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Fatty acid composition and $\delta^{13}C$ isotopic ratio characterisation of pumpkin seed oil

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ABSTRACT

This study aimed to verify the authenticity and geographical origin of pumpkin seed oil using chemical analysis by gas chromatography (GC) and isotopic characterisation by gas chromatography combustion stable isotope ratio mass spectrometry (GC/C/IRMS) combined with chemometric analysis. Thirty-eight pumpkin seed samples from various parts of the world were collected and authentic oils were prepared. Pumpkin seed oils are highly unsaturated, oleic acid varies from 26.8 to 43.6% and the content of linoleic acid is between 37.2 and 54.9%. The average $\delta^{13}C$ values of the main fatty acids are $-29.2 \pm 1.2‰$, $-30.3 \pm 1.6‰$, $-27.9 \pm 1.7‰$ and $-28.1 \pm 1.7‰$ for $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, respectively. To determine adulteration, rapeseed, sunflower and soybean oil, were added to pumpkin seed oil in varying percentages (1–10%). A 100% correct classification of both geographical and botanical origin was achieved based on the composition and $\delta^{13}C$ values of fatty acids. Principal component analysis (PCA) and regularised discriminant analysis (RDA) analysis gave comparable results.

1. Introduction

Pumpkin (*Cucurbita pepo* L.) seed oil is a commonly used and produced salad oil in eastern Slovenia and southern Austria (Procida et al., 2012). It is valued for its nutritional qualities (high contents of polyunsaturated fatty acids, vitamin E and carotenoids, plant sterols) and beneficial health effects against prostate cancer, macular degeneration and high cholesterol levels (Procida et al., 2012; Fruhwirth and Hermetter, 2008; Murkovič et al., 2004). All these facts including production costs, contribute to the higher price of pumpkin oil compared to other seed oils. Consequently, adulteration with cheaper seed oils is expected (Butinar et al., 2010).

The identification of triacylglycerols by chromatography combined with chemometric methods is suggested as an excellent tool for detecting changes in edible oil composition due to adulteration and for determining geographical origin (Bosque-Sendra et al., 2012; Ruiz-Samblás et al., 2012). Since the natural differentiation in fatty acid composition may conceal the adulteration of edible oils (Spangenberg and Ogrinc, 2001), stable carbon isotope analysis was additionally used in this study.

Stable isotope ratio analysis is a useful method for controlling the geographical origin and botanical authenticity of vegetable oils, especially when mixing C3 and C4 plant oils (Osorio et al., 2014; Meier-Augenstein, 2002). The carbon isotope ratios are related to the processes of atmospheric CO_2 fixation by photosynthesis, in which plant cells differentiate between the heavier ^{13}C and the lighter ^{12}C stable isotope. Other factors that can influence the carbon isotope ratio are plant variety, geographic origin, year of harvest, local atmospheric CO_2 concentration, enzyme levels, plant growth rate, and water-use efficiency (Meier-Augenstein, 2002; Spangenberg and Ogrinc, 2001). Stable isotope analysis has already been successfully used to olive oil (Spangenberg and Ogrinc, 2001; Spangenberg et al., 1998), sesame oil (Kim et al., 2015) and some other edible seed oils (Guo et al., 2010). The results of these studies have shown that $\delta^{13}C$ values together with the fatty acid composition of oils can give a reliable classification of vegetable oils when analysing the data using chemometrics (Spangenberg and Ogrinc, 2001).

Abbreviations: GC/C/IRMS, gas chromatography combustion isotope ratio mass spectrometry; PCA, principal component analysis; RDA, regularised discriminant analysis; FID, flame ionisation detection; IRMS, isotope ratio mass spectrometry; BHT, butylated hydroxytoluene; FAME, fatty acid methyl ester; VPDB, Vienna Pee Dee Bee Belemnite limestone standard.

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To the authors' knowledge, only two studies of pumpkin seed oil authenticity have used stable isotope analysis (Guo et al., 2010; Spangenberg and Ogrinc, 2001), where only two and four pumpkin seed oil samples were used as comparison with a larger group of other edible oil samples. Until recently, adulteration studies of pumpkin seed oil were based on a specific phytosterol determination (Wenzl et al., 2002; Mandl et al., 1999), trace elemental characterisation (Juranovic Cindric et al., 2007) and stereospecific analysis of triacylglycerols, known as the ΔECN42 method (Butinar et al., 2010). Classical analysis of chemical components differs from IRMS (isotope ratio mass spectrometry) in the level of detection – i.e. IRMS is on atomic level while classical methods are on the molecular level.

Knowing the origin of food is becoming important to customers. Among the reasons for this are the specific organoleptic properties associated with regional products, patriotism, and decreased trust in the quality and the safety of imported products (Luykx and Van Ruth, 2008). Despite high instrumental costs and moderate speed of the analysis, IRMS is an effective tool for determining the geographical origin of numerous food products (Luykx and Van Ruth, 2008), including forcamelina oil (Hrastar et al., 2009) and olive oil (Camin et al., 2010; Angerosa et al., 1999; Royer et al., 1999; Spangenberg et al., 1998). For geographic origin of pumpkin seed oil elemental fingerprinting of rare earth elements has been used (Bandoniene et al., 2013; Joebstl et al., 2010).

In this study the determination of fatty acids and their $\delta^{13}C$ values combined with chemometric methods was used to verify the authenticity and geographical origin of pumpkin seed oil. For this purpose, 38 samples from 14 countries were collected. An authenticity check was performed by adding sunflower, rapeseed and soybean oil to authentic pumpkin seed oil, since they are cheaper and belong to different botanical families (Asteraceae, Brassicaceae and Leguminosae). All pumpkin seed oils were pressed from *Cucurbita pepo* subsp. *pepo* (family Cucurbitaceae). Other pumpkin families were not included in this research.

2. Materials and methods

2.1. Samples

Dry pumpkin seeds were collected from local farmers in Slovenia, Croatia and Austria. Twenty pumpkin seed samples were obtained from the U.S. Department of Agriculture, representing other geographical areas (Table 1). Sunflower, rapeseed and soybean oil, used in adulteration experiments, were obtained from various oil producers. Pumpkin oil, extracted from one sample of seeds obtained from a local farmer, was gravimetrically adulterated with sunflower, rapeseed and soybean oil in the following mass percentages from 1% to 10% (1%, 2%, 3%, 4%, 5%, and 10%).

2.2. Chemicals and reagents

All chemicals, sodium chloride, butylated hydroxytoluene (BHT), sodium hydroxide, boron trifluoride (14% methanol solution), hydroquinone, potassium hydroxide and Supelco 37 Component Fatty Acid Methyl Ester (FAME) Mix were purchased from Sigma Aldrich (St. Louis, MO). All solvents (analytical-reagent grade or GC grade), methanol, heptane, hexane, were also from Sigma Aldrich.

2.3. Extraction and methylation

Oilseeds were extracted according to the method of Hrastar et al. (2009). In short, after milling, each sample was extracted twice, once with 0.17 M NaCl in methanol and heptane (1:3) at 80 °C with the addition of BHT (0.01% w/v), and again with heptane at room temperature. The solvent was then removed from the combined extracts by rotary evaporation. The free fatty acids were converted into their FAMEs using methylation procedure as described by Hrastar et al. (2009). In brief, 100 μL of oil were mixed with 0.5 M NaOH in methanol. After heating for 5 min at 80 °C the samples were allowed to cool, before adding 1 mL of a 20% solution of boron trifluoride and 0.5 mL of 0.1% hydroquinone in methanol. After heating and cooling the sample again, 2 mL of 0.73% NaCl solution were added. The FAMEs were then extracted with hexane. After removing the water phase, an aliquot was transferred to two vials, one for gas chromatography-flame ionisation detection (GC-FID) analysis and one for gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) analysis.

2.4. Gas chromatography

For the analysis of FAMEs, we used gas chromatography (GC; Agilent 6890 Series, Hoofddorp, The Netherlands) equipped with a flame ionisation detector and a Select FAME fused silica capillary column (100 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Waldbronn, Germany) with helium (purity 5.0) as the carrier gas (1 mL/min). The injection volume was 1 μL, the split ratio was 1:50, and the inlet and detector temperatures were 220 °C and 250 °C, respectively. The oven programme was as follows: 80 °C for 1 min, 20 °C/min to 160 °C, 1 °C/min to 198 °C, 5 °C/min to 250 °C, held 5 min. Chromatograms were processed using Agilent Chemstation software. FAMEs in oil samples were

Table 1
Minimum, maximum, average, and standard deviation values of the four main fatty acids and $\delta^{13}C$ of bulk oil and pumpkin seed oils from different countries.

Country	Fatty acids (%, w/w)				$\delta^{13}C$ (‰, VPDB)				
	palmitic (16:0)	stearic (18:0)	oleic (18:1)	linoleic (18:2)	bulk oil	palmitic (16:0)	Stearic (18:0)	oleic (18:1)	linoleic (18:2)
Slovenia	10.4–11.9	5.1–7.3	38.2–49.1	31.0–44.5	−28.1 to −26.5	−31.4 to −26.1	−31.5 to −28.5	−30.5 to −27.2	−31.2 to −25.6
($n = 7$)	11.1 (0.5)	6.5 (0.8)	43.6 (4.2)	37.2 (4.9)	−27.1 (0.6)	−29.0 (1.8)	−29.2 (1.5)	−27.9 (1.2)	−27.1 (1.9)
Croatia	11.0–12.6	5.0–6.3	33.4–38.1	42.5–49.2	−28.9 to −27.0	−29.1 to −27.3	−31.7 to −28.4	−28.5 to −25.2	−33.5 to −26.1
($n = 5$)	11.7 (0.8)	5.4 (0.5)	34.8 (2.0)	46.7 (2.5)	−28.0 (0.8)	−28.2 (0.7)	−29.8 (1.2)	−27.0 (1.4)	−28.4 (3.0)
Austria	10.7–12.5	4.5–5.8	15.2–48.9	30.8–65.8	−28.0 to −26.7	−30.2 to −26.5	−30.8 to −27.8	−31.8 to −25.7	−30.5 to −28.1
($n = 6$)	11.6 (0.6)	5.1 (0.5)	26.8 (9.7)	54.9 (8.9)	−27.5 (0.6)	−29.0 (1.3)	−29.0 (1.2)	−28.6 (2.5)	−29.3 (0.9)
Other*	10.4–12.2	3.8–7.0	21.0–46.5	35.5–62.5	−29.0 to −26.7	−31.2 to −27.6	−33.4 to −28.8	−30.9 to −25.2	−30.5 to −25.4
($n = 20$)	11.4 (0.5)	5.5 (0.7)	32.3 (6.2)	48.7 (6.5)	−28.0 (0.6)	−29.6 (1.0)	−31.2 (1.1)	−27.9 (1.6)	−27.9 (1.2)

* Other: $n = 3$ (Russia, Macedonia, Turkey, Spain), $n = 2$ (Syria), $n = 1$ (Netherlands, Greece, Sweden, Poland, Ukraine, Moldova); VPDB, Vienna Pee Dee Belemnite limestone standard.