ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 16(3) 2017, 293–301
www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://dx.doi.org/10.17306/J.AFS.2017.0494

ORIGINAL PAPER

Received: 30.04.2017
Accepted: 23.06.2017

ROASTING PUMPKIN SEEDS AND CHANGES IN THE COMPOSITION AND OXIDATIVE STABILITY OF COLD-PRESSED OILS

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ABSTRACT

Background. Pumpkin seed oil is valuable oil for its distinctive taste and aroma, as well as supposed health-promoting properties. The aim of this study was to investigate how roasting pumpkin seeds influences the physicochemical properties of cold-pressed oils.
Materials and methods. The fatty acid composition, content of phytosterols, carotenoids and tocopherols, oxidative stability and colour were determined in oils after cold pressing and storage for 3 months using GC-FID, GCxGC-ToFMS, HPLC, Rancimat and spectrophotometric methods.
Results. The results of this study indicate that the seed-roasting and storage process have no effect on the fatty acid composition of pumpkin seed oils, but does affect phytosterols and tocopherols. The carotenoid content decreased after storage. The colour of the roasted oil was darker and changed significantly during storage.
Conclusion. Pumpkin oil obtained from roasted seeds shows better physicochemical properties and oxidative stability than oil from unroasted seeds.

Keywords: pumpkin seed oil, roasting, sterols, fatty acid, tocopherols, carotenoids

Abbreviations: URPO – cold-pressed pumpkin oil from unroasted seeds; RPO – cold-pressed pumpkin oil from roasted seeds; URPOS – cold-pressed pumpkin oil from unroasted seeds stored for 3 months at room temperature; RPOS – cold-pressed pumpkin oil from roasted seeds stored for 3 months; PV – peroxide value; AV – acid value; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

INTRODUCTION

In recent years, people have become more and more interested in healthy diets. A healthy and balanced diet has an important role to play in the prevention of many diseases. Atherosclerosis, cardiovascular disease, urinary tract infections, high blood pressure and obesity are just a few of the illnesses which may be prevented with a proper diet (Willett et al., 2006). Oil from pumpkin (*Cucurbita pepo* L.) seeds, obtained by cold pressing, is considered to be a source of many bioactive components, positively affecting human health.

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The pumpkin belongs to the family *Cucurbitaceae*, which includes approx. 90 orders and 750 species (Schaefer and Renner, 2011). The species of squash known as the pumpkin (*Cucurbita pepo* L.) is one of the 5 cultivated and approx. 10 wild species of the genus *Cucurbita* L., from the Cucurbita family (*Cucurbitaceae*). The oil content of pumpkin seeds is around 50% (Hilledbrand et al., 1996). Seeds with or without husks are used in the production of oil. There are several ways to obtain oil from pumpkin seeds. One of them is pressing from dry, unroasted seeds in temperatures up to 50°C. In this way, a greenish oil with a characteristic pumpkin taste is obtained. The other method begins with the seeds being roasted at 100–120°C and, after pressing, the oil has a dark green/brown colour and a nutty flavour (Nederal-Nakić et al., 2006).

Pumpkin seed oil is most commonly used in Austria, Slovenia and Hungary, and is also popular in other regions worldwide. Hot- and cold-pressing procedures are used to produce oil from seeds. In the traditional pressing method, dried ground seeds, fresh water and salt and mixed to form a pulp. The pulp is roasted for up to 60 min at 100–130°C, which leads to the coagulation of the protein fraction and enables the lipid fraction to be conveniently separated by pressing. The pressing process is carried out under isothermal conditions at pressures between 300 and 600 bar, yielding dark green oil (Fruhwirth and Hermetter, 2007). During the roasting process, the characteristic aroma and colour of the final product are formed. Roasting the seeds affects the physicochemical properties and oxidative stability of the oils. A decrease in the content of tocopherols, as well as an increase in peroxide value (PV), total phenolic content and oxidative stability of the oils can be observed (Nederal et al., 2012). Phenolic compounds, e.g. isoflavones and lignans, have been detected in pumpkin seeds (Adlercreutz and Mazur, 1997; Murkovic et al., 2004). Murkovic and Pfannhauser (2000) suggested that the oxidative stability of pumpkin oil is governed by the ratio of linoleic acid to oleic acid. The fatty acid composition is influenced by the type, geographical origin, growing conditions and maturity of the seeds (Murkovic et al., 1996; Wentzel, 1987). The typical fatty acid composition of pumpkin oil comprises palmitic (9–15%), stearic (3–7%), oleic (21–47%) and linoleic acids (36–61%). As well as fatty acids, triacylglycerols, vitamins, phytosterols, minerals, carotenoids and polyphenols (flavonoids) can be found (Nederal et al., 2012; Velickovska et al., 2015; Vorobyova et al., 2014). Because of the high content of squalene, γ-tocopherol, carotenoids and D7-phytosterols, pumpkin oil may be used as a pharmaceutical remedy to treat lipid-associated diseases such as benign prostatic hyperplasia and malignant neoplasms e.g. myeloma (Vorobyova et al., 2014).

Roasting and storing pumpkin seeds can influence the content and composition of bioactive compounds, thus the aim of this study was to assess these changes under controlled conditions.

MATERIALS AND METHODS

Chemicals

Sterol standards, anhydrous pyridine, 1M methanolic KOH solution, potassium iodide, sodium thiosulfate and all solvents of HPLC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). FAME standards and Sylon BTZ were purchased from Supelco (Bellefonte, PA, USA). Standards of tocopherols (>95% purity) were purchased from Merck (Darmstadt, Germany).

Materials

Seeds of *Cucurbita pepo* var. *oleifera* were purchased from a local warehouse of oil seeds from the 2015 harvest. The chemical composition of the seeds provided by the producer is presented in Table 1. Half of the

Table 1. Chemical composition of pumpkin seeds provided by the producer

Parameters	Composition, g/100 g
Moisture	8.16 ±0.34
Protein	22.13 ±1.55
Fat	34.45 ±3.88
Carbohydrates	0.44 ±0.03
Ash	4.82 ±0.50
Fibre	10.27 ±3.39
Energy value	500 kcal/100 g

seeds were roasted at 110°C for 40 min in the oven. After roasting, a water was added to determine the water content (8.2%) of the seeds. Then the pumpkin seeds were pressed at room temperature using a screw expeller (Miramar Nowa Wieś, Dziećmorowice, Poland). The temperature inside the press was 60 ±10°C and the temperature of the oil produced was 39 ±1°C. Oil pressed from unroasted seeds was used as a control. The obtained oils were analysed directly after cold pressing, and again after 3 months of storage in dark glass bottles at 20°C. Seed roasting and oil pressing were performed in duplicate. 0.5 kg of seeds were taken for each oil pressing.

Moisture content

Moisture content in the pumpkin seeds before and after roasting was determined according to the Polish standard PN ISO 712/2002. After milling, 5 g of pumpkin seeds were heated at 130°C to constant weight.

Acid value (AV)

The acid value was determined and calculated as mg of potassium hydroxide necessary to neutralise free fatty acids in 1 g of sample according to AOCS Official Methods Te 1a-64 (2009c).

Peroxide value (PV)

Peroxide value was determined and calculated as milliequivalents of peroxide per 1000 grams of sample, according to AOCS Official Methods Cd 8b-90 (2009a).

Fatty acid composition

The percentage composition of fatty acids was estimated by GC-FID. Fatty acid methyl esters were prepared according to the AOCS Official Method Ce 2-66 (1997). Gas chromatography separation was performed in a Hewlett-Packard 5890 II instrument on a Supelcowax 10 capillary column (30 m × 0.25 mm × 0.25 μm) under programmed temperature conditions: from 60°C, at 12°C/min to 200°C held for 25 min. The injector and detector were heated at 240°C. Separated FAMEs were identified by comparing retention data of separated compounds in the analysed samples with those obtained for the standard solution. Analyses were performed in triplicate.

Phytosterols and squalene

Phytosterols and squalene were identified using a multidimensional Pegasus IV LECO GCxGC-ToFMS equipped with two capillary columns: ZB-5MS (30 m × 250 μm × 0.25 μm) and Supelcowax 10 (1 m × 100 μm × 0.1 μm). The flow rate of helium as a carrier gas was 0.8 ml/min. Injection 1 mL was set in the 1:20 split mode. Operating conditions for the first column were as follows: initial oven temperature 45°C (1 min), raised by 6°C/min to 200°C and by 25°C/min to 235°C (5 min). Operating conditions for the second column were 15°C higher than for the first column. The transfer line and injection port were heated up to 260°C. The ion source was heated up to 200°C, modulation time was optimized and set at 3 s, mass spectra were collected at a rate of 150 scans/s, while the range of collected mass was 100–550 m/z. The derivatives of phytosterols were identified by comparing the mass spectra with the NIST (The National Institute of Standards and Technology) library (Gaithersburg, MD, USA) and respective standards.

Sterol contents were determined by GC following the procedure described by the AOCS Official Method Ch 6–91 (2009b). Briefly, lipids (50 mg) were saponified with 1 M KOH in methanol and the unsaponifiables were extracted three times with hexane/methyl tert-butyl ether (1:1, v/v). The solvent was evaporated under a stream of nitrogen. Dry residues were dissolved in 0.2 mL pyridine and silylated with 0.8 mL of Sylon BTZ (Supelco, Bellefonte, PA, USA). Derivatives of the sterols were separated on an Agilent Technologies 6890 Plus GC Version A.03.08 equipped with a DB-35MS capillary column (25 m × 0.20 mm, 0.33 mm; Agilent J&W, USA). Samples of 1.0 mL were injected into the splitless mode. The column temperature was maintained at 100°C for 5 min, then raised by 25°C/min to 250°C, kept constant for 1 min, then further raised by 3°C/min to 290°C and held for 20 min. The detector temperature was set at 300°C. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL/min. An internal standard, 5a-cholestane, was used to quantify sterols.

Tocopherols

Tocopherols were qualitatively and quantitatively identified using a Waters HPLC system (Waters, Milford, MA) consisting of a pump (Waters 600), a fluorimetric

detector (Waters 474), a photodiode array detector (Waters 2998 PDA), an autosampler (Waters 2707), a column oven (Waters Jetstream 2 Plus), and a LiChrosorb Si 60 column (250 × 4.6 mm, 5 mm) from Merck (Darmstadt, Germany). The mobile phase was a mixture of n-hexane with 1,4-dioxane (96:4 v/v). The flow rates were 1.0 mL/min. To detect fluorescence of tocopherols, the excitation wavelength was set at l = 295 nm and the emission wavelength at l = 330 nm.

Carotenoids

The oil samples were mixed with acetone, partitioned to petroleum ether and saponified (10% KOH in methanol) according to the method described by Rodriguez-Amaya and Kimura (2004). Identification and quantification were performed using an Agilent Technologies 1200 Rapid Resolution system (Waldbronn, Germany) equipped with Agilent 1260 Infinity DAD VL+ (Waldbronn, Germany). Separation was carried out on a Poroshell 120 SB-C18 column (4.6 × 150 mm, 5 µm) (Agilent Technology Inc., Palo Alto, USA), operated at 25°C at a flow rate of 0.5 mL/min. The mobile phase was composed of acetonitrile:methanol:ethyl acetate in a gradient from 95:5:0 to 60:20:20 in 40 min, with the latter concentration being maintained until the end of the run (50 min). Acetonitrile contained 0.5 g/L of triethylamine, as recommended by Hart and Scott (1995), to improve carotenoid recovery from the chromatographic column. Carotenoids were quantified at 454 nm. Quantification of carotenoids was performed by the external standard method, related to β-carotene (Sigma Aldrich Chemie GmbH, Steinheim, Germany).

Colour

The colour parameters of the oil were determined using a Konica-Minolta CR-3600d spectrophotometer (Osaka, Japan). Instrumental colour data was expressed as CIE L*a* b* coordinates, which define colour in a three-dimensional space: L* (dark–light), a* (redness–green) and b* (yellowness–blueness). The colour coordinates of the samples were determined using the D65 illuminant, 10° observer angle and 25 mm aperture diameter.

Oxidative stability index

The oxidative stability of the oils was determined by automated Metrohm Rancimat model 743 (Herisau, Switzerland). A stream of purified air was passed through 3 g of oil at 120 ±0.1°C at a flow rate of 20 L/h. The induction period (IP) in hours was automatically recorded and taken as the break point of the plotted curves.

Statistical analysis

Results are presented as means ±standard deviation from three replicates of each experiment. The differences between mean values were determined by analysis of variance (ANOVA). The post-hoc analysis was performed using Tukey's test. All tests were considered significantly different at $p < 0.05$. The statistical analysis was performed using Statistica 10.0 software (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

The effect of roasting pumpkin seeds on the quality of cold-pressed oil

The unroasted pumpkin seeds contained 8.2% water and, after roasting at 110°C for 40 min, this decreased to 4.6%. The cold-pressed oils were analysed directly after pressing and after storage in dark bottles at room temperature for 3 months.

Pumpkin oil pressed from unroasted seeds was characterized by a slightly higher acid value than oil from roasted seeds, but PV was lower in oil obtained from roasted seeds (Table 2). Similar results were recorded for roasted walnuts and peanuts (Kita and Figiel, 2007; Özdemir et al., 2001). Probably, the temperature and duration of the seed-roasting process were the most important parameters influencing the AV and PV of the obtained oils (Siger et al., 2016). It may be stated that the parameters of seed roasting used in this experiment have no influence on the hydrolytic and oxidative changes in the lipids contained in the seeds.

The content of squalene in pumpkin oils pressed from unroasted and roasted seeds was 272 mg/100 g and 258 mg/100 g respectively, with no significant statistical differences observed (Table 2). The obtained results are much lower than results reported Rabrenović et al. (2014). A similar squalene content was found in oils produced from unroasted husked and de-husked pumpkin seeds by Nederal-Nakić et al. (2006), ranging from 225 mg to 351 mg per 100 g. The method

Table 2. Acid value, peroxide value and chemical composition of pumpkin oils pressed from unroasted and roasted seeds before and after storage

	URPO	RPO	URPOS	RPOS
Acid value, mg KOH/g of oil	1.7 ±0.1[b]	1.4 ±0.1[a]	2.5 ±0.1[c]	3.6 ±0.3[d]
Peroxide value, mEq O_2/kg	5.6 ±0.1[b]	3.8 ±0.1[a]	12.2 ±0.1[d]	10.6 ±0.2[c]
Induction period at 120°C, h	4.62 ±0.07	5.16 ±0.06		
Phytosterols, mg/100 g	173.9[a]	216.8[d]	165.6[a]	181.5[c]
Stigmasterol	1.2 ±0.1[b]	2.4 ±0.1[c]	0.8 ±0.1[a]	2.2 ±0.2[c]
24-Methyl-cholest-7-en-3β-ol	3.2 ±0.2[a]	7.1 ±0.3[b]	3.8 ±0.2[a]	7.3 ±0.4[b]
Δ7,22,25-Stigmastatrien-3β-ol	34.6 ±1.1[b]	43.8 ±1.2[c]	29.0 ±4.9[a]	42.2 ±1.8[c]
α-Spinasterol	51.4 ±2.3[b]	54.9 ±2.8[b]	48.8 ±2.1[a]	52.4 ±1.9[b]
Δ7,25-Stigmastadien-3β-ol	40.8 ±1.6[a]	56.2 ±2.1[c]	42.4 ±1.8[a]	49.5 ±2.7[b]
Δ7-Stigmasterol	12.1 ±0.6[b]	15.0 ±0.6[d]	13.8 ±0.5[c]	10.1 ±0.9[a]
Δ7-Avenasterol	30.6 ±1.3[c]	37.4 ±1.8[d]	27.0 ±1.5[b]	17.8 ±0.8[a]
Squalene, mg/100 g	272.0 ±12.1[a]	258.0 ±11.2[a]	255.0 ±10.5[a]	245.0 ±10.2[a]
Carotenoids, mg/100 g	0.29	0.54	0.21	0.21
Lutein	0.12 ±0.04[c]	0.27 ±0.02[d]	0.10 ±0.01[a]	0.10 ±0.02[b]
Cryptoxanthin	0.03 ±0.01[c]	0.05 ±0.02[d]	0.02 ±0.01[a]	0.02 ±0.01[b]
α-Carotene	0.02 ±0.02[d]	0.01 ±0.01[c]	0.01 ±0.01[a]	0.01 ±0.01[b]
β-Carotene	0.12 ±0.01[b]	0.21 ±0.03[c]	0.08 ±0.02[a]	0.08 ±0.02[a]
Tocopherols, mg/100 g	60.1	54.2	49.6	51.6
α-Tocopherol	5.5 ±0.3[d]	4.7 ±0.2[c]	1.1 ±0.2[a]	2.2 ±0.2[b]
β-Tocopherol	0.1 ±0.0[a]	0.1 ±0.0[a]	Traces	Traces
γ-Tocopherol	54.1 ±0.2[b]	49.0 ±0.2[a]	48.1 ±0.2[a]	49.0 ±0.2[a]
δ-Tocopherol	0.4 ±0.1[a]	0.4 ±0.1[a]	0.4 ±0.1[a]	0.4 ±0.1[a]
Fatty acid composition, %				
C12:0	0.1 ±0.05[a]	0.1 ±0.03[a]	0.1 ±0.10[a]	0.1 ±0.04[a]
C16:0	10.4 ±0.09[a]	10.4 ±0.08[a]	10.4 ±0.07[a]	10.5 ±0.11[a]
C16:1	0.1 ±0.07[a]	0.1 ±0.04[a]	0.1 ±0.03[a]	0.1 ±0.09[a]
C18:0	6.4 ±0.10[a]	6.4 ±0.11[a]	6.9 ±0.06[a]	7.3 ±0.06[b]
C18:1	37.7 ±0.04[a]	38.3 ±0.06[a]	37.8 ±0.10[a]	37.8 ±0.12[a]
C18:2	44.9 ±0.12[a]	44.3 ±0.11[a]	44.3 ±0.09[a]	43.7 ±0.11[a]
C20:0	0.4 ±0.07[a]	0.4 ±0.01[a]	0.5 ±0.03[a]	0.5 ±0.05[a]
∑ SFA	17.3	17.3	17.8	18.4
∑ MUFA	37.8	38.4	37.9	37.9
∑ PUFA	44.9	44.3	44.3	43.7

Values (means ±SD) bearing different superscripts are statistically significantly different ($P < 0.05$).

used for extracting oil from pumpkin seeds influenced the content of squalene (Nederal-Nakić et al., 2006). A lower squalene content was detected in oils from industrially-roasted pumpkin seeds than in those produced under laboratory conditions. The content of this non-glyceride component could be influenced by seed ripeness and origin, as well as by the temperature applied during production (Cherif et al. 2013; Fruhwirth and Hermetter, 2007). Squalene is a natural organic compound produced by bacteria, fungi, algae, plants, animals and humans. It is a triterpene precursor of sterols, steroid hormones and vitamins in the human body. It has been suggested that it is an important antioxidant, anti-carcinogenic and anti-inflammatory component of the Mediterranean diet (Smith, 2000). Recently, it has become common practice to hunt sharks and process their livers to obtain squalene, to be used as a functional additive to food products. Vegetable sources of squalene could be an alternative to shark hunting.

The total content of phytosterols in oil pressed from unroasted seeds was 173.9 mg/100 g, including stigmasterol at 1.2 mg/100 g, 24-methyl-cholest-7--en-3β-ol at 3.2 mg/100 g, D7,22,25-stigmastatrien--3β-ol at 34.6 mg/100 g, α-spinasterol at 51.4 mg/100 g, D7,25-stigmastadien-3β-ol at 40.8 mg/100 g, D7--stigmasterol at 12.1 mg/100 g and D7-avenasterol at 30.6 mg/100 g (Table 2). When comparing this to the content of phytosterols in the oil from roasted seeds, we observed average 25% increase in the amount of these compounds after roasting. No differences were found in the total contents of phytosterols in lipids pressed from rapeseeds roasted at 160 and 180°C, but lower contents were recorded in oil from seeds roasted at 140°C (Siger et al., 2016). The phytosterol fraction of pumpkin seeds contains high levels of D7-sterols, which have beneficial effects in the treatment and prevention of prostate and bladder diseases. The roasting process has a significant influence on the content of Δ7,22,25-stigmastatrien-3β-ol, α-spinasterol, Δ7-avenasterol as well as the amount of squalene.

Roasting pumpkin seeds was found to have the greatest influence on the content of carotenoids. Their total content increased from 0.29 mg/100 g in URPO to 0.54 mg/100 g in RPO. The main compound was lutein, which accounted for about 44% of the total amount of carotenoids and, after roasting, this increased to 50%. β-carotene was the second most common carotenoid; the percentage of β-carotene in URPO and RPO was 43% and 39% respectively. The content of cryptoxanthin in URPO was two times lower than in RPO (Table 2). Literature data concerning carotenoids in pumpkin seed oil is rather scarce. Matus et al. (1993) described lutein (53%) and β-carotene (10%) as the main carotenoids detected in pressed pumpkin oil. Among the 600 carotenoids isolated from natural products, β-carotene, lutein, α-carotene and cryptoxanthin are the most prevalent in human serum (Gerster, 1997). These molecules have attracted interest due to their provitamin A and antioxidant properties. Epidemiological studies have demonstrated the protective effect of carotenoids against cancer and cardiovascular disease.

The presence of carotenoids in food products is shown by their colour. URPO exhibited an average of 6.6 for L*, 9.3 for a* and 7.5 for b*. In this evaluation, the L* value represents lightness, ranging from 0 (black) to 100 (white), the a* value ranges from –100 (green) to +100 (red) and the b* value ranges from –100 (blue) to +100 (yellow). All colour values were lower in RPO, with L* = 5.7, a* = 7.7, and b* = 3.6. During the roasting of walnuts, the lightness of the pressed oil decreased, while redness and yellowness (a* and b* colour values) increased (Vaidya and Eun, 2013). The increase in redness was probably connected with the formation of the Maillard reaction and caramelization products during roasting. In this experiment pumpkin seeds were roasted at a lower temperature (110°C) than walnuts or rapeseed (140–160°C; Siger et al., 2016).

Analyses of URPO and RPO detected α-, β-, γ- and δ-tocopherols, and their total content in oil was 60.1 and 54.2 mg/100 g respectively. Tocotrienols were not detected. The main isomer present in both oils was γ-tocopherol, which accounted for 90% of tocopherols, followed by α-tocopherol at 9% in both pumpkin seed oils, while δ- and β-isomers were recorded at below 1%. Tocopherol content in oil produced from rapeseed cake roasted at 140°C and 160°C was slightly lower for α-tocopherol, at 1 mg/100 g (Siger et al., 2016). Seed roasting at high temperatures generated or released antioxidants such as phenolic compounds or Maillard reaction products, which act as antioxidants and protect tocopherols from degradation.

In food systems, antioxidant activity decreases in the following order: γ > δ > β > α isomers (Hilledbrand et al., 1996). The high level of γ-tocopherols in the analysed oils plays an important role in the antioxidative stabilization of unsaturated fatty acids. Oxidative stability results are presented as an induction period (Table 2). The oxidative stability of both oils was analysed at 120°C. The oxidative stability of URPO was about 10% lower than that of RPO. Pumpkin oil is often recommended for its high antioxidant activity and oxidative stability (Murkovic and Pfannhauser, 2000). However, roasting conditions have a major effect on the content of antioxidants and induction period. Depending on roasting conditions, a significant increase in the content of phospholipids, phenolic compounds and tocopherols in the oil was detected, thus increasing oxidative stability.

Changes in pumpkin oils pressed from roasted and unroasted seeds after storage

After 3 months of storage at room temperature in brown bottles, the AV and PV of URPO and RPO increased, with the AV of URPO being lower than that of RPO, whereas the opposite was found to be true of PV (Table 2). Nevertheless, both these parameters were found to be below the limits (4.0 mg KOH/g and 15 meq O_2/kg, respectively) recommended by Codex Alimentarius (2015) for virgin cold-pressed pumpkin oils. Nederal et al. (2012) observed no changes in these parameters throughout the storage of three pumpkin oils at 20°C for 12 months. The fatty acid composition was stable and only slight differences were observed after storage, while the content of squalene, phytosterols, carotenoids and tocopherols decreased.

Linoleic acid was the main fatty acid found in the stored oils, amounting to 44% of the total fatty acids in URPO and RPO. It was followed by oleic acid, the content of which in both stored oils was 38%. Palmitic acid, at 10%, and stearic acid, at 7%, were found in lesser amounts. The share of SFA increased in URPOS and RPOS by only 0.5 and 1.1 percentage points, PUFA decreased by 0.6 percentage points in both oils, while the level of MUFA was unchanged. It may be stated that neither roasting the pumpkin seeds prior to oil pressing nor the storage of the oils had any effect on the fatty acid composition of the oils.

The content of squalene decreased during storage by 6% in URPOS and 5% in RPOS. The content of squalene in cold-pressed pumpkin oil and roasted pumpkin oil showed an 8–14% decrease in the first 3 weeks of storage at 42°C, which increased to 22–28% after 9 weeks. A similar effect was observed in both types of oil (Nederal et al., 2012). Our data indicates that the loss of squalene during storage was affected by the storage conditions (temperature, time, etc.) rather than production technology (unroasted vs. roasted).

Phytosterol content decreased in URPO and RPO by 5% and 16% respectively. Nevertheless, RPO was still more abundant in phytosterols than URPO. The drop in phytosterol is probably caused by autoxidation and the formation of oxidation derivatives, such as oxyphytosterols and volatile compounds. There is little data to be found in literature concerning changes in phytosterols in stored pumpkin oils. Tests performed on the effect of aging (heating at 50°C for several weeks and at 100°C for 1 h) on different plant oils and fats showed no significant variation in the phytosterol content (Thanh et al., 2006).

During storage, the total content of tocopherols decreased in URPO by 17% to 50 mg/100 g. Smaller losses of these compounds (5%) were observed in RPO, which after storage contained 52 mg of tocopherols per 100 g oil. The rate of tocopherol degradation in walnut oil during storage was lower in roasted samples than unroasted samples (1.2% vs. 2.2% per day) (Vaidya and Eun, 2013). This implies that the stability of tocopherols increased when the seeds were roasted prior to oil production.

The greatest changes in the colour of the stored oils were connected with the decomposition of carotenoids. The content of these compounds decreased by 30% in URPO and 62% in RPO (Table 2). Colour parameters (L*, a*, b*) of both oils after storage were very similar (Table 3). Carotenoids are known to be very important to human health, particularly for skin and eye functionality. Within food matrices, carotenoids can play roles as pro-oxidants or antioxidants, depending on the conditions to which they are exposed. Roasted pumpkin oils could be a good source of these compounds in the human diet, but their decomposition during storage requires special methods to protect them against pro-oxidative factors such as oxygen or light.

Table 3. Parameters of pumpkin oil colour

Oils	L*	a*	b*
URPO	6.6 ±0.1[b]	9.3 ±0.1[c]	7.5 ±0.1[c]
RPO	5.7 ±0.1[a]	7.7 ±0.1[b]	3.6 ±0.1[b]
URPOS	15.6 ±0.1[c]	0.6 ±0.1[a]	–1.0 ±0.1[a]
RPOS	15.9 ±0.4[c]	0.5 ±0.1[a]	–0.9 ±0.2[a]

L* – lightness, a* – share of the red colour, b* – share of yellow colour.
Values (means ±SD) bearing different superscripts are statistically significantly different ($P < 0.05$).

CONCLUSIONS

Studies on the production of cold-pressed oils from unroasted and roasted pumpkin seeds and 3 months of storage at room temperature in dark bottles showed that roasting the pumpkin seeds caused changes in the chemical composition and oxidative stability of the obtained oils. The content of squalene and tocopherols was lower in roasted than in unroasted oil, but the level of phytosterols and especially carotenoids increased after roasting the pumpkin seeds. The storage of the analysed oils under conditions typical for vegetable oils influenced various quality attributes and bioactive compounds in both the oils. The loss of squalene content was similar in both oils. A greater decomposition of tocopherols was detected in unroasted oil, while the degradation of phytosterols and carotenoids was greater in roasted oil. The roasting process and storage of pumpkin oils have no effect on their fatty acid composition.

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