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Variance of quality parameters and fatty acid composition in pumpkin seed oil during three crop seasons

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ABSTRACT

Roasted and cold-pressed pumpkin seed oil obtained from pumpkin seeds (*Cucurbita pepo*, L.) from the 2010/2011 to 2012/2013 crop seasons were analyzed to determine the basic quality parameters (free fatty acids and peroxide value) as well as content of *cis* and *trans* fatty acids. Free fatty acids and peroxide values were within the legal limits in all except one of the roasted samples and were influenced by climate conditions of a particular crop season, while the significance of influences due to processing conditions was not determined. Gas chromatography revealed a total of 12 *cis* and 3 *trans* fatty acids in pumpkin seed oil. The dominant fatty acids in all seed oil samples were linoleic, oleic, palmitic, and stearic (98.8 ± 0.18%). Their content was significantly affected by the crop season and processing. Higher amounts of saturated and monounsaturated fatty acids were noted in oil from a crop season with higher air temperature and in cold-pressed oil. All pumpkin seed oil contained palmitoleic fatty acid (0.10–0.14%) which is listed as "not detected" in the authenticity requirements for pumpkin seed oil in regulations of various EU countries. The content of *trans* fatty acids was significantly higher in roasted seed oil (0.03%–0.39%) than in cold-pressed oil (0.03%–0.05%).

1. Introduction

Pumpkin seed oil is a specific salad oil made from the seeds of several *Cucurbita* varieties. Thanks to its high nutritive value, good oxidative stability (Szterk et al., 2010) and its characteristically very pleasant sensory properties, it is becoming an increasingly popular unrefined oil. Cultivars of pumpkin which is currently being used for the production of pumpkin seed oil (*Cucurbita pepo* var. *styriaca*) were obtained by natural mutation which led to a very thin outer hull on the seed (naked seeds), resulting in facilitated production of this oil (Fruhwirth and Hermetter, 2007). Further selections, made in order to increase parameters like yield, seed, and oil content or to introduce the resistance to field pests and viruses, generated several cultivars and hybrids among which the "Gleisdorfer" is the best known (Teppner, 2000). The properties of this species' oil differ from the oil of other *Cucurbita* varieties e.g. *Cucurbita maxima* L. (Rezig et al., 2012). Pumpkin seeds are usually planted in early May and the fruits reach their full physiological maturity in late August or early September. Traditional pumpkin seed oil is made by pressing pumpkin seeds which were roasted at 110–130 °C for 30–60 min, and according to Codex Alimentarius (CODEX STAN, 1999) belongs to the virgin oil category. Nowadays, cold-pressed oil from unroasted pumpkin seeds is also emerging on market shelves (Vujasinović et al., 2010; Rabrenović et al., 2014). These two oils differ mainly in their sensory properties, i.e. color, taste, and odor characteristics (Siegmund and Murkovic, 2004). In addition, roasted pumpkin seed oil has a higher oxidative stability and phenol content (Andjelkovic et al., 2010) but lower content of tocopherols and sterols (Nederal Nakić et al., 2006). Even though the mentioned bioactive compounds possess an important role in human nutrition, the major composition of pumpkin seed oil is fatty acids. The dominant fatty acid in pumpkin seed oil is linoleic acid (43–52%) followed by oleic acid (28–38%) (Murkovic et al., 1996). The influence of the roasting process on the differences between fatty acid composition of roasted and cold-pressed pumpkin seed oil was also investigated in the work of Murkovic et al. (2004) and Nederal et al. (2012). They state that in roasted pumpkin seed oil a small decrease of linoleic fatty acid occurs. However, regardless of the processing conditions, the oil's fatty acid profile mainly depends on the fatty acid composition in the seed. Therefore, this profile may

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be influenced by geographical latitude, climate and growth conditions as is evident for many of different seed crops (Lajara et al., 1990; Harris et al., 1978). To our knowledge, the influence of a specific crop season on the fatty acid profile of pumpkin seed oil has not been researched. The aim of this study was to determine the basic quality and variance of fatty acid content of pumpkin seed oil influenced by local climatic conditions (temperature and rainfall) at the time of germination, growth, blooming, and maturing in both roasted and cold-pressed pumpkin seed oils gathered during the Pumpkin Seed Oil Exhibition in the years of 2011, 2012, and 2013. In addition, because most of the research on pumpkin seed oil is done in laboratory conditions, this study will give insight into the effects of commercial production on the quality of pumpkin seed oil.

2. Materials and methods

2.1. Pumpkin seed oil

The samples used in this study were commercial – roasted and cold-pressed pumpkin seed oil gathered during the Pumpkin Seed Oil Exhibition of North-Western Croatia in 2011, 2012, and 2013. Samples were therefore produced from Styrian oil pumpkin seeds (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) from the 2010/2011, 2011/2012, and 2012/2013 crop seasons grown in the area of northwest subregion (45°54′–46°32′ lat. N; 15°36′–16°52′ long. E) located in the Pannonian agricultural region of Croatia (Bašić, 2013). Seeds were grown under agronomic management standards for this region and culture. In autumn, the soil was fertilized with 28–31.5 kg ha^{-1} of nitrogen, 80–90 kg ha^{-1} of phosphorus, and 120–135 kg ha^{-1} of potassium (NPK) fertilizer, and in spring before seeding with 150–200 kg ha^{-1} of calcium and ammonium nitrate (CAN) fertilizer (27% nitrogen). Seed was planted 2.5–4 cm deep with 140 cm of spacing between rows and 30–40 cm of spacing within the row. Herbicides such as metolachlor, clomazone, and pethoxamid including the insecticides phoxim and chlorpyrifos were used. Harvested pumpkin seeds were stored at an ambient temperature in dry storage, protected from molds, yeasts, and insect infestation, and were used for the commercial production of oil. At the beginning of the following year, fresh batches of pumpkin seed oil were prepared for the exhibition. Pumpkin seed oil samples were received at the laboratory in 250 mL dark glass bottles and kept at 4 °C until the analysis. The number of oil samples was as follows: 15 roasted and 3 cold-pressed in 2011, 27 roasted and 7 cold-pressed in 2012, and 25 roasted and 5 cold-pressed pumpkin seed oil samples in 2013.

2.2. Reagents and standards

All chemicals and solvents were of analytical grade. Ethanol, diethyl ether peroxide free, glacial acetic acid, isooctane, and methanol gradient grade were purchased from J.T. Backer (Deventer, Holland). Potassium iodide, starch, thymolphtaleine indicator, potassium hydroxide, and sodium hydrogensulphate monohydrate were purchased from Kemika (Zagreb, Croatia) together with 0.1 mol L^{-1} of potassium hydroxide solution and 0.1 N of sodium thiosulfate solution. Analytical standard Food Industry Fatty Acid Methyl Esters (FAME) containing 37 components in methylene chloride was obtained from Restek (Bellefonte, PA, USA).

2.3. Determination of the free fatty acids

Free fatty acid (FFA) values were determined using the ISO method (ISO 660, 2009). A test portion of 5 g was weighted into a 250 mL conical flask and dissolved in 50 mL of neutralized solvent mixture (ethanol: diethyl ether 1:1) and was then gentle warmed. A few drops of thymolphthalein solution in ethanol (0.2 g L^{-1}) were added as an indicator, and the solution was titrated with a standard volumetric solution of potassium hydroxide (0.1 mol L^{-1}). The end point of the titration was reached when the addition of a single drop of potassium hydroxide produced a color change persisting for at least 15 s. The values were expressed in milligrams of potassium hydroxide per gram of sample.

2.4. Determination of the peroxide value

Peroxide values (PV) of samples were determined in milliequivalents of active oxygen per kilogram of sample using ISO method (ISO 3960, 2007). In brief, 5 g of sample was weighed into a nitrogen rinsed 250 mL Erlenmeyer flask with a stopper, and the sample was dissolved in 50 mL of solvent mixture (glacial acetic acid: isooctane 3:2) by gently swirling. After that, 0.5 mL of saturated potassium iodide solution was added, the flask was closed and mixed manually for 1 min. Then 100 mL of deionized water and 0.5 mL of a starch solution were added and coloured the solution a violet color. The liberated iodine was titrated with 0.01 N sodium thiosulfate standard solution. The end point of the titration was reached when the addition of a single drop of sodium thiosulphate solution turned the violet solution to a colourless solution and it remained colourless for at least 30 s.

2.5. Determination of the fatty acid composition

The samples were converted to corresponding FAMEs directly by trans-esterification with a methanolic solution of potassium hydroxide (EN ISO, 5509). Approximately 60 mg of the sample was weighed into plastic test tube and dissolved using 4 mL of isooctane. After that, 200 μL of derivatization reagent (potassium hydroxide solution in methanol, 2 mol L^{-1}) was added and the test tube was vigorously shaken two times for 30 s. The solution was then neutralized by the addition of 1 g of sodium hydrogen sulphate monohydrate and transferred into a 2 mL vial. The fatty acid composition was determined on a CP-3800 gas chromatograph (Varian, Palo Alto, CA, USA) using a capillary column DB-23 60 m × 0.25 mm, with a 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA, USA), split/splitless injector, and flame ionization detector. The temperatures of the injector and detector were 230 °C and 250 °C respectively. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1} and the injections were performed in a split ratio 1:30. The temperature program of the column oven was set at 60 °C (1 min) rising to 220 °C at the rate of 7 °C min^{-1}. FAMEs were identified by comparing the retention times of each fatty acid with retention times obtained in the Food Industry FAME standard chromatogram, and the results were calculated through a normalization procedure. A detailed description of the method and its suitability was given previously (Petrović et al., 2012).

2.6. Statistical analyses

All analyses were done in duplicate and Statistica 12 (StatSoft, Tulsa, USA) used for statistical evaluation of the results. Based on the values obtained from the analysis, the mean, standard deviation, and range were calculated. To get an insight into the relationship between the variables and in order to determine their influence on the results, one-way ANOVA, correlation and principal component analysis were performed. Correlations were calculated by comparing the sums of the average monthly air temperatures and average monthly rainfall (Table 2) with fatty acid profiles of the particular years.