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The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds

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ABSTRACT

The composition and content of certain bioactive components of the cold pressed oil obtained from six samples of pumpkin seeds (*Cucurbita pepo* L.) cultivated in Serbia were analyzed by GC and GC/MS. The composition and content of fatty acids, tocopherols and phytosterols, and the total content of squalene were determined. The results indicate oil's excellent quality, with high contents of monounsaturated fatty acids (37.1 ± 0.70–43.6 ± 0.69 g/100 g of total fatty acids), total tocopherols (38.03 ± 0.25 –64.11 ± 0.07 mg/100 g of oil), sterols (718.1 ± 6.1–897.8 ± 6.8 mg/100 g of oil) and especially squalene (583.2 ± 23.6–747 ± 16 mg/100 g of oil). High content of squalene, phytosterols and monounsaturated fatty acids recommend the use of this type of the oil in the nutritional and medical purposes.

1. Introduction

It is evident that both the production of cold pressed pumpkin seeds oil (PSO) and its consumption have been on a constant increase in the last few years (Dimić, 2005; Vujasinović, Đilas, Dimić, Romanić, & Takači, 2010). Cold pressed PSO is a relatively new product on the Serbian market. In fact, the establishment of a chain of small oil mill plants for producing cold pressed oils was initiated in the late 1990s.

The majority of these plants for processing different oilseeds use small capacity screw presses (6–40 kg/h). In this case, cold pressed PSO is produced by pressing of raw, dried, mainly hull-less seeds, on a continuous screw press. An important factor in the cold pressed oil manufacturing process is the temperature of the oil leaving the press. Due to friction, pressing is accompanied by the release of heat. However, modern screw presses are constructed in a way that reduces the heat released per unit mass of raw material processed by the press, thus decreasing the temperature and enabling a better temperature control during the process. According to literature 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 (Dimić, 2005). This method of production preserve bioactive components, such as vitamins, provitamins, phytosterols, phospholipids and squalene, which are, together with some fatty acids, key PSO nutritional value factors. In addition, it has been proven that these components have a positive effect on human health in many different ways: they have anti-inflammatory and diuretic properties, alleviate negative symptoms of benign prostatic hyperplasia, help lower cholesterol levels, bind free radicals, and others (Caili, Huan, & Quanhong, 2006; Fruhwirth & Hermetter, 2007; Sener et al., 2007).

Researchers have so far focused mostly on the composition and content of fatty acids and tocopherols in roasted PSO and their positive health effects, but there is insufficient literature data on the phytosterol content of cold pressed PSO. It is a known fact that sterols may help reduce total and LDL cholesterol, decrease the risk of certain forms of cancer, and improve treatment of prostate disorders (Moreau, Whitaker, & Hicks, 2002; Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007; Woyengo, Ramprasath, & Jones, 2009). One of the specific characteristic of PSO is the predominance of Δ^7 sterols, while the majority of other vegetable oils have the highest levels of Δ^5 sterols (Breinhölder, Mosca, & Lindner, 2002; Nedjeral-

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Nakić et al., 2006). Squalene, the richest sources of which are shark liver oil and, to a smaller extent, olive oil, is indicated as a component with preventive properties against certain types of cancer (Dessi et al., 2002; Spanova & Daum, 2011).

The objective of this research was to determine and compare the composition and content of fatty acids, tocopherols and sterols, as well as the squalene content in cold pressed PSO obtained from different pumpkin seeds (*Cucurbita pepo* L.) cultivated in Serbia.

2. Material and methods

2.1. Seed material

For oil extraction by cold pressing, six samples of oil pumpkin seeds were used, four of which were naked and two of which were with husk. Four samples were provided by the Institute for Field and Vegetable Crops (Novi Sad, Serbia), Saatzucht Gleisdorf GmbH (Gleisdorf, Austria) and DUDU Bt (Debrecen, Hungary), while two commercial samples were randomly selected from the Serbian market (Table 1).

All seeds that served as samples had intact kernels and no visible damages, and were dried to safe storage humidity. The coat was manually removed from the husk seeds immediately before analysis of seeds' chemical characteristics. The seeds were stored in closed plastic bags in dark at 4 °C, until the oil extraction.

2.2. Chemicals

Standards for fatty acid methyl esters (FAME), standards for individual tocopherols, cholesterol standard, boron trifluoride solution and methanol of HPLC grade were obtained from Sigma–Aldrich (St Louis, MO, USA). Extraction solvents (analytical-grade), hexane, diethyl ether, petroleum ether, methanol, ethanol, methylene chloride, were from Merck (Darmstadt, Germany). All other chemicals (analytical grade) i.e. potassium hydroxide, sodium chloride, sodium sulfate and pyrogallol were also purchased from Merck (Darmstadt, Germany). Silylating agents pyridine, hexamethyldisilazane and chlorotrimethylsilane were obtained from Sigma–Aldrich (Steinhelm, Germany), Merck (Schuchardt, Germany) and Merck (Darmstadt, Germany), respectively.

2.3. Methods

2.3.1. Oil extraction by cold pressing

Oil was extracted from sampled seeds by the cold pressing. Pressing is a technological process of extracting (draining) oil from the prepared seeds exclusively by mechanical means, i.e. by pressure. Cold pressing is performed by directly pressing raw/dried seeds on a continuous screw press at low temperature. In this research, a screw press with a capacity of 40 kg/h of seed, equipped with an engine of 4.0 kW (Kern Kraft, Germany), was used.

Since the cold pressed oil technology excludes thermal processing of material during the preparation phase, this may cause some problems in the initial stage of pressing on a screw press. In order to avoid difficulties and increase efficiency, the head of the screw press is heated to a temperature between 80 and 100 °C before starting the pressing process. For that purpose, a specially designed heating ring, connected to an automatic temperature control device, was attached to the head of the screw press. Heating process does not reflect on the temperature of extracted oil since the heaters are turned off after optimal seeds flow has been achieved (Martinez, Penci, Ixtaina, Ribotta, & Maestri, 2013; Zengh, Weisenborn, Tostenson, & Kangas, 2003). First samples of pressed oil were not collected for safety reasons. Temperature of extracted oil was measured during the whole experiment with digital thermometer and it was continuously at below 45 °C.

After 24-hour sedimentation, the extracted oil was separated from the sediment by decantation and stored in dark green glass bottles with metal screw caps. The bottles were kept in the refrigerator, at the temperature of 4 °C, until the moment of analysis.

Table 1
Characterization of pumpkin seed samples.

Sample number	Name of the sample	Self-fertile/hybrid	Seed type	Origin
1.	F1 Olinka × G	F1 hybrid	Naked	Novi Sad
2.	F1 Olinka × 371B2	F1 hybrid	Naked	Novi Sad
3.	Gleisdorfer Express	F1 hybrid	Naked	Austria
4.	K2	Commercial sample	Naked	Market
5.	Daki 802	Self-fertile	Husk	Hungary
6.	K1	Commercial sample	Husk	Market

2.3.2. Fatty acid composition

Fatty acid composition was determined by gas chromatography and for that purpose they were transformed to volatile fatty acid methyl esters by direct transesterification for the neutral samples and with boron trifluoride solution for samples with higher content of free fatty acids (ISO 5509, 2000). Gas chromatograph (GC; VARIAN chromatograph, model 1400; Varian Associates, Walnut Creek, CA), was equipped with a flame ionization detector and a 3.0 m × 0.32 cm steel column, packed with LAC-3R-728 (20%; Cambridge Ind. Co., Cambridge, UK) on ChromosorbW/AW (80–100 mesh; Merck, Darmstadt, Germany). Nitrogen was used as a carrier gas (flow rate, 24 mL/min) (ISO 5508, 1990). Fatty acids were identified by comparison of their retention times (Rt) with those of standards (SupelcoTM FAME Mix). All determinations were carried out in triplicates.

2.3.3. Determination of tocopherols

Determination of tocopherols was carried out using HPLC (Waters M600E, USA) on a reversed phase Nucleosil 50-5 C18 column with fluorescence detection using a method based on the procedure of Carpenter (1979) with some modifications. The following procedures were applied: 20 mL of 96 mL/100 mL of ethanol, 0.12 g of pyrogallol, and 3 mL KOH solution (8.9 mol/L) were added to 0.5 mL of oil, after which the solution was heated for 30 min at 60 °C with a reflux and stirring. Once the saponification process was completed, the content was cooled, transferred to the volumetric flask (50 mL) and topped with ethanol. An aliquot of 5 mL was then transferred to the separation funnel. Volume of 5 mL of cold deionized water and 5 mL of hexane were added as well. The mixture was shaken using vortex for 3 min and 4 mL of the solution was then dried under nitrogen. The dry matter was then dissolved in 4 mL of methanol. The sample was then filtered using a membrane syringe filter and injected into the HPLC system. The mobile phase was 95 mL/100 mL methanol at a flow rate of 1.2 mL/min. Detection was done by the fluorescence detector (Shimadzu RF-535, Japan) operated with the excitation wavelength at $\lambda = 290$ nm and the emission wavelength at $\lambda = 330$ nm. The relative retention time and maximum values of absorption at the given relative retention time were used for identification of tocopherol in the oil samples.

2.3.4. Determination of sterols and squalene

The sterol fraction was determined by GC of the complete unsaponifiable fraction according to the method described by Verleyen (2002). A 7.5 mL volume of internal standard solution (1.33 mg/mL of cholesterol in methylene chloride) was added to