Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2018, 46(1):161-166. DOI:10.15835/nbha46110845

Original Article

Antioxidant Activity and other Quality Parameters of Cold Pressing Pumpkin Seed Oil

Jurgita KULAITIENĖ*, Judita ČERNIAUSKIENĖ, Elvyra JARIENĖ, Honorata DANILČENKO, Dovilė LEVICKIENĖ

*Aleksandras Stulginskis University, Institute of Agriculture and Food Sciences, Studentu str. 11, LT-53361 Akademija, Kaunas distr., Lithuania; jurgita.kulaitiene@asu.lt (*corresponding author); judita.cerniauskiene@asu.lt; elvyra.jariene@asu.lt; honorata.danilcenko@asu.lt; dlnlulgl@gmail.com*

Abstract

Pumpkin seeds oil are rich in biologically active substances such as a source of tocopherols, carotenoids, especially β-carotene, lutein and other compounds. Also four fatty acids – palmitic, stearic, oleic and linoleic, dominated in the oil of pumpkin seeds oil. The study mainly aimed to evaluate the fatty acid contents and antioxidant parameters of seed oils isolated from different pumpkin cultivars. Investigations of seeds oil from *Cucurbita pepo* L. 'Miranda', 'Golosemianaja', and 'Herakles' pumpkin cultivars grown in Lithuania revealed that crude fat contents ranged from 44.4% to 47.3%, although 'Miranda' cultivar seeds consistently and significantly had the lowest content. The seed oils contained appreciable amounts of unsaturated fatty acids (approximately 83%), of which polyunsaturated fatty acids, particularly palmitic, stearic, oleic and linoleic acids, were dominant with values ranging from 64.29% to 66.71% of the total amount of fatty acids. The seeds oil were a particularly rich source of linoleic acid (66%), among which 'Miranda' cultivar seeds had the significantly highest content. Our investigation identified that pumpkin seeds oil as a good source of phenolic compounds, particularly cvs. 'Golosemianaja' and 'Miranda' oil. Methanolic seed oil extracts were characterized by statistically significant differences in their antioxidant activity, with the highest antioxidant activity found in cultivar 'Miranda', followed by 'Golosemianaja'. The antioxidant activity level increased proportionally with the total phenolic content, thus establishing a linear relationship between DPPH-radical scavenging activity and total phenolic content.

Keywords: DPPH, fatty acids, pumpkin seed oil, total phenolic

Introduction

The medicinal features of the pumpkin (*Cucurbita pepo* L., Cucurbitaceae family) including anti-diabetic, anti-hypertensive, anti-tumour, immunomodulatory, anti-bacterial, intestinal anti-parasitic, and anti-inflammatory activities have been described in several studies (Caili *et al*., 2006; Fruhwirth and Hermetter, 2007; Danilcenko *et al*., 2011). Notably, pumpkin seeds are rich in proteins and biologically active substances, including essential and non-essential amino acids, tocopherols, carotenoids (especially β-carotene and lutein), mineral elements, fibre, and other compounds considered to exhibit valuable dietetic and medicinal properties (Caili *et al.,* 2006; Glew *et al*., 2006; Nakic *et al*., 2006; Fruhwirth and Hermetter, 2007; Stevenson *et al*., 2007; Kreft *et al*., 2009; Badr *et al*., 2011).

Pumpkin seeds oil belongs to the group of very expensive and good quality edible oils. Usually pumpkin seed oil is known as cold press oil, but according Codex Alimentarius Standard for Named Vegetable Oils, this oil could be "virgin oil". Standard defines that "virgin oils" is oil obtained without altering the nature of the oil, by mechanical procedures, e.g. expelling or pressing and the application of heat only. According Murkovic *et al*. (2004) and Fruhwirth and Hermetter (2007) indicated that the most important quality parameters such as fatty acids, vitamins, minerals, and polyphenols present in Styrian pumpkin oil obtained from roasted seeds. The important factor influence cold pressed oil quality is the temperature of the oil leaving the press (Rabrenovic *et al*., 2014). According to Dimic (2005) data, the temperature of the oil leaving the press during the process of pressing oilseeds, with the aim of producing cold pressed oils, should not exceed 50 °C. Pumpkin seed oil belongs to the group of oils of high nutritive value due to its favourable fatty acid composition and different components which have certain beneficial effects on the human organism.

Received: 10 May 2017. Received in revised form: 21 Aug 2017. Accepted: 22 Aug 2017. Published online: 19 Sep 2017.

Pumpkin seeds and seed oils are excellent sources of nutrition for humans due to the beneficial and synergistic effects of the above-mentioned components. Four fatty acids – palmitic, stearic, oleic and linoleic, dominated in the oil of pumpkin seeds oil and make about 96-99% of the total amount of fatty acids. Linoleic acid, or omega 6 polyunsaturated fatty acid, is a primary fat component of pumpkin seeds oil that produces a hormone-like substance which promotes blood clotting, inhibits inflammatory responses, and enhances the immune system. Pumpkin seeds oil are also rich in the monounsaturated oleic acid omega 9, which may improve cardiac health and lower the risk of cardiovascular disease. These fats act to lower dangerous LDL cholesterol levels and discourage arterial plaque build-up (Aronson *et al*., 2001; Iso *et al*., 2002).

An important trait related to food quality is biological activity, especially antioxidative activity (Gajewski *et al*., 2008). Antioxidant compounds protect cells against the damaging effects of reactive oxygen species, and are known to effectively prevent several current diseases (e.g. cancer and cardiac disease) because of their ability to inhibit the actions of oxidants (free radicals), which bind to otherwise healthy tissues and cause cellular destruction and can lead to tumour development and cancer. Furthermore, antioxidants help to prevent the build-up of arterial plaque and therefore ward off future cardiovascular problems (Al-Saleh *et al*., 2006; Jeznach *et al*., 2012). Vegetables are well-known for high antioxidant agents' content, and vegetable products containing high levels of vitamins A, C, E, and β-carotene are believed to be most beneficial (Gajewski *et al*., 2008).

Naturally occurring plant phenolics include several groups of compounds with health-promoting properties. These phenolics may act as antioxidants, thereby reducing the risks of atherosclerosis and coronary heart disease caused by the oxidation of low-density lipoproteins. Plant phenolics also may protect against some forms of cancer (Emmons and Peterson, 2001). Descriptions of the phenolic compounds isolated from pumpkin, pumpkin seeds, or seed oil and their characteristics have been previously published (Andjelkovic *et al*., 2010). The present study mainly aimed to evaluate the fatty acid contents and antioxidant parameters of seed oils isolated from different pumpkin cultivars.

Materials and Methods

Plant material

All the experiments were conducted at the Experimental Station of Aleksandras Stulginskis University during the years 2013 to 2014. Plants were grown in soil with the following characteristics: limnoglacial loam on moraine loam, carbonate deeper gleyic luvisol (*Calearie Luvisol*), slightly neutral and neutral, medium humus content, phosphorus rich and potassium rich. The experimental field was not fertilized. Seeds from the following cultivars were chosen for investigation: *Cucurbita pepo* L. 'Miranda', 'Golosemiannaja', and 'Herakles'. Of particular interest of pumpkins oil of consumers growing up, but there are no selection of pumpkin cultivars in Lithuania. So the cultivars were chosen from neighbouring countries and investigated how they grow under Lithuanian conditions. 'Golosemiannaja' cultivar was bred in Ukraine. A fruits weighs about 3-5 kg. Seeds are oval, green, without a stiff seed coat. 'Herakles' cultivar was bred in Germany. Fruits are round weighing 2-3 kg. Seeds are green with a lilac hue and without a stiff seed coat. 'Miranda' was bred in Poland. At an average, a fruits weighs about 3-4 kg. Seeds are big of a yellowish green colour.

Prior to laboratory analysis, seeds were removed from pumpkin fruits, washed, dried to a moisture content of 7-8%, and maintained in a chilled, air-tight container.

Pumpkin seed oil production

Cleaning and pressing of oil seeds are the main steps for processing plant oil (Fig. 1). The seeds were cleaned from extraneous impurities such as debris, plant parts and damaged seeds. This provides more constant oil. The seed is pre-warmed to about 25 °C by a special unit or by a heat exchanger that makes use of the heat from the warm press cake. Pre-heating the seed to over 25 °C has no additional benefit. The screw presses CA 59 G type (IBG Monforts Oekotec GmbH) was used for pressing for production of cold pressed pumpkin oil (Fig. 2). Screw rotation speed was 20 rpm.

Determination of total phenolic contents

The total phenolic contents of methanol extracts of seeds oil (1 g seeds oil with liquid nitrogen and diluted with 10 ml of 80% methanol) were determined using a calorimetric method (Ragaee *et al*., 2006). Each extract was shaken for 30 min, followed by centrifugation at 2012 × g

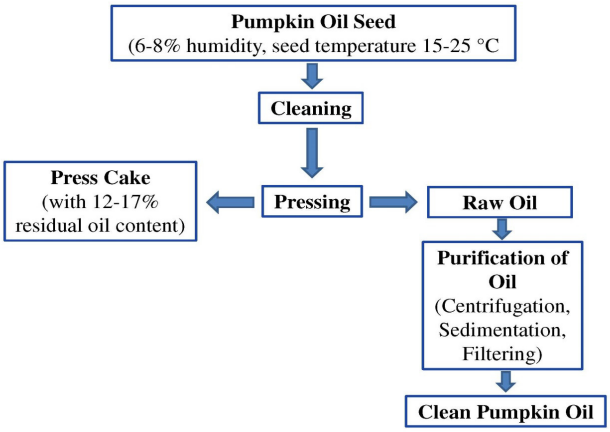

Fig. 1. The scheme of cold pressing oil

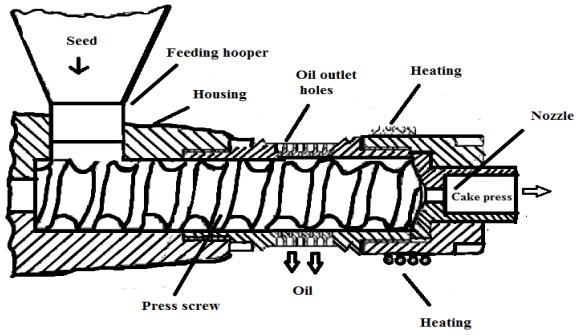

Fig. 2. Screw press hole cylinder type CA 59 G type (IBG Monforts Oekotec GmbH)

for 20 min. Then, 1 ml of sample extract was diluted with 1 ml of 10% (w/v) Folin-Ciocalteu reagent (diluted with twice-distilled water) and 2 ml of 1 M Na_2CO_3 solution. Finally, the absorbance at 765 nm was measured using a Genesys 6 spectrophotometer (Thermo Spectronic, Rochester, NY, USA) against a water and other chemicals as blank. Gallic acid was used as a standard to generate a calibration curve, which was used to extrapolate the total phenolic contents of samples.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity

The antioxidant activities of seeds oil by to assess their 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging capacity ability (μmol g^{-1}) was determined by spectrophotometric method according to Sreerama *et al.* (2010). The extracts were prepared from 5 g of pumpkin seeds oil with 25 ml of methanol for 2 h with constant shaking than was mixed with 2.7 mL of a methanol solution of DPPH. The samples were analysed on a Genesys 6 spectrophotometer (Thermo Spectronic), and the absorbance at 517 nm was measured over a 30 minute period and used to calculate the ability of seed oil material to scavenge DPPH free radicals (μmol g^{-1}).

Determination of crude fat contents

The crude fat contents of seeds were determined by refluxing weighed, powdered, hexane-treated seeds in a Soxhlet extractor for 16 h (Methodenbuch, 1983-1999). After removing the solvent with a rotary evaporator, each oil sample was placed in a vacuum oven at 60 °C for 30 min; subsequently, the samples were accurately weighed and percentage yields were calculated. The reported values are the means of three determinations.

Determination of the fatty acid composition of seed oils by gas chromatography

Gas chromatography and the data processing program 'GCsolution' were used to determine the fatty acid compositions of samples (% of total fatty acids). The Folch method was used to extract fats from a total volume of 20 ml; briefly, 1 g of sample in methanol was combined with chloroform at a 1:2 ratio to yield 10 ml of mixture. Then, 0.5 g of vegetable fat was poured over this mixture for extraction, which occurred over 12 hours at room temperature. The sample was then filtered, mixed with 20-40 ml of 0.74% KCl solution, vigorously agitated for 1-2 minutes, and left for 10-12 hours to allow complete stratification. The lower layer was then transferred via syringe to a 20-ml tube and evaporated in a vacuum thermostat at 50 °C. According to Christopherson and Glass (1969), the isolated fat was methylated using a freshly made 2% sodium methylate (NaOMe) solution. After adding 5 ml of NaOMe, the tube was shaken and left for 1 hour at room temperature. Subsequently, 7 ml of 1 N HCl, 5 ml of hexane, and 2 ml of H_2O were added, and the tube was closed and allowed to stratify after 1 min of agitation. The upper layer was transferred to a conical tube and evaporated, and the resulting fatty acid methyl ester mixture was analysed using a gas chromatograph (GC-2010 SHIMADZU) with a hydrogen flame detector and an Alltech capillary column (ATTM-FAME; 30 m, ID: 0.25 mm). The column temperature was set to shift from 150 °C to 240 °C, with an inlet temperature if 240 °C and detector temperature of 240 °C. Nitrogen was used as the carrier gas at a flow rate of 63.0 ml/min. The total analysis time was 60 min.

The quantitative ratios of the following fatty acids were estimated in pumpkin seed oils: myristic $C_{14:0}$, palmitic $C_{16:0}$, palmitoleic $C_{16:1}$, margaric $C_{17:0}$, stearic $C_{18:0}$, oleic $C_{18:1}$, linoleic $C_{18:2}$, linolenic $C_{18:3}$, arachidic $C_{20:0}$, eicosaenoic $C_{20:1}$, eicosatetraenoic $C_{20:4}$, docosatetraenoic $C_{22:4}$, docosapentaenoic $C_{22:5}$, docosahexaenoic $C_{22:6}$, and lignoceric $C_{24:0}$.

Statistical analysis

The experimental data were statistically analysed using the dispersion analysis method (ANOVA) and STATISTIKA 7.0 software (StatSoft, Tulsa, OK, USA). Data were subjected to a one-way Fisher's least squares difference test. A P value ≤0.05 was considered to indicate statistical significance. Values are presented as means ± standard deviations. The correlation regression analysis was done to establish the nature and strength of the correlation between the variables.

Results and Discussion

The hull-less seeds of the Styrian oil pumpkin are enriched in their oil content as compared to other *Cucurbita pepo* spp., which crude oil contents ranging from 41 up to 59%, depending on the genetic diversity (Murković *et al*., 2004). The literature indicates that in seeds, component ratios fluctuate widely depending on growth conditions, although specific crop characteristics remain. In addition, the crude fat amount and fatty acid composition also depend on the cultivar, climate, and ripeness (Murković *et al*., 2004; Abdel-Rahman, 2006). In the pumpkin cultivars investigated herein, the crude fat amounts fluctuated from 44.4% to 47.3 % (Table 1), and seeds of the 'Miranda' cultivar consistently exhibited the lowest amounts ($p < 0.05$).

Fats comprise an integral part of the human diet and supply both calories and essential fatty acids; however, gram for gram, saturated fats elevate the levels of serum cholesterol twice as rapidly as polyunsaturated fats lower these levels. Mensik and Katan (1989) demonstrated that replacing dietary saturated fatty acids with monounsaturated fatty acids led to in reductions in LDL cholesterol that were equal to, less than, or greater than those achieved with polyunsaturated fatty acids. Table 1 presents the proportions of saturated, monounsaturated, and polyunsaturated fatty acids in various types of pumpkins seed oil. The current research data demonstrated that saturated fatty acid percentages ranged from 15.5% to 15.92% and monounsaturated fatty acid levels ranged from 16.19% to 18.49%. In contrast, polyunsaturated fatty acids were dominant in seed oils from the investigated pumpkins, with amounts ranging from 64.29% to 66.71% of the total amount of fatty acid, depending on cultivars (Table 1). The total unsaturated fatty acid content was similar to that of other studies of pumpkin seed oil (Murković *et al*., 2004; Fruhwirth and Hermetter, 2007).

Table 1. Concentrations of crude fats and fatty acids in pumpkin seed oil (% of total fatty acids)

Cultivars	Crude fats (% DM)	Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids
'Golosemianaja'	47.43 ± 1.2[a*]	15.92	18.49	64.29
'Herakles'	47.24 ± 0.95[a]	15.71	17.31	66.20
'Miranda'	44.4 ± 0.25[b]	15.50	16.19	66.71

*Note: Values (means ± standard deviations) with different index letters are significantly different (P <0.05)

Table 2. Concentrations of fatty acids in pumpkin (*Cucurbita pepo* L.) seeds oil (% of total fatty acids)

Fatty acids	Cultivars		
	'Golosemianaja'	'Herakles'	'Miranda'
Myristic	0.09 ± 0.00[a*]	0.09 ± 0.01[a]	0.09 ± 0.00[a]
Palmitic	11.87 ± 0.49[a]	11.83 ± 0.16[a]	11.87 ± 0.46[a]
Palmitoleic	0.10 ± 0.00[a]	0.11 ± 0.01[a]	0.11 ± 0.00[a]
Margaric	0.06 ± 0.00[a]	0.63 ± 0.01[a]	0.058 ± 0.00[a]
Stearic	3.58 ± 0.09[a]	3.44 ± 0.02[b]	3.18 ± 0.06[c]
Oleic	18.33 ± 1.61[a]	17.13 ± 1.04[a]	16.01 ± 1.6[a]
Linoleic	64.65 ± 1.24[b]	66.05 ± 1.06[ab]	67.24 ± 1.6[a]
Linolenic	0.28 ± 0.06[a]	0.24 ± 0.03[a]	0.34 ± 0.06[a]
Arachidic	0.25 ± 0.01[a]	0.24 ± 0.01[a]	0.24 ± 0.01[a]
Eicosenoic	0.11 ± 0.01[a]	0.11 ± 0.01[a]	0.13 ± 0.01[a]
Eicosatetraenoic	0.06 ± 0.00[a]	0.06 ± 0.00[a]	0.06 ± 0.00[a]
Docosatetraenoic	0.23 ± 0.01[b]	0.25 ± 0.07[ab]	0.36 ± 0.01[a]
Docosapentaenoic	0.03 ± 0.01[b]	0.12 ± 0.08[a]	0.06 ± 0.02[ab]
Docosahexaenoic	0.20 ± 0.01[a]	0.19 ± 0.01[a]	0.19 ± 0.01[a]
Lignoceric	0.05 ± 0.00[b]	0.05 ± 0.00[b]	0.06 ± 0.00[a]

*Note: Values (means ± standard deviations) with different index letters are significantly different (P <0.05)

Table 3. Total phenolic contents (μg g–1) and antioxidant activities (μmol g–1) of pumpkin seed oil

Cultivars	Total phenolic content ($\mu g\ g^{-1}$)	DPPH ($\mu mol\ g^{-1}$)
'Golosemianaja'	52.6 ± 11.95[a*]	2.49 ± 0.90[ab]
'Herakles'	37.0 ± 1.47[b]	1.64 ± 0.36[b]
'Miranda'	60.6 ± 3.84[a]	3.28 ± 1.44[a]

*Note: Values (means ± standard deviations) with different index letters are significantly different (P <0.05)

As shown in Table 2, linoleic, oleic, palmitic, and stearic acids were the major fatty acid components, and these in constituted >98% of the total fatty acid content in pumpkin seeds oil, in accordance with previous studies (Murković *et al*., 2004; Nakic *et al*., 2006). The predominant fatty acid, linoleic acid (Table 2), accounted for almost two thirds of the total amount in oils from the present study, in contrast to previous studies wherein it accounted for one third or less of the total amount of fatty acids (Abdel-Rahman, 2006; Glew *et al*., 2006; Badr *et al*., 2011). The highest and lowest linoleic acid levels were identified in 'Miranda' and 'Golosemianaja' cultivar seeds oil, respectively. The experimental analysis found no essential difference of oleic acid content in different pumpkin cultivars, which fluctuated from 16.01% to 18.33% (Table 2). As in other researcher investigation of the fatty acid composition of *C. pepo* (El-Adawy and Taha, 2001), we observed a higher percentage of linoleic acid (43.1–55.6%) relative to oleic acid (20.4-37.8%). Furthermore, saturated palmitic and stearic fatty acids were dominant (Table 2), although the analysis revealed no essential differences of palmitic acid content among the different cultivars (11.83-11.87%, Table 2). In contrast, the stearic fatty acid contents differed significantly, with the highest and lowest values observed in seeds oil from the 'Golosemianaja' and 'Miranda' cultivars, respectively (3.58% and 3.18%, respectively).

The seed of oil crops which containing a high percentage of polyunsaturated fatty acids are usually rich in antioxidants. The phenolic content is closely dependent on the oil extraction process. The higher amount of phenolic compounds is released from the seeds when the oil is extracted at higher temperatures and higher pressure (Zhao *et al*., 2006). Pumpkin seeds oil have been recognized as a good source of phenolic compounds, and the cultivar, extraction method, and processing and storage conditions are known critical factors with regard to phenolic compound profiles (Boskou, 2006). Xanthopoulou *et al*. (2009) investigated samples of pumpkin seeds of different origins and observed total phenolic compound concentrations ranging from 33.71 to 41.66 μmol Gallic acid/g. In the present study, it can be observed that the total phenolic content concentrations were cultivar-dependent. The lowest total phenolic content 37.0 $\mu g\ g^{-1}$ was found in the 'Herakles' seeds, and the highest in seeds of cv. 'Golosemianaja' and 'Miranda' (Table 3).

The methanolic seed extracts exhibited statistically significant differences in antioxidant activity, as measured using the DPPH method. The highest antioxidant activity was observed in the 'Miranda' extract from seed oil, followed by the 'Golosemianaja' extract (respectively 3.28 μmol g^{-1} and 2.49 μmol g^{-1} (Table 3). Antioxidant activity increased proportionally with the phenolic content, allowing the establishment of a linear relationship between DPPH-radical scavenging activity and total phenolic content ($r = 0.823$, $p<0.05$). Other researchers have reported a similar relationship between these parameters (Fruhwirth and Hermetter, 2007; Okada *et al*., 2010). According scientists the high concentration of total phenol content implies that the antioxidant capacity of the oil significantly increases due to its capacity to block free radicals (Morelló *et al*., 2005; Baiano *et al*., 2009).

In the correlation test A. Prescha and other scientists (2014) observed the effect of the antiradical activity of oils on inhibition of monounsaturated fatty acids deterioration ($r = -0.548$) during 12 months storage. But before storage no correlation was observed in pumpkin seed oil DPPH and in monounsaturated fatty acids ($r = 0.199$, $p<0.05$).

Conclusions

The seeds oil from the pumpkin cultivars 'Golosemianaja' and 'Miranda' reliably yielded the highest total phenolic amounts. Furthermore, polyunsaturated linoleic fatty acid was the predominant fat component in pumpkin seed oil, and the significantly highest concentration was observed in 'Miranda'. Among saturated fatty acids, palmitic and stearic acids predominated, whereas stearic fatty acid concentrations differed significantly with the highest content detected in 'Golosemianaja' cultivar seeds oil. The antioxidant activity increased proportionally with the phenolic content, allowing the establishment of a linear relationship between DPPH-radical scavenging activity and total phenolic content.

References

Abdel-Rahman MK (2006). Effect of pumpkin seed (*Cucurbita pepo* L.) diets on benign prostatic hyperplasia (BPH): Chemical and morphometric evaluation in rats. World Journal of Chemistry 1(1):33-40.

Al-Saleh IA, Billedo G, El-Doush II (2006). Levels of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. Journal of Food Comparative Analysis 19(2-3):167-175.

Andjelkovic M, Van Camp J, Trawka A, Verhé R (2010). Phenolic compounds and some quality parameters of pumpkin seed oil. European Journal of Lipid Science and Technology 112(2):208-217.

Aronson WJ, Glaspy JA, Reddy ST, Reese D, Heber D, Bagga D (2001). Modulation of omega-3/omega-6 polyunsaturated ratios with dietary fish oils in men with prostate cancer. Urology 58(2):283-288.

Badr SEA, Shaaban M, Elkholy YM, Helal MH, Hamza AS, Masoud MS, El Safty MM (2011).Chemical composition and biological activity of ripe pumpkin fruits (*Cucurbita pepo* L.) cultivated in Egyptian habitats. Natural Product Research 25(16):1524-1539.

Baiano A, Gambacorta G, Terracone C, Previtali MA, La Notte E (2009). Characteristics of drupes, phenolic content and antioxidant capacity of Italian olive fruits. The Journal of Food Lipids 16:209-226.

Boskou D (2006). Culinary applications. In: Olive oil chemistry and technology. AOCS Press Champaign, Illinois pp 243-248.

Caili F, Huan S, Quanhong LA (2006). Review on pharmacological activities and utilization technologies of pumpkin. Plant Foods for Human Nutrition 61(2):73-80.

Christopherson SW, Glass RL (1969). Preparation of milk fat methylesters by alcoholysis in an essentially nonalcoholical solution. Journal of Dairy Science 52(8):1289-1290.

Danilcenko H, Jariene E, Gajewski M, Cerniauskiene J, Kulaitiene J, Sawicka B, Aleknaviciene P (2011). Accumulation of elements in some organically grown alternative horticultural crops in Lithuania. Acta Scientiarum Polonorum Hortorum Cultus 10(2):23-31.

Dimic E (2005). Cold-pressed oils, monograph. University of Novi Sad, Faculty of Technology, Novi Sad, Serbia.

El-Adawy TA, Taha KM (2001). Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. Journal of Agriculture and Food Chemistry 49(3):1253-1259.

Emmons CL, Peterson DM (2001). Antioxidant activity and phenolic content of oat as affected by cultivar and location. Crop Science 41(6):676-1681.

Fruhwirth GO, Hermetter A (2007). Seeds and oil of the Styrian oil pumpkin: Components and biological activities. European Journal of Lipid Science and Technology 109(11):1128-1140.

Gajewski M, Radzanowska J, Danilcenko H, Jariene E, Cerniauskiene J (2008). Quality of pumpkin cultivars in relation to sensory characteristics. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 36(1):73-79.

Glew RH, Glew RS, Chuang LT, Huang YS, Millson M, Constans D, Vanderjagt DJ (2006). Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita* spp.) and *Cyperus esculentus* nuts in the Republic of Niger. Plant Foods for Human Nutrition 61(2):51-56.

Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, Imano H, Okamura T, Naito Y, Shimamoto T (2002). Linoleic acid, other fatty acids, and the risk of stroke. Stroke 33(8):2086-2093.

Jeznach M, Danilcenko H, Jarienė E, Kulaitienė J, Černiauskienė J (2012). Accumulation of antioxidative vitamins and minerals in seeds of oil pumpkin (*Cucurbita pepo* L. var. *styriaca*) cultivars. Journal of Food, Agriculture & Environment 10(1):245-247.

Kreft M, Zorec R, Janes D, Kreft S (2009). Histolocalisation of the oil and pigments in the pumpkin seed. Annals of Applied Biology 154(3):413-418.

Mensik RP, Katan MB (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoproteins cholesterol in healthy women and men. New England Journal of Medicine 321(7):436-441.

Methodenbuch (1983-1999). Band III. Die chemische Untersuchung von Futtermitteln. Verlag-Darmstadt.

Morelló JR, Vuorela S, Romero MP, Motilva MJ, Heinonen M (2005). Antioxidant activity of olive pulp and olive oil phenolic compounds of the Arbequina cultivar. Journal of Agricultural and Food Chemistry 53(6):2002-2008.

Murković M, Piironen V, Lampi AM, Kraushofer T, Sontag G (2004). Changes in chemical composition of pumpkin seeds during roasting process for production of pumpkin seed oil (Part 1: non-volatile compounds). Food Chemistry 84(3):359-365.

Nakic SN, Rade D, Skevin D, Strucelj D, Mokrovcak Z, Bartolic M (2006). Chemical characteristics of oils from naked and husk seeds of *Cucurbita pepo* L. European Journal of Lipid Science and Technology 108(11):936-943.

Okada Y, Okada M, Sagesaka Y (2010). Screening of dried plant seed extracts for adiponectin production activity and tumor necrosis factor-alpha inhibitory activity on 3T3-L1 adipocytes. Plant Foods Human Nutrition 65(3):225-232.

Prescha A, Grajzer M, Dedyk M, Grajeta H (2014). The antioxidant activity and oxidative stability of cold-pressed oils. Journal of the American Oil Chemists Society 91(8):1291-1301.

Rabrenovic BB, Dimic EB, Novakovic MM, Tesevic VV, Basic ZN (2014). The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds. Food Science and Technology 55:521-527.

Ragaee S, Abdel-Aal EM, Maher N (2006). Antioxidant activity and nutrient composition of selected cereals for food use. Food Chemistry 98(1):32-38.

Sreerama YN, Sashikala VB, Pratape VM (2010). Variability in the distribution of phenolic compounds in milled fractions of chickpea and horse gram: evaluation of their antioxidant properties. Journal of Agricultural and Food Chemistry 58(14):8322-8330.

Stevenson DG, Eller FJ, Wang L, Jane JL, Wang T, Inglett GE (2007). Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. Journal of Agriculture and Food Chemistry 55(10):4005-4013.

Xanthopoulou MN, Nomikos T, Fragopoulou E, Antonopoulou S (2009). Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. Food Research International 42(5-6):641-646.

Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L, Lin Y, Fan W, Gu G (2006). Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). Journal of Agriculture and Food Chemistry 54(20):7277-7286.