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PHYSICOCHEMICAL PROPERTIES AND COMPOSITION OF LIPID FRACTION OF SELECTED EDIBLE NUTS

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Abstract

The study presents the characteristics of oil fraction of 8 types of edible nuts available on the Polish market. All tested nuts were characterized with high content of dry matter. Fatty acid and sterol composition was analyzed by GC-MS. Squalene and tocopherol profiles were examined by HPLC with diode array (DAD) and fluorescence detectors (FLDs). The highest level of fat was found in macadamia (75.4 g/100 g) and the lowest in cashew nuts (46.9 g/100 g). Fatty analysis showed that nuts were rich in unsaturated fatty acids. Monounsaturated fatty acids were predominant in most cases, with the exception of Brazilian nuts, walnuts and pine nuts which were richer in polyunsaturated fatty acids. β-Sitosterol was the main sterol of nuts, and its content ranged from 96.9 mg/100 g of oil (in macadamia) to 474.8 mg/100 g of oil (in pistachio). γ-Tocopherol homologue was predominant among its fraction with the largest content determined in pistachio (8.3 mg/100 g of oil) and walnuts (8.6 mg/100 g of oil). The presence of squalene was confirmed in seven types of nuts, and the richest source of it were Brazilian nuts (145.8 mg/100 g of oil). The study proofs the variation of nut oil composition, especially phytosterol and tocopherol content and can be used for better characterization of nuts derived from different geographic areas or cultivars.

Introduction

Nuts are known as a source of bioactive compounds as well as macro- and micro-nutrients. They contain high amounts of fat, with almost ¾ of their fatty acids (FA) being unsaturated. Monounsaturated fatty acids contribute to about 60% of fat energy. These constituents exhibit a cardioprotective effect. Moreover, diets high in monounsaturated fatty acids cause lowering of low density lipoprotein (LDL) levels without effecting the level of high density lipoproteins (HDL), therefore such diets are beneficial in protection against coronary heart disease (CHD) (Shad *et al*., 2012; Win *et al,*. 2011). Even though the role of unsaturated fatty acids in reducing the risk of CHD is clear, some researchers believe that other bioactive compounds of nuts e.g. phytosterols, tocopherols and squalene enhance this effect (Maguire *et al.,* 2004, Ciecierska & Obiedzinski, 2013; Hassan & Ahmed, 2012). Basing on those facts, Food and Drug Administration (FDA) stated in 2003 that consumption of 42 g of nuts daily reduces the risk of CHD (Miraliakbari & Shahidi, 2008).

Phytosterols are compounds chemically very similar to cholesterol, they differ in the structure of the side chain, e.g. β-sitosterol and stigmasterol have ethyl group at C_{24}, and campesterol and brassicasterol contain a methyl group. Another distinguishing feature is the presence of a double bond between C_{22} and C_{23} of the side chain of stigmasterol and brassicasterol. Compatibility of phytosterols to cholesterol cause their hypocholesterolemic effect and their role in antioxidant processes. High consumption of phytosterols (about 2-3 grams per day) leads to the reduction of LDL level in serum (Wolfs *et al.,* 2006).

Vitamin E consists of eight natural compounds: α-, β-, γ-, δ-tocopherols, and α-, β, γ-, δ-tocotrienols. All those tocochromanols are built of a polar chromanol ring and a long prenoid side-chain. The difference between tocopherols and tocotrienols is in the degree of saturation of the hydrophobic chain. Tocotrienols contain 3 double bonds at C_3, C_7 and C_{11} in the side-chain (Strzałka *et al.,* 2009). The most abundant tocochromanols detected in nuts are α-tocopherol and γ-tocopherol (Anwar *et al.,* 2011). Vitamin E is a very efficient antioxidant and has been shown to lower the risk of CHD by inhibiting LDL oxidation (Yang, 2009).

Squalene is a hydrocarbon, based on a thirty-carbon chain with six double bonds. It is a steroid precursor for cholesterol and steroid hormones. Squalene can be produced by animal and plant cells and in plants is converted to phytosterols (Maguire *et al.,* 2004). Squalene is reported to have antioxidant activity and is used as a component of skin-care products. It is mainly obtained from shark liver, fish and also plant sources like olive oil and amaranth oil (Sun *et al.,* 1997).

The aim of the present study was the evaluation of fat composition and chosen bioactive compound contents in most popular nuts: hazelnuts, walnuts, cashew nuts, pecans, macadamias, Brazilian nuts, pines and pistachios. The composition of lipid fraction has been discussed to show differences between cultivars of nuts and also their geographic origin.

Materials and Methods

Raw material: Hazelnuts (from Georgia), walnuts (Ukraine), Brazilian nuts (Peru), pines (China), pistachios (Iran), cashew nuts (USA), pecans (USA), macadamias (Australia) were purchased in a local supermarket in Warsaw (Poland).

Reagents: Reagent grade chemicals: methanol, hexane, potassium hydroxide, ascorbic acid, sulfuric acid and HPLC grade methanol were purchased from POCh (Gliwice, Poland). The derivatisation reagent *N, O*-Bis (trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane) was acquired from Sigma-Aldrich Co. (Poznań, Poland) and pyridine from Riedel-de Haën (Seelze, Germany).

Standards: The following fatty acid methyl esters (FAME) were used: GLC-85 (Nu-Check-Prep, Inc., Elysian, MN, USA) and FAME Mix GLC-90 (Supelco, Bellefonte, PA, USA). Other standards were: 5α-cholestane (Supelco, Bellefonte, PA, USA), squalene

(Sigma-Aldrich Co., Poznań, Poland), and α-tocopherol (Chemie GmbH, Germany).

GC and HPLC columns: DB5ms capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, 5% diphenyl polysiloxane and 95% dimethyl polysiloxane) came from Phenomenex (Torrance, CA, USA); 007-23 column (30 m x 0.20 mm i.d. x 0.25 µm film thickness, (78% cyanopropyl) methylpolysiloxane) from Quadrex (Woodbridge, USA) and Discovery®-C8 (15cm × 4.6mm × 5µm) from Supelco (Bellefonte, PA, USA).

Equipment: The GC instrument equipped with a mass spectrometer (GCMS-QP2010S), HPLC with DAD and FLD detectors (Shimadzu LC20 series) and HPLC with mass spectrometer (LCMS 2010) with Shimadzu SIL-10ADVP autosampler were obtained from Shimadzu Corporation (Shim-Pol A. M. Borzymowski, Poland). Other instruments were: Soxtec 2055 from FOSS Tecator (Höganäs, Sweden) and MPW-210 centrifuge afrom Mechanika Precyzyjna (Poland).

Dry matter content: Samples were chopped in an electric knife mill. Dry matter was determined gravimetrically by drying in 130ºC for 2 hours, after this time samples were dried again at 105ºC to obtain constant weight. Three replicates per each sample were analyzed.

Oil content: Lipid extraction for total fat determination was made in a Soxhlet glass apparatus using hexane as a solvent, 7 grams of dried at 105ºC, homogenized samples were placed in extraction cells and extracted for 1 hour at 155ºC, afterwards the solvent was evaporated. The lipid content was determined gravimetrically. Three replicates per each sample were analyzed.

Oil extraction: Lipid extraction for chemical analysis was also performed with hexane. About 1,5 g of homogenized nuts were extracted by vigorous shaking with 10 ml of solvent for 1 hour. Hexane was then removed from the extract under nitrogen. The obtained oils were kept in tubes and stored in a refrigerator until analyzed (in three replications).

Analytical Methods

FA analysis: Fatty acids were analyzed by GC-MS. The procedure was identical to method described by O'Fallon *et al*., (2007). Briefly, oil samples were subjected to alkaline saponification by addition of 700 µl of 10 $mol \cdot l^{-1}$ KOH in water and then extracted with 5.3 ml methanol. Afterwards 580 µl of 24 $mol \cdot l^{-1}$ H_2SO_4 in water were added. Both procedures were performed in 55ºC (90 minutes) and after transestrification the samples were shaken vigorously for 5 min. Capillary column type 00723 from Quadrex was used to separate FAMEs with helium as a carrier gas at a flow rate of 0.47 ml/min. The injector temperature was 245°C, and the column temperature was programmed as follows: 60ºC for 1 min with subsequent increase to 230ºC at the rate of 4ºC/min and the final temperature was maintained for 10 min. The interface temperature for GC-MS was 225°C, the temperature of ion source was 200°C, ionization energy was 70V. Total ion monitoring (TIC) was used for fatty acid detection (m/z range: 50-400). Identification of fatty acids was made on the basis of mass spectral libraries (NIST 47, NIST 147 and Wiley 175), as well as by comparison of their retention times with those of authentic standards (GLC-85 and FAME Mix GLC-90). Results were expressed as w/w (%) of total fatty acids (in three replications).

Sterol analysis: Sterols were analyzed by GC-MS. Oils samples were saponified following the procedure by O'Fallon *et al*., (2007) using potassium hydroxide, sulfuric acid and 5α-cholestane as an internal standard. Sterols were determined after trimethylsilyl derivatization using N, O-bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane as derivatization agents. Separation of sterols was performed with DB5ms capillary column, the carrier gas was helium (flow rate 1.18 ml/min). Split (50:1) injection at 275ºC was applied. The column temperature was programmed as follows: 65ºC for 2 min subsequently increased to 250ºC at the rate of 15ºC/min, then to 310ºC at the rate of 5ºC/min, final temperature was maintained for 10 min. The interface temperature for GC-MS was 260°C. The temperature of ion source was 250°C, ionization energy was 70eV. Total ion monitoring (TIC) was applied for sterols detection (m/z range: 100-600). Sterols were identified by the comparison of their mass spectral patterns with those of authentic reference compounds. When standards were not available, the components were identified by mass matching using Wiley mass spectra library and published data. Three replicates per each sample were analyzed.

Tocopherols and squalene analysis: Tocopherols and squalene were analyzed by HPLC according to the procedure of Maguire *et al*., (2004). The extracted fat (80 mg) was dissolved in 600 µL of 50% KOH in water (w/v) and 2 ml of ethanolic ascorbic acid. The tubes were kept in a water bath at 70ºC for 30 minutes. Afterwards the tubes were cooled in cold water, and 4 ml of hexane and 1 ml of water were added. The tubes were shaken vigorously for 5 minutes. The upper hexane layer was removed and the extraction repeated with 2 ml of hexane. Combined hexane layers were dried under nitrogen and the residue was dissolved in 2 ml of methanol. Obtained samples were filtered through PTFE filters (0.45 µm pore size). For tocopherol and squalene analysis the samples (15 µL) were injected with the usage of autosampler onto Supelco Discovery®-C8 column. The mobile phase was 90% aqueos methanol at a flow rate of 1.5 ml/min. In a single run tocopherol detection was made using a fluorometric detector (λ_{ex}=295, λ_{em}=330 nm) and squalene was detected using DAD detector at 215 nm. Tocopherols and squalene were identified by the comparison of authentic standard retention times. Three replicates per each sample were analyzed.

Data analysis: Data were analyzed using Statistica 8.0 software. To appraise the significance of the differences between means of particular compound contents, Tuckey's test was used at significance level α=0.05.

Results and Discussion

Dry matter and fat content: The results of dry matter content analysis are shown in Fig. 1. Nuts investigated in this study were hulled and packed into packages. All nuts were characterized by high contents of dry matter from 95.4 (cashew) to 98.2 g/100 g of product (macadamia). In fresh macadamia nuts the water content of about 30% can be achieved. Due to preserving those nuts, they are dried to a moisture content of about 2% (Kaijser *et al.,* 2000). Kornsteiner *et al*., (2006) found that dry matter content in cashews was 96.0% and in macadamias 98.1%, all those results are in accordance with obtained in present study. Also a high content of fat was determined in nuts (Fig. 2), the highest level was found in macadamias (75.4 g/100 g of product) and pecans (73.2 g/100 g) and the lowest in cashews and pistachios (46.9 g/100 g of product). All presented results are similar to those obtained by Kornsteiner *et al*., (2006) and others (Kaijser *et al.,* 2000; Amaral *et al*., 2003, Amaral *et al.,* 2006). Mean fat content in 22 different cultivars of pecans investigated by Toro-Vazquez *et al*., (1997) was 73.9 g/100 g of product. Amaral *et al*., (2003, 2006) observed that the mean content of fat in nineteen hazelnut cultivars grown in Portugal and six cultivars of walnuts was 63.97 g/100 g and 64.58 g/100 g product, respectively. Amaral *et al*., (2006) also found that Portuguese hazelnuts contained more fat than samples from Spain (56.1%), New Zealand (58.4%) or Turkey (59.8%). A lower content in Brazilian nuts (60.8%) than in present study was reported by Ryan *et al*., (2006). Among all analyzed in present study nuts, pines had the lowest content of fat (52.3 g/100 g of product). Kornsteiner *et al*., (2006) observed higher content of fat in pines (67.3 g/100 g product), on the other hand Nergiz & Dönmez (2004) reported that pines contained only 44.9% of fat. Nergiz & Dönmez (2004) suggested that such differences are the results of climatic conditions and species diversity. Comparing fat content of different cultivars of one type of nut other authors also stated that the place of origin and species had strong influence on fat content and its composition (Savage *et al.,* 1997, Tsantili *et al.,* 2010).

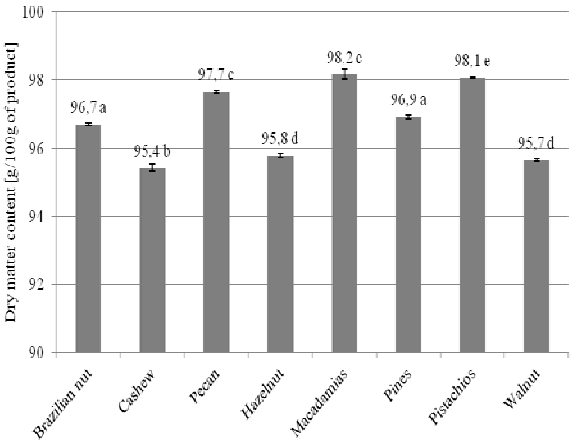

Fig. 1. Dry matter content in nuts. Values with different letters are significantly different (α≤0.05) Results are the mean values of triplicate determinations.

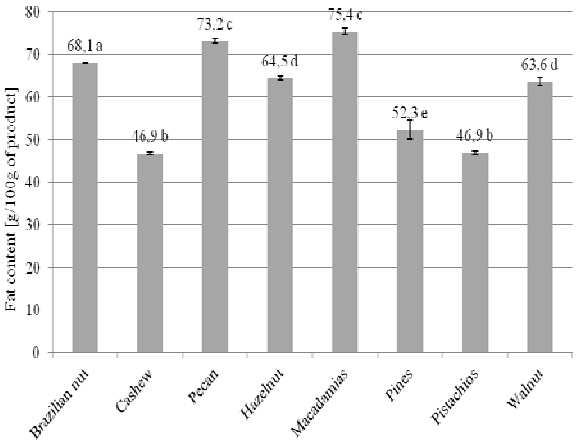

Fig. 2. Fat content in nuts. Values with different letters are significantly different (α≤0.05) Results are the mean values of triplicate determinations.

Fatty acid composition: Fatty acid composition of nuts is presented in Table 1. SFAs in nuts ranged from 6.8 to 28.4% with highest content of palmitic and stearic acid. Predominant group of fatty acids in nuts were monounsaturated fatty acids (MUFAs) (18.68-77.46%), rich in oleic acid. Among polyunsaturated fatty acids (PUFAs), highest contents of linoleic acid and α-linolenic were noted. Total content of PUFAs in nuts ranged from 1.9 to 73.0%.

The main fatty acid in hazelnuts was oleic acid (75.8%), followed by palmitic (10.1%) and linoleic acid (8.4%). Bada *et al*., (2004) and Amaral *et al*., (2006) also observed that oleic acid was predominant in hazelnut oil (78.5-83.5 and 76.7-82.8%, respectively). They reported however, that hazelnuts contained higher than in present study amounts of linoleic acid (10.3 and 9.1%, respectively) and palmitic acid (5.3 and 5.5%, respectively).

Walnuts were characterized by the highest content of linoleic (62.5%) and α-linolenic acid (10.5%). This finding is in agreement with results reported by Bada *et al*., (2010) for fifteen cultivars of walnuts grown in Spain and by Amaral *et al*., (2003) for six cultivars of walnuts from Portugal. Dogan & Akgul presented different results, they analyzed Turkish walnuts, which contained 15.9% of α-linolenic acid and only 52.1% of linoleic acid. At the same time Dogan & Akgul (2005) noted higher content of oleic acid (23.9%) in walnuts in comparison with results presented in this paper (17.8%) and other authors, e.g. 16.3% and 15.3% reported by Amaral *et al*., (2003) and Bada *et al*., (2010), respectively.

Predominant fatty acids of Brazilian nuts were linoleic (38.8%) and oleic acid (31.9%). Brazilian nuts were the richest source of stearic and palmitic acid among tested nuts (17.9% and 9.9%, respectively). The data are in accordance with results determined by Neto *et al*., (2009). Higher content of oleic acid and lower of linoleic acids in Brazilian nuts were noted by Miraliakbari & Shahidi (2008), the values were 37.5 and 35.6%, respectively.

Table 1. Fatty acids composition [%] of nuts.

	Walnut	Hazelnut	Pistachios	Cashew	Pecan	Pines	Maca damias	Brazilian nut
SFA	8.3±0.6a	14.2±1.3b	14.0±0.7b	18.9±1.5c	11.4±1.6b	6.8±0.2a	21.2±0.8c	28.4±0.7d
C 14:0	nd	0.4±0.1a	nd	nd	nd	nd	0.9±0.1b	nd
C 16:0	5.9±0.3ad	10.1±1.2bc	11.7±0.6c	10.3±1.4bc	8.3±1.5bd	4.2±0.2a	12.1±0.5c	17.9±0.7e
C 18:0	2.3±0.3ab	3.72±0.25c	2.3±0.2a	7.9±0.24e	3.2±0.1bc	2.0±0.0a	5.2±0.3d	9.9±0.5f
C 20:0	nd	nd	nd	0.70±0.04a	nd	0.5±0.1a	3.0±0.1b	0.6±0.05a
MUFA	18.7±0.3a	77.5±1.5b	59.9±0.3c	63.5±0.8d	49.4±0.6e	25.4±0.3f	76.9±1.0g	33.2±0.5h
C 16:1 Δ^{9}	nd	nd	0.4±0.05a	nd	nd	nd	15.0±0.3b	0.6±0.1a
C 18:1 Δ^{9}	17.8±0.2a	75.8±1.7b	57.3±0.1c	63.5±0.8d	47.7±0.7e	23.9±0.3f	56.2±0.5c	31.2±0.6g
C 18:1 Δ^{11}	0.9±0.2ab	1.7±0.2cd	2.2±0.2d	nd	1.7±0.4cd	0.6±0.0a	3.7±0.3e	1.4±0.1bc
C 20:1 Δ^{11}	nd	nd	nd	nd	nd	0.9±0.1a	2.0±0.2b	nd
PUFA	73.0±0.8a	8.4±0.3b	26.1±0.7c	17.7±0.7d	39.1±1.3e	67.9±0.5f	1.9±0.2g	38.8±0.6e
C 18:2 $\Delta^{9,12}$	62.5±0.7a	8.4±0.3b	26.1±0.7c	17.7±0.7d	37.6±1.3e	47.1±0.4f	1.9±0.2g	38.8±0.6e
C 18:2 $\Delta^{5,9}$	nd	nd	nd	nd	nd	3.5±0.1	nd	nd
C 18:3 $\Delta^{9,12,15}$	10.5±0.2a	nd	nd	nd	1.5±0.1b	nd	nd	nd
C 18:3 $\Delta^{5,9,12}$	nd	nd	nd	nd	nd	15.5±0.2	nd	nd
C 20:2 $\Delta^{11,14}$	nd	nd	nd	nd	nd	0.4±0.1	nd	nd
C 20:3 $\Delta^{5,11,14}$	nd	nd	nd	nd	nd	1.3±0.1	nd	nd

nd= Not detected. Values within rows with different letters are significantly different ($\alpha \leq 0.05$)
Results are the mean values of triplicate determinations

Pines except for a high content of linoleic acid (47.1%), the only contained Δ5-olefinic acids. These acids were C_{18} Δ5-olefinic acids (18:2 *cis, cis*-5, 9 and 18:3 *cis, cis, cis*- 5, 9, 12-acids) and C_{20} Δ5-olefinic acids (20:2 *cis, cis*-5, 11 and 20:3 *cis, cis, cis*-5, 11, 14 acids). In this group the most abundant was pinoleic acid (18:3 *cis, cis, cis*-5, 9, 12), although its content may vary considerably in different cultivars of pines as it was reported by Wolff *et al*., (2000) and Destaillats *et al*., (2011).

Despite similar physical appearance of pecans and walnuts, those nuts had different fatty acid compositions. It was characterized by high content of oleic acid (47.7%) and linoleic acid (37.6%). Similar results were presented by Miraliakbari & Shahidi (2008). Ryan *et al*., (2006) reported higher content of linoleic acid in pecans -50.3%, and lower amounts of oleic acid -40.6%.

Macadamia nuts' fatty acid profile was specific due to a very low content of PUFAs, the amount of linoleic acid was only 1.9%. On the contrary to most nuts (except for cashews and Brazilian nuts), a high content of palmitoleic acid (15.0%) was determined. Maguire *et al*., (2004) stated that macadamia contained 2% and 10% of palmitoleic and oleic acid, respectively. Studies of Kaijser *et al*., (2000) showed that levels of palmitoleic and oleic acid in 7 cultivars of macadamia nuts grown in New Zealand were much higher: 16.9-33.7% and 40.5-59.0%, respectively.

Fatty acid profiles of tested pistachios and cashew are in accordance with other author results (Miraliakbari & Shahidi, 2008; Ryan *et al*., 2006; Tsantili *et al.,* 2010). In pistachio the reported content of palmitic acid was 8.5-10.2%, stearic 1.4-2.1%, oleic 58.4-61.9% and linoleic 25.1-27.0% (Miraliakbari & Shahidi, 2008; Tsantili *et al.,* 2010). In cashew the amount of palmitic acid was 9.9%, stearic 8.7%, oleic 57.2% and linoleic 20.8% (Tsantili *et al.,* 2010).

Sterol content: Total content of sterols in nuts ranged from 124.1 mg/100 g (macadamia oil) to 563.5 mg/100 g (pistachio oil) (Table 2). In all nut samples β-sitosterol, campesterol and Δ^{5}-avenasterol were identified. Also 4-methylsterols (citrostadienol) and 4,4-dimethysterol (cycloartenol and 24-methylcycloartenol) were detected. Moreover, in some samples stigmasterol, Δ^{7}-avenasterol, Δ^{7}-sitosterol and $\Delta^{5,24}$-stigmastadienol were also identified.

The most abundant phytosterol in nuts was β-sitosterol. Mean β-sitosterol content in walnuts, hazelnuts and Brazilian nuts was about 110.0 mg/100 g of oil. Higher levels of β-sitosterol were detected in cashews and pecans (about 145.0 mg/100 g of oil). As in the case of total sterols, the lowest content of β-sitosterol was noted in macadamia (96.9 mg/100 g of oil) and the highest in pistachios (474.8 mg/100 g of oil).

Table 2. Sterol content in nuts [mg/100 g of oil].

	Walnut	Hazelnut	Pistachios	Cashew	Pecan	Pines	Maca damias	Brazilian nut
campesterol	10.1±2.0^{a}	5.4±0.4^{bd}	24.5±1.0^{c}	11.4±0.2^{a}	6.5±0.6^{b}	26.5±0.7^{c}	6.7±0.2^{b}	2.9±0.7^{d}
stigmasterol	nd	1.3±0.05^{a}	nd	nd	2.6±0.5^{a}	nd	nd	7.9±0.9^{b}
β-sitosterol	111.8±20.3^{ab}	114.3±4.7a^{b}	474.8±7.9^{d}	141.4±6.3^{bc}	152.0±13.0^{c}	224.1±10.0^{e}	96.9±2.q^{a}	111.9±12.5^{ab}
Δ^{5}-avenasterol	14.4±1.5^{a}	6.0±0.3^{bc}	23.1±1.8^{e}	13.6±1.1^{a}	22.0±2.1^{d}	52.3±3.9^{d}	13.0±0.8^{ac}	2.0±0.5^{b}
$\Delta^{5.24}$-stigmastadienol	nd	nd	nd	nd	2.5±0.3	nd	nd	nd
Δ^{7}-sitosterol	nd	nd	nd	nd	nd	nd	2.2±0.6^{a}	2.9±0.4^{a}
Δ^{7}-avenasterol	nd	4.4±1.7^{a}	nd	nd	nd	nd	1.5±0.03^{b}	nd
cycloartenol	27.9±4.9^{a}	nd	13.0±0.5^{b}	7.6±0.6^{bc}	9.4±1.8^{bc}	nd	nd	3.3±0.9^{c}
	nd	nd	14.6±1.4^{a}	4.5±0.3^{b}	nd	nd	1.3±0.4^{c}	nd
	7.0±0.9^{a}	5.3±1.9^{ac}	13.5±1.6^{b}	2.4±0.2^{c}	11.6±2.1^{b}	19.6±1.2^{d}	2.7±0.8^{ac}	4.0±1.9^{ac}
	171.1±29.6^{ab}	136.6±9.0^{ab}	563.5±14.2^{b}	180.8±8.8^{ab}	206.5±20.4^{ab}	322.4±15.8^{ab}	124.1±4.8^{ab}	134.9±17.8^{a}

nd= Not detected. Values within rows with different letters are significantly different ($\alpha \leq 0.05$)
Results are the mean values of triplicate determinations

The second abundant phytosterol in hazelnuts, cashews, macadamias, pecans and pines was Δ^5-avenasterol that ranged between 13.0 and 52.3 mg/100 g of oil. In pistachios, walnuts and Brazilian nuts the second abundant phytosterol was campesterol, cycloartenol and stigmasterol, respectively. Remaining phytosterols (such as Δ^7-avenasterol, Δ^7-sitosterol, $\Delta^{5,24}$-stigmastadienol) were present only in certain types of nuts in smaller amounts that ranged from 1.5 to 4.4 mg/100 g of oil.

The individual sterol contents were generally in agreement with other author results. Maguire *et al*., (2004) determined vary similar β-sitosterol content in walnuts (112.9 mg/100 g of oil), lower content in hazelnuts (99.1 mg/100 g of oil) and higher in macadamia (150.6 mg/100 g). In our study stigmasterol was found in hazelnuts, pecans and Brazilian nuts (1.3 to 7.9 mg/100 g of oil). Maguire *et al*., (2004) noted stigmasterol content only in hazelnuts (3.8 mg/100 g of oil). Sterol profile in macadamia nuts investigated in present study was similar to the results given by Kaijsera *et al*., (2000). Those authors stated that the content of such sterols as β-sitosterol, Δ^5-avenasterol and campesterol was 104.6, 13.3 and 8.7 mg/100 g of oil, respectively. The same results for β-sitosterol in walnuts and hazelnuts as reported in our study were shown by Amaral *et al*., (2003, 2006). β-Sitosterol levels in hazelnuts and walnuts was 112.9 and 109.8 mg/100 g of oil, respectively (Amaral *et al.,* 2003, 2006). The content of sterols is also influenced by the cultivar of nuts. Yorulmaz *et al*., (2009) investigated 17 different cultivars of hazelnuts from Turkey. β-Sitosterol content in their samples ranged from 110.3 to 193.2 mg/100 g of oil, and total sterol content was from 118.0 to 223.9 mg/100 g of oil.

Tocopherol content: In all samples except for macadamia, α- and γ-tocopherols were identified (Fig. 3). γ–Tocopherol content ranged from 3.1 μg/g of oil in hazelnuts to 88.2 μg/g of oil in pistachios. α–Tocopherol predominated in hazelnuts and in pines (226.3 and 96.6 μg/g of oil, respectively). Hazelnuts contained the highest level of α-tocopherol, the most active form of vitamin E. On the other hand pistachios and walnuts had the highest content of γ-tocopherol, 88.2 and 86.5 ug/g of oil, respectively.

Very similar results of γ-tocopherol content in pecans in comparison to our study (70.8 μg/g of oil) showed Villarreal-Lozoya *et al*., (2007). Their results ranged from 72 to 135 μg/g of oil. All presented tocopherol profiles of other nuts were lower that found in literature. α-Tocopherol content in hazelnuts was reported on the level of 350-400 μg/g of oil, and γ–tocopherol content in walnuts - 300-350 μg/g of oil (Maguire *et al.,* 2004; Miraliakbari & Shahidi, 2008; Bada *et al*., 2004, Villarreal-Lozoya *et al.,* 2007). Also higher γ-tocopherol content in pines, pecans, cashew and pistachios was reported by Ryan *et al*., (2006) from 57.2 to 275.4 μg/g of oil. The content of α-tocopherol ranged between 3.6-124.3 μg/g of oil (Ryan *et al*., 2006). The results of tocopherol levels in nuts given by Kornsteiner *et al*., (2006) are slightly different. For instance α-tocopherol content was 157-421 μg/g of oil in hazelnuts and 22-60 μg/g of oil in pines. γ-Tocopherol level was between 124-328 μg/g of oil in walnuts, 100-434 μg/g of oil in pistachios, 64-98 μg/g of oil in pines, and 21-238 μg/g of oil in pecans. Kornsteiner *et al*., (2006) and Wall (2010) did not identify tocopherols in macadamia, contrary to present study.

Tocopherol contents are influenced by varietal differences of nuts, stage of maturity of the plant at harvest, as well as geographic and climatic conditions under which the nuts have been grown (Toro-Vazquez *et al.,* 1997). Different results may also be an effect of various methods used. Delgado-Zamarreño *et al*., (2001) compared the content of vitamin E in seed of sunflower and some nuts, depending on methodology, and found that saponification of samples increased the content of α-tocopherol and decreased other homologue contents.

Squalene content: Squalene was identified in six nut samples (Fig. 4). Squalene content in Brazilian nuts was the highest (1458.2 μg/g of oil) while the lowest was in pistachios (82.1 μg/g of oil) and cashews (116.0 μg/g of oil). In pines, hazelnuts and macadamia squalene content was between 208.2 and 383.4 μg/g of oil.

The literature is not rich in information about squalene content in nuts. Wall (2010) investigated the composition of fat fraction of seven varieties of macadamia nuts, in which squalene was determined on the level of 72.4-171.3 ug/g of oil. Bada *et al*., (2004) reported that squalene content in fifteen different hazelnut samples derived from Spain was between 93.6 and 392.5 μg/g. Maguire *et al*., (2004) also detected squalene in walnuts, macadamia nuts and hazelnuts (9.4, 185.0 and 186.4 μg/g of oil, respectively). The results given by Bada *et al*., (2004) were in agreement with results presented in our study.

Conclusions

The present study documents the variation of nut oils' composition. Our and other author results show that composition of nut lipids can be very different and is influenced by varietal differences, as well as geographic and climatic conditions. These observations can be used to build up a database for further studies on the characterization of nut oils, derived from different cultivars and countries.

Moreover, consumption of nuts may help preventing from inflammations, cardiovascular disorders and cancer development, but on the other hand nuts contain high level of fat, which is not recommended for people suffering from overweight and obesity. Nuts should be often consumed especially by children, due to their high content of n-6 fatty acids (mainly linoleic acid in all types of nuts) and n-3 fatty acids (in walnuts). Although nuts vary in fat composition, all have a beneficial fat profile taking into account growing risk of coronary heart disease in modern population.

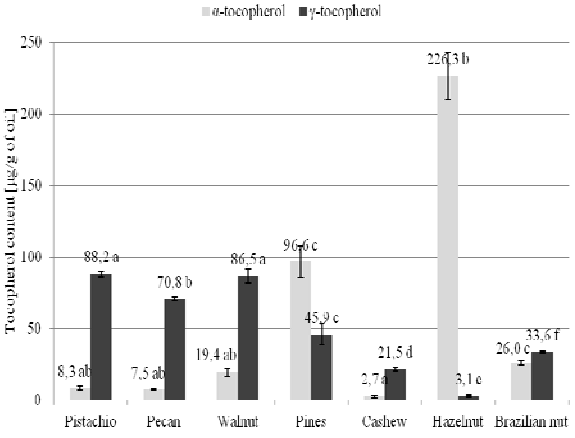

Fig. 3. Tocopherol content in nuts. Values for each compound with different letters are significantly different (α≤0.05) Results are the mean values of triplicate determinations.

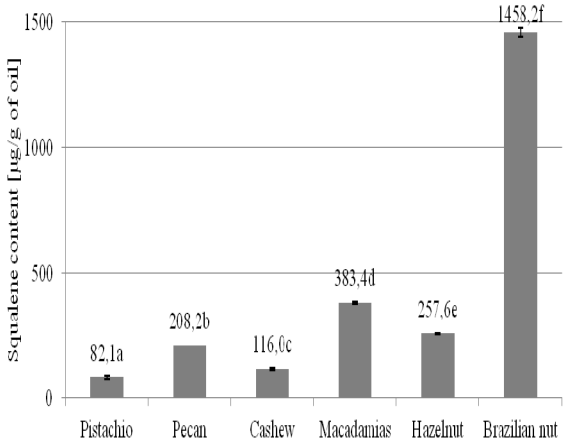

Fig. 4. Squalene content in nuts. Values with different letters are significantly different (α≤0.05) Results are the mean values of triplicate determinations

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