezig *et al*., 2012). Bardaa *et al.* (2016) also reported that PSO had high antioxidant activity using β-carotene/linoleic acid bleaching. PSO revealed lipid peroxidation inhibition than butylhydroxytoluene (BHT) as positive control. PSO exhibited important antioxidant activities due to tocopherols contained.

The antioxidant activity using DPPH radical scavenging activity of PSO from three cultivars of Miranda, Golosemianaja, and Herakles grew in Lithuania and its correlation with phenolics content has been investigated by Kulaitienė *et al*. (2018). The highest antiradical activity was found in cultivar Miranda accounting of 3.28 μmol/g, followed by cultivar Golosemianaja (2.49 μmol/g) and Herakles (1.64 μmol/g). DPPH antiradical activity of studied PSO increased with the phenolic content, allowing for establishing the linear correlation between DPPH-radical scavenging activity and total phenolic content with correlation coefficient (r) of 0.823 ($p<0.05$). The high levels of phenolic compounds and tocopherols have been correlated to its potent antioxidant capacities in vivo, as indicated the increased levels hepatic enzymatic activities of superoxide dismutase, catalase and glutathione peroxidase *in vivo* and decreased level of malonaldehyde (Bora, 2018).

5. Authentication of pumpkin seed oil

Some methods have been proposed, developed and used for the authentication of PSO which include molecular spectroscopy (ultraviolet-visible, near-infrared, mid-infrared spectroscopy), chromatographic based technique (gas and liquid chromatography), carbon isotope analysis of the individual fatty acids by the use of gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS) (Spangenberg, 2001).

5.1 Chromatography methods

Gas chromatography has been used for the analysis of Δ7-phytosterols (Δ7-stigmastenol, Δ7,25-stigmastadienol, and Δ7,22,25-Stigmastatrienol) in PSO for authentication purpose (Mandl *et al*., 1999). The column used was fused silica capillary column of HP 35 (30 m length and 0.25 mm i.d.), coated with a 0.25 mm layer of crosslinked 65% dimethyl-35% diphenyl polysiloxane. The temperatures of injector and detector were 300^{o}C. The oven temperature was isothermally set at 280^{o}C using helium as a carrier gas with a flow rate of 1.1 mL/min. PSO has different profiles of Δ7-phytosterols from sunflower oil, used as the adulterant model in this study, even at low concentrations (2% PSO mixed with sunflower oil).

HPLC using detector refractive index combined with chemometrics of principal component analysis has been used for authentication of PSO. The stereospecific triacylglycerol (TAG) composition data was used for the differentiation of PSO and 29 TAGs were analyzed. Among these, 25 TAGs were identified using TAGs standard and 4 TAGs were could not be identified marked as NAS1, NAS2, NAS3, and NAS4. Based on TAGs composition, PCA could classify PSO from different region of origin as group I comprising of TAGs of LnLnLn, LLnLn, NAS1, LLLn, OLnLn; group II LLL, OLLn, PLLn, OLL, OOLn; group III, PLL, LnLS, POLn, LOO, SLL; group IV, PLO, PLP, OOO, NAS2, SOL; group V, POO, SPL, POP, NAS3, SOO; and group VI, SLS, POS, NAS4, PPS (Butinar *et al*., 2010).

5.2 Molecular spectroscopy

UV-vis, near-infrared, and mid-infrared in combination with chemometrics have been employed for classification of PSO. UV-vis and near-infrared (NIR) spectra were used for classification PSO as "good'' and ''bad'' oils, while and mid IR was used for classification PSO into three classes namely "excellent" (oils with highest-quality scores), "satisfactory" (medium score) and "bad" (not acceptable score). The classification of PSO into these categories was performed visually by expert panelist using parameters of smell, taste, visual, odour, and colour. Some spectral treatments including correlation analysis and feature selection were employed for optimum classification. UV-vis spectra using absorbances in 52 wavelengths and NIR spectra with variables of 62 absorbance values could classify 100% successful classification, while FTIR spectra using absorbances in 59 wavenumbers could classify 98.8% classification. Using these variables and corresponding techniques, classification with 100% accuracy into three classes was obtained. The wavelengths at 382-388, 410-420 and 586-94 nm (UV-vis), 3074.0, 3758.6, 3791.4, 3095.2, 3197.4, 3824.1 and 4339.0 nm (NIR spectra), and wavenumbers at 1738-1741, 1750-1754, 2849-2859, 2904-2910, 2915-2917, 2940-2948 and 3201-3202 cm^{-1} (FTIR spectra) contributed significantly in classification (Lankmayr *et al*., 2004).

FTIR spectroscopy using attenuated total reflectance as sampling handling technique in combination with linear discriminant analysis (LDA) has been employed for discrimination of PSO from different species namely *Cucurbita maxima, C. pepo,* and *C. moschata.* The different cultivars of pumpkin seeds may exhibit significant differences in PSO composition terms of fatty acid compositions of linoleic, oleic, and stearic acids and tocopherols composition. LDA could discriminate PSO

from different species and the main wavenumbers contributing for such discrimination using LDA model was 2952-2900 cm^{-1} corresponding to CH_2, symmetrical stretching, 1418-1361 cm^{-1} (-C—H in CH_3 bending), 1147-1128 cm^{-1} (C—O stretching), 887-804 cm^{-1} (-CH_2, wagging), and 665-639 cm^{-1} (O-H, bending out of plane) (Saucedo-Hernandez *et al*., 2011).

FTIR spectroscopy coupled with chemometrics and fatty acid composition as determined by gas chromatography-flame ionization detector was reported to analyze PSO as an adulterant in olive oil (Rohman *et al*., 2015). The absorbance values at combined wavenumbers of 3020-2995 and 1070-900 cm^{-1} were used as variables during quantification of PSO using partial least square (PLS) model with coefficient of determination (R^2) obtained of >0.99. The calibration and validation errors were relatively low, i.e. 0.166 and 1.32% (vol/vol), respectively. The chemometrics of Discriminant analysis could discriminate PSO in olive oil and pure olive oil. In addition, using fatty acid composition change, the presence of PSO in olive oil could be investigated by decreased levels of oleic with the increasing concentration of PSO with R^2 of 0.946. In addition, the level of linoleic acid was increased with the increasing level of PSO with R^2 of 0.978.

6. Conclusion

Pumpkin seed oil (PSO) is valuable oil having functional oil properties and exhibits several biological activities, mainly antioxidant due to high contents of phenolic compounds and tocopherols. The high price of PSO in the market make it to be adulterated with lower price oils, and some analytical methods have been successfully used for authentication of PSO. Besides, the characteristic properties of PSO including physico-chemical constants and fatty acid compositions can assist the authentication of PSO from any adulteration practice.

Acknowledgement

The authors acknowledged the Ministry of Research and Higher Education, the Republic of Indonesia for financial support during preparing this review article through scheme *Penelitian Dasar Unggulan Perguruan Tingi* (PDUPT) 2019.

References

Bardaa, S., Halima, N.B., Aloui, F., Mansour, R.B., Jabeur, H., Bouaziz, M. and Sahnoun, Z. (2016). Oil from pumpkin (*Cucurbita pepo* L.) seeds: evaluation of its functional properties on wound healing in rats. *Lipids in Health and Disease*, 15, 73. https://doi.org/10.1186/s12944-016-0237-0

Bora, N.S. (2018). Beneficial Properties of Pumpkin Seed Oil as an Antioxidant Nutraceutical. *EC Pharmacology and Toxicology*, 6(7), 498-499.

Butinar, B., Bučar-Miklavčič, M., Valenčič V. and Raspor, P. (2010). Stereospecific Analysis of triacylglycerols as a useful means to evaluate genuineness of pumpkin seed oils: lesson from virgin olive oil analyses. *Journal of Agricultural and Food Chemistry*, 58(9), 5227–5234. https://doi.org/10.1021/jf904542z

Can-Cauich, C.A., Sauri-Duch, E., Moo-Huchin, V.M, Betancur-Ancon, D. and Cuevas-Glory, L.F. (2019). Effect of extraction method and species on the content of bioactive compounds and antioxidant activity of pumpkin oil from Yucatan, Mexico. *Food Chemistry*, 285, 186–193. https://doi.org/10.1016/j.foodchem.2019.01.153

Cuco, R.P., Cardozo-Filho, L. and da Silva C. (2019). Simultaneous extraction of seed oil and active compounds from peel of pumpkin (Cucurbita maxima) using pressurized carbon dioxide as solvent. *The Journal of Supercritical Fluids*, 143, 8–15. https://doi.org/10.1016/j.supflu.2018.08.002

Durante, M., Lenucci, M.S., D'Amico, L., Piro, G. and Mita, G. (2014). Effect of drying and co-matrix addition on the yield and quality of supercritical CO_2 extracted pumpkin (*Cucurbita moschata* Duch.) oil. *Food Chemistry*, 148, 314–320. https://doi.org/10.1016/j.foodchem.2013.10.051

Elfiky, S.A., Elelaimy, I.A., Hassan, A.M., Ibrahim, H.M. and Elsayad, R.I. (2012). Protective effect of pumpkin seed oil against genotoxicity induced by azathioprine. The *Journal of Basic and Applied Zoology*, 65(5), 289–298. https://doi.org/10.1016/j.jobaz.2012.10.010

Fruhwirth, G. O., Wenzl, T., El-Toukhy, R., Wagner, F.S. and Hermetter, A. (2003a). Fluorescence screening of antioxidant capacity in pumpkin seed oils and other natural oils. *European Journal of Lipid Science and Technology*, 105(6), 266–274. https://doi.org/10.1002/ejlt.200390055

Fruhwirth, O.G., Wenzl, T., El-Toukhy, R., Wagner, F.S. and Hermetter, A. (2003b). Fluorescence screening of antioxidant capacity in pumpkin seed oils and other natural oils. *European Journal of Lipid Science and Technology*, 105(6), 266–274. https://doi.org/10.1002/ejlt.200390055

Fu, C., Shi, H. and Li, Q. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods for Human Nutrition*, 61(2), 73–80. https://doi.org/10.1007/s11130-006-0016-6

Habib, A., Biswas, S., Siddique, A.H., Manirujjaman, M., Uddin, B., Hasan, S., Khan, M.M.H., Uddin, M., Islam, M., Hasan, M., Rahman, M., Asaduzzaman, M., Sohanur Rahman, M., Khatun, M., Islam, M.A. and Rahman, M. (2015). Nutritional and lipid composition analysis of pumpkin seed (*Cucurbita maxima* Linn.). *Journal of Nutrition and Food Science*, 5(4), 374–379.

Hernández-Santos, B., Rodríguez-Miranda, J., Herman-Lara, E., Torruco-Uco, J.G., Carmona-García, R., Juárez-Barrientos, J.M., Chávez-Zamudio, R. and Martínez-Sánchez, C.E. (2016). Effect of oil extraction assisted by ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (*Cucurbita pepo*). *Ultrasonics Sonochemistry*, 31, 429–436. https://doi.org/10.1016/j.ultsonch.2016.01.029

Jiao, J., Li, Z-G., Gai, Q-Y., Li, X-J., Wei, F-Y., Fu, Y-J. and Ma, W. (2014). Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. *Food Chemistry*, 147, 17–24. https://doi.org/10.1016/j.foodchem.2013.09.079

Kouba, M., Mhemdi, H., Barba, F.J., Roohinejad, S., Greiner, R. and Vorobiev, E. (2016). Oil seed treatment by ultrasounds and microwaves to improve oil yield and quality: An overview. *Food Research International*, 85, 59–66. https://doi.org/10.1016/j.foodres.2016.04.007

Kulaitienė, J., Černiauskienė, J., Jarienė, E., Danilčenko, H. and Levickienė, D. (2018). Antioxidant Activity and other Quality Parameters of Cold Pressing Pumpkin Seed Oil. *Notulae Botanicae Horti Agrobotanici,* 46(1), 161-166. https://doi.org/10.15835/nbha46110845

Lankmayr, E., Mocakb, J., Serdtc, K., Ballab, B., Wenzl, T., Bandoniene, D., Gfrerer, M. and Wagner, S. (2004). Chemometrical classification of pumpkin seed oils using UV–Vis, NIR and FTIR spectra. *Journal of Biochemistry and Biophysics Methods*, 61 (1-2), 95-106. https://doi.org/10.1016/j.jbbm.2004.04.007

Latif, S. and Anwar, F. (2011). Aqueous enzymatic sesame oil and protein extraction. *Food Chemistry*, 125(2), 679–684. https://doi.org/10.1016/j.foodchem.2010.09.064

Mandl, A., Reich, G. and Lindner W. (1999). Detection of adulteration of pumpkin seed oil by analysis of content and composition of specific Δ7-phytosterols. *European Food Research and Technology*, 209(6), 400–406. https://doi.org/10.1007/s002170050516

Mitra, P., Ramaswamy, H.S. and Chang, K.S. (2009). Pumpkin (*Cucurbita maxima*) seed oil extraction using supercritical carbon dioxide and physicochemical properties of the oil. *Journal of Food Engineering*, 95(1), 208–213. https://doi.org/10.1016/j.jfoodeng.2009.04.033

Montesano, D., Blasi F., Simonetti, M.S., Santini, A. and Cossignani, L. (2018). Chemical and Nutritional Characterization of Seed Oil from *Cucurbita maxima* L. (var. Berrettina) Pumpkin. *Foods*, 7(3), 30. https://doi.org/10.3390/foods7030030

Nawirska-Olszańska, A., Kita A., Biesiada, A., Sokół-Łe¸ Towska, A. and Kucharska, A.Z. (2013). Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. *Food Chemistry*, 139, 155–161. https://doi.org/10.1016/j.foodchem.2013.02.009

Nawirska-Olszańska, A., Kita, A., Biesiada, A., Sokół-Łe¸ Towska, A. and Kucharska, A.Z. (2013). Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. *Food Chemistry*, 139(1-4), 155–161. https://doi.org/10.1016/j.foodchem.2013.02.009

Naziri, E., Mitić, M.N. and Tsimidou, M.Z. (2016). Contribution of tocopherols and squalene to the oxidative stability of cold-pressed pumpkin seed oil. *European Journal of Lipid Science and Technology*, 118(6), 898–905. https://doi.org/10.1002/ejlt.201500261

Nederal, S., Skevin, D., Kraljić, K., Obranović, M., Papeša, S. and Bataljaku, A. (2012). Chemical Composition and Oxidative Stability of Roasted and Cold Pressed Pumpkin Seed Oils. *Journal of the American Oil Chemists' Society*, 89(9), 1763-1770. https://doi.org/10.1007/s11746-012-2076-0

Nishimura, M., Ohkawara, T., Sato, H., Takeda, H. and Nishihira, J. (2014). Pumpkin seed oil extracted from *Cucurbita maxima* Improves urinary disorder in human overactive bladder. *Journal of Traditional and Complementary Medicine*, 4(1), 72-74. https://doi.org/10.4103/2225-4110.124355

Perez-Serradilla, J.A., Priego-Capote, F. and Luque de Castro, M.D. (2007). Simultaneous ultrasound-assisted emulsification-extraction of polar and nonpolar compounds from solid plant samples. *Analytical Chemistry*, 79, 6767–6774. https://doi.org/10.1021/ac0708801

Potočnik, T., Cizej, M.R. and Košir, I.J. (2018). Influence of seed roasting on pumpkin seed oil tocopherols, phenolics and antiradical activity. *Journal of Food Composition and Analysis*, 69, 7–12. https://doi.org/10.1016/j.jfca.2018.01.020

Rabrenović, B.B., Dimić, E.B., Novaković, M.M.,

Tešević, V.V. and Basić, Z.N. (2014). The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds. *LWT-Food Science and Technology*, 55(2), 521–527. https://doi.org/10.1016/j.lwt.2013.10.019

Rezig, L., Chouaibi, M., Msaada, K. and Hamdi, S. (2012). Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. *Industrial Crops and Products*, 37(1), 82–87. https://doi.org/10.1016/j.indcrop.2011.12.004

Rezig, L., Chouaibi, M., Ojeda-Amador, R.M., Gomez-Alonso, S., Salvador, M.D., Fregapane, G. and Hamdi, S. (2018). *Cucurbita maxima* pumpkin seed oil: From the chemical properties to the different extracting techniques. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 46, 663-669. https://doi.org/10.15835/nbha46211129

Rohman, A., Che Man, Y.B. and Nurrulhidayah, A.F. (2015) Fourier-transform infrared spectra combined with chemometrics and fatty acid composition for analysis of pumpkin seed oil blended into olive oil. *International Journal of Food Properties*, 18(5), 1086-1096, https://doi.org/10.1080/10942912.2012.654564

Romano, R., Santini, A., Le Grottaglie, L., Manzo, N., Visconti, A. and Ritieni, A. (2014). Identification markers based on fatty acid composition to differentiate between roasted Arabica and Canephora (Robusta) coffee varieties in mixtures. *Journal of Food Composition and Analysis*, 35(1), 1–9. https://doi.org/10.1016/j.jfca.2014.04.001

Saucedo-Hernandez, Y., Lerma-García, M.J., Herrero-Martínez, J.M., Ramis-Ramos, G., Jorge-Rodríguez, E. and Simo-Alfonso, M.F. (2011). Classification of Pumpkin Seed Oils According to Their Species and Genetic Variety by Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy. *Journal of Agricultural and Food Chemistry*, 59, 4125–4129. https://doi.org/10.1021/jf104278g

Siano, F., Straccia, M.C., Paolucci, M., Fasulo, G., Boscaino, F. and Volpe, M.G. (2016). Physico-chemical properties and fatty acid composition of pomegranate, cherry and pumpkin seed oils. *Journal of Science and Food Agriculture*, 96(5), 1730–1735. https://doi.org/10.1002/jsfa.7279

Spangenberg, E. (2001). Authentication of vegetable oils by bulk and molecular carbon isotope analyses with emphasis on olive oil and pumpkin seed oil. *Journal of Agricultural and Food Chemistry*, 49, 1534−1540. https://doi.org/10.1021/jf001291y

Stevenson, D.G., Eller, F.J., Wang, L., Jane, J.L., Wang, T. and Inglett, G.E. (2007). Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *Journal of Agricultural and Food Chemistry*, 55, 4005–4013. https://doi.org/10.1021/jf0706979

Zhang, S., Zu, Y. G., Fu, Y. J., Luo, M., Liu, W. and Li, J. (2010). Supercritical carbon dioxide extraction of seed oil from yellow horn (*Xanthoceras sorbifolia* Bunge.) and its anti-oxidant activity. *Bioresource Technology*, 101(7), 2537–2544. https://doi.org/10.1016/j.biortech.2009.11.082

Zuhair, H.A., Abd El-Fattah, A.A. and El-Sayed, M.I. (2000). Pumpkin-seed oil modulates the effect of feloipine and captopril in spontaneously hypersensitive rats. *Pharmacology Research*, 41(5), 555–563. https://doi.org/10.1006/phrs.1999.0622

eISSN: 2550-2166