Research Article

Comprehensive sterol and fatty acid analysis in nineteen nuts, seeds, and kernel

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Abstract

Consumption of nuts and seeds is considered to have many beneficial health effects, as lowering cholesterol, incidence of cancer, or enhancement of immunity system. Among the most important lipid constituents of oils present in nuts and seeds are fatty acyls in lipid classes and phytosterols. The aim of the study was to quantify wide profile of fatty acids and phytosterols in 19 nuts, seeds, and kernel commonly available in the Czech Republic. Samples were extracted by the modified Folch procedure. Fatty acid analyses were conducted by gas chromatography. The sterols were derivatized and subsequently measured by HPLC–MS/MS procedure allowing for the separation of isobaric compounds including sterol $\Delta 5$ – $\Delta 7$ isomers. Nuts and seeds contained predominantly oleic (18:1n-9) and linoleic (18:2n-6) acids, rich sources of α -linoleic acid (18:3n-3) were linseed, chestnut, walnuts and hempseed, the last being the best source of stearidonic acid (18:4n-3). The phytosterol analyses revealed majority of β -sitosterol and campesterol with the exception of pumpkin and melon seeds, rich in $\Delta 7$ sterols, which were separable from $\Delta 5$ isomers. Correlation of sterols and fatty acids content revealed positive correlation between monounsaturated/saturated fatty acids (16:1n-9, 18:1n-9, 20:1n-9, 20:0) and stigmasterol and negative correlation between fatty acids (16:1n-9, 18:1n-9) and sitostanols. In summary, our study revealed the wide profile of fatty acids and phytosterols between different nuts and seeds. Further studies are needed to confirm the positive effect of each nut and seeds for health.

Keywords Sterols · Fatty acids · Oils · Nuts · HPLC–MS

Mathematics Subject Classification 92C80

1 Introduction

Nuts and seeds are one of the most important sources of unsaturated fatty acids (FA) and phytosterols in our diet. Nowadays, their consumption is claimed to have many beneficial health effects as lowering cholesterol, incidence of cancer, protect against coronary heart disease, insulin resistance, metabolic syndrome, or enhancement

of immunity system [1–5]. Analysis of phytosterols and FA in various seeds and nuts could contribute to elucidate which of them has a protective health effect.

Sterols are an important part of either plant and animal liquids and tissues. Phytosterols are found in plants and cannot be synthesized in humans [2]. Their structure resembles cholesterol molecule, apart from the side chain differences. In the plant species, there were identified

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more than 200 various kinds of phytosterols [6]. The most abundant sterols are β -sitosterol, campesterol, and stigmasterol, which represent up to 98% of total sterols [7]. Further minor sterols, divided according to number of carbons, are C30 sterols—lanosterol, amyirin, lupeol, C29 sterols—fucosterols, avenasterols, β -sitosterol, and sitostanol, C28 sterols—brassicasterol, crinosterol, campesterol, stigmasterol, as well as C27 sterols—cholesterol, lathosterol and desmosterol [3, 6, 8]. Their content differs in connection with their location [7]. Phytosterols are present in plants as esters, free alcohols, or glycosides [7].

In the plant cell membranes, the role of phytosterols is analogous to that of cholesterol in animals [9]. Sterols play an essential role in fluidity and permeability of membrane, even in the synthesis of steroid hormones [7]. Phytosterols also serve as substrates for brassinolids which protect plants from both pathogen and oxidative stress [10]. Phytosterols are completely saturated phytosterols present in higher amounts in, e.g., cereal species [11].

On the other hand, level of sterols, especially cholesterol, in human body was positively correlated with the occurrence of some types of cancer [12, 13], coronary heart disease, metabolic syndrome, and enhancement of immune function [6, 13, 14]. The effect of supplementation by diet rich in phytosterols on lowering cholesterol absorption in intestine was previously described [15]. Phytosterols compete with free cholesterol in the intestine by reduction in its solubility and subsequently absorption. As surrogate markers of cholesterol absorption, campesterol, and β -sitosterol are measured in blood together with markers of cholesterol synthesis (lathosterol). Interestingly, lathosterol is an intermediate of synthesis of cholesterol from lanosterol, but also a less abundant phytosterol in nuts and seeds [16].

The nut and seed oils contain low level of saturated FA (range 4–16%), and almost half of the total fat is unsaturated FA [17]. The fatty acids are usually bound in triacylglycerols of oils and serve also as a carbon/energy source [18]. Unsaturated FA is known to have pro- and anti-inflammatory actions. They play a role also in the regulation of gene expression, immune response, enzymatic activity, and gluconeogenesis [19, 20].

Sterols are measured by liquid chromatography combined with various types of detection as mass spectrometry or UV/VIS [5, 21]. Mass detection of isobaric compounds relies most often either on their different chromatographic properties or specific fragmentation of the parent molecule. The content of FA in nutrition supplements is analyzed by gas chromatography equipped with flame ionization detector [22–24]. The studies concerning both FA and phytosterol composition of various nuts and seeds are scarce [25]. The aim of the study was to analyze FA as well as sterol content in samples of

selected commercially available nuts and seeds on the market in the Czech Republic and to develop HPLC–MS method for the separation of isobaric sterols.

The knowledge on the content of various dietary components is important for the nutritionists, and nut/seeds being increasingly used for various purposes due to their high and variable lipid content. In this study, we analyzed FA profiles ranging from medium to very long polyunsaturated FA and proved that there are types of nuts/seeds with unique FA composition. Moreover, the analyses of content of sterols, minor lipid components in these samples, revealed that there are unusual sterols in pumpkin/melon seeds.

2 Methods

2.1 Chemicals and standards

Standards of campesterol, β -sitosterol, α -amyirin, brassicasterol, fucosterol, desmosterol, stigmasterol, 7-dehydrocholesterol, cholesterol, lathosterol, lanosterol, epicoprostanol as internal standard (IS), ursolic acid, erythrodiol, and lupeol were purchased from Sigma-Aldrich (USA). Standard mixture of FA methyl esters (FAME) (GLC-566) was obtained from Nu-Chek (Elysian, U.S.A.). Other chemicals and solvents of chromatographical purity were from Sigma and Chromservis (Czech Republic).

2.2 Sample preparation

We collected 11 kinds of nuts (Brazil nut, cashew, chestnut, coconut, hazelnut, macadamia, peanut, pecan, pine, pistachio, walnut), 7 kind of seeds (almond, hemp, linseed, musk melon, pumpkin, sesame, sunflower), and apricot kernel (details are presented in supplementary material Table S1). Samples were extracted at least in triplicates by the following modified Folch procedure [26].

The sample was homogenized and aliquot of circa 20 mg was weighed into the glass extraction test tube, 2.5 ml of methanol with butylated hydroxytoluene (BHT) as antioxidant added and vortexed (Ultra Thorax T 10b (IKA®, Germany)). Then, 2.5 ml of dichloromethane was added, vortexed, and the blades rinsed with the same volume of dichloromethane. The suspension was filtered, the filter rinsed with 2 × 1 ml of methanol/dichloromethane (1/2 v/v) mixture and water added to ensure the separation of phases. After centrifugation (10 min at 720×g), the lower phase was aliquoted and evaporated under nitrogen at 40 °C, weighed, and stored at –20 °C until further analyses.

2.2.1 Analyses of sterols

For calibration and validation purposes, we used mixed standards in absolute concentrations ranging from 0.625 to 50.000 ng/ml. Validation parameters are presented in Supplementary Table S2. We adapted the method of Jonker [27] for acid hydrolysis with subsequent saponification of samples. To the 1 mg aliquot of the lipid extract (or standards), the internal standard (0.5 µg of epicoprostanol in 10 µl heptane) was added, hydrolyzed with 2 ml of 0.5 N HCl in methanol under nitrogen atmosphere in dark at 60 °C for overnight. Then, 2 ml of 1 N KOH in methanol was added, mixed, purged with nitrogen, and left for 1 h at 65 °C in dark. The liberated sterols were extracted twice (if formation of foams complicated the extraction, lesser part of the aliquot had to be processed) with 2.5 ml of hexane and evaporated residue derivatized to carbamates according to Ayciriex [28]. The residue was resuspended in 0.2 ml of 4-(dimethylamino)phenyl isocyanate solution (10 mg/ml in dichloromethane), vortexed, then 30 µl of triethylamine was added and left in dark at 65 °C for 2 h. The reaction was stopped with 150 µl of phosphate buffer (pH=8) and carbamates extracted with 3 ml of hexane, the hexane layer was removed and evaporated under nitrogen at 40 °C. The sample was dissolved in 2 ml of acetonitrile/isopropanol mixture (10/1 v/v) and 5 µl injected into the LC–MS/MS platform. The LC–MS/MS was equipped by HPLC system (Dionex Ultimate 3000, Dionex Softron GmbH, Germany) with Hypersil GOLD column type C18 (USP cat. L1) (150 × 2.1 mm, 3 µm, Thermo Scientific, USA) and Security Guard column (Phenomenex, USA), coupled to triple quadrupole mass spectrometric detector TSQ Quantum Access Max (Thermo Scientific, USA). Binary mobile phase gradient consisted of acetonitrile/methanol/water (40/40/20 v/v/v) mixture with 0.1% acetic acid (A) and acetonitrile/methanol/water (45/45/10 v/v/v) mixture with 0.1% acetic acid (B) at constant flow rate of 0.3 ml/min. The column chamber was set to 50 °C. The initial conditions (100% A) were continuously shifted to 100% B in 20 min, kept for 19 min, and then shifted back to 100% A with 11 min washout period. To reduce the contamination of the detector, the flow from HPLC was allowed to the detector from 5 to 45 min only.

As an MS detector, TSQ Quantum Access Max with HESI-II probe (Thermo Fisher Scientific, Inc., USA) was used to operate in SRM mode. The heated HESI-II probe for MS detector was run under the following conditions: spray voltage +4300 V, vaporizer temperature 350 °C, sheath gas 34 arbitrary units (au), auxiliary valve flow 15 au, ion sweep gas pressure 1.2 au, and capillary temperature 320 °C. Tube lens voltage varied depending on the compound analyzed. Skimmer offset voltage was not used. The tuning of MS/MS transitions was performed by combined

infusion of standards (10 mg/L in mobile phase, 20 µl/min) and the mobile phase (300 µl/min), and collision gas (Ar) pressure was set to 1.0 mTorr. The SRM is presented in Supplementary Table S3.

2.2.2 Analyses of fatty acids

An aliquot of 5 mg of lipid extract was analyzed from one single batch. Samples were converted directly to FAME as previously described [29] and analyzed by capillary gas chromatography using DB23 60 m × 0.25 mm (0.25 µm d.f.) column. The evaporation step was omitted in the samples of coconut oil, as the resulting hexane phase was directly injected into GC without evaporation to prevent loss of short-chain FAMES.

2.3 Statistics

Data were processed by Xcalibur® software ver. 2.2 (ThermoFisher Scientific, U.S.A.) Limits of quantification (LOQ) were determined using signal/noise approach. Concentrations of sterols and FA in each sample are presented as a mean in mol % ± standard deviation (SD). The hierarchical clustering and univariate analysis were performed using MetaboAnalyst 4.0. Pearson's coefficient of correlation was used to identify a linear relationship.

3 Results and discussion

The method for analysis of sterols in different matrices was developed including validation. Furthermore, sterols and FA were analyzed in 19 various kinds of nuts, seeds, and kernel.

The method for 15 different sterols (7-dehydrocholesterol, cholesterol, lathosterol, campesterol, β-sitosterol, stigmasterol, lupeol, α-amyrin, erythrodiol, ursolic acid, desmosterol, brassicasterol, lanosterol, fucosterol, and cholestanol) was optimized and validated by using standards. The carbamates for all analyzed compounds produced stable parent ion signal in positive mode $[M + H]^+$ that gave product ions formed from the derivatization agent (m/z 181 and 166), see Supplementary Fig. S1 for example. As the tuning of these compounds gave different maxima for both tube lens and collision energies, we used SRM approach instead of parent ion scans. The recoveries of known spiked amounts of sterols ranged from 80 to 116% and no matrix effects were found. Analytical parameters of analyzed sterols are presented in Supplementary Table S2 and examples for chromatographic separation in Supplementary Figs. 4a–4d.

Limits of quantification for sterols varied from 0.625 to 3.500 ng/ml. LOQ for lupeol, erythrodiol, and ursolic

acid (3.500 ng/ml) was too high to use this method for analysis of these sterols in seed and nut samples. On the other hand, $\Delta 7$ -stigmasterol, $\Delta 7,25$ -stigmastadienol, $\Delta 7$ -stigmastenol, and $\Delta 7$ -campesterol, for which the commercial standards were not available, were detectable in samples. For these sterols, due to the lack of commercial standards, the response factors were set at unity for quantitative purposes.

We grouped the analyzed samples to obtain more illustrative results. The state in which the nuts or seeds were provided did not affect the amount of FA and sterol content (data not shown). Tables 1 and 2 present the content of sterols and FA in nuts, seeds, and kernel.

3.1 The sterol content of analyzed samples

The majority of papers define the total content of phytosterols as the sum of β -sitosterol, campesterol, and stigmasterol only [8]. The sterol composition of the seeds and the nuts are shown in Table 1 and graphic presentation by heat map in Fig. 1. The total content of phytosterols was the highest in sesame seeds 6.59 mg/g oil and pistachio 5.85 mg/g oil contrary to low sterol contents in pumpkin seeds and coconut oil which were found to have low sterol content of 1.57 mg/g oil (0.31 mg/g oil, respectively). It is known that pistachio contains high amounts of the total phytosterols contrary to other tested nuts and seeds [5] and the content of the total phytosterols in coconut oil was reported to be as low as 0.30–0.33 mg/g [30].

The predominant sterol in seeds and nuts was β -sitosterol, whose content varied between 55 wt% of sterols (linseed) and 86 wt% of sterols (pistachio) (Table 1). Exceptions were pumpkin/melon seeds, where β -sitosterol was a minor constituent (cca 9 wt% of sterols). The major occurrence of β -sitosterol is in agreement with other publications [5, 31–34]. β -sitosterol plays an important role in fluidity of plant cell membranes, and its accumulation in seeds and nuts reflects phytosterol importance in cellular differentiation and proliferation [35].

The next mostly occurring sterols were stigmasterol and campesterol. Sesame seeds were the richest stigmasterol source (8 wt% of sterols). Stigmasterol content varied from 8 wt% of sterols (sesame seeds) to 1.4 wt% of sterols (Brazil nuts). Stigmasterol was described as a potential preventive compound against osteoarthritis and inhibitor of several pro-inflammatory factors [36]. The levels of campesterol were highest in linseed group (11 wt% of sterols), sesame seeds (8 wt% of sterols), and peanuts (7 wt% of sterols). Some authors [37, 38] reported even higher proportions of

campesterol in linseed (up to 25 wt%). On the other hand, the content of campesterol was less than 1 wt% of sterols in pumpkin seeds and Brazil nuts. Cashews, macadamias, almonds, pecans, pine nuts, pistachios, and walnuts showed a higher average content of stigmasterol than campesterol and sitosterols. In accordance with these results, Phillips et al. reported that pistachio and sesame seeds contained a majority of β -sitosterol with high content of campesterol in sunflower seeds and linseed and considerable amounts of stigmasterol in sunflower seeds. Moreover, they described low content of campesterol in Brazil nut and stigmasterol in hazelnuts and considerably low content of β -sitosterol in pumpkin seeds [34]. The high content of β -sitosterol in majority of analyzed nuts and seeds and its favorable effects on colon cancer can advocate the nuts/seeds as a part of a healthy diet, while campesterol, usually a minor phytosterol, had no effect [13, 39].

Another important group of phytosterols are $\Delta 7$ sterols, e.g., $\Delta 7$ -stigmastenol, $\Delta 7$ -avenasterol, spinasterol, $\Delta 7,22$ -stigmastadienol, and $\Delta 7,22,25$ -stigmastatrienol. For distinguishing of these sterols from their $\Delta 5$ isobaric isomers, we developed a method separating critical $\Delta 5/\Delta 7$ pairs. Separation of the lathosterol/cholesterol pair (see Supplementary Figure S4a for example) is particularly important for analysis of animal matrices, as lathosterol can be used as a surrogate marker for cholesterol biosynthesis. In our study, we used only plant matrices, where the cholesterol content is negligible and not highly abundant to lathosterol as in animals. Other $\Delta 5/\Delta 7$ pairs represent campesterol/ $\Delta 7$ -campesterol, β -sitosterol/ $\Delta 7$ -stigmastenol, and avenasterol/fucoesterol, which are present in plant matrices (see Supplementary Figures S4c–d for examples). Some optical isomers could be also separated: as an internal standard, we used epicoprostanol (5 β -cholesta-3 β -ol) which was baseline separated (see Supplementary Figure S4a) from cholestanol (5 α -cholesta-3 β -ol). Other R/S isomers, such as brassicasterol/crinosterol or campesterol/24-epi-campesterol, were seen as coeluting compounds. Derivatization of sterols for HPLC–MS-based methods usually increases the sensitivity of the detector [28] thus considerably decreasing column load. Underivatized sterol molecules are not easily ionizable with electrospray in some instrumental platforms [40] and we observed signal intensity of free sterols lower by several orders of magnitude using our HPLC–MS platform in comparison to sterol carbamates (data not shown).

Pumpkin seeds differ from other nuts and seeds by characteristic content of $\Delta 7$ -phytosterols. The concentrations of $\Delta 7$ sterols were 5–10 times higher than in other tested

Table 1 Sterol content in analyzed samples

	Peanut	Pumpkin/ melon	Cashew	Coconut	Chestnut	Hempseed	Linseed	Hazelnut	Macadamia	Almond	Apricot	Brazil nut	Pecan	Pine nuts	Pistachio	Sesame	Sunflower	Walnut
Oil content (g/100 g sample)	45.8±3.8	40.5±3.7	41.5±5.6	50.7±0.8	2.2±1.6	44.4±5.3	38.4±1.6	56.1±10.0	64.9±4.6	48.8±2.2	40.8±0.3	61.2±1.4	67.9±0.9	54.3±5.5	50.4±3.8	50.3±1.1	48.6±8.0	65.3±4.5
Total sterols (mg/g oil)	2.25±0.58	1.57±0.07	2.34±0.38	0.31±0.07	4.54±0.86	2.82±0.26	3.59±0.61	2.30±0.49	2.46±0.38	3.77±0.52	4.62±0.35	1.80±0.61	1.94±0.21	4.38±1.04	5.86±2.22	6.59±1.83	3.70±0.89	2.02±0.17
Sterol content (mg/100 g sample)	103±26	64±3	97±16	15±3	10±2	124±12	138±23	129±27	160±25	184±25	188±14	110±37	132±14	238±56	295±111	331±92	180±43	132±11
Lanosterol	0.3±0.1	<0.2	0.4±0.3	11.8±2.2	0.9±0.2	0.4±0.4	4.0±0.5					0.4±0.4	0.5±0.4		0.5±0.3	0.3±0.3	0.8±0.3	1.0±1.1
Amyrins	0.8±0.5	1.7±1.6	0.4±0.4	1.5±0.3	2.5±0.1	1.0±0.3	1.3±1.5	1.0±0.4	0.8±0.7	0.8±0.2	3.1±0.4	5.0±3.8	1.9±0.6	1.3±1.5	1.3±1.1	0.9±0.5	2.9±3.0	1.0±0.9
Sitosterols	2.7±0.8	3.0±1.9	3.1±0.7		3.4±0.7		2.3±0.9	4.8±1.2	2.5±0.7	3.9±0.4		6.3±0.3	2.9±0.5	3.1±1.5	3.4±0.4	2.4±0.1	2.6±0.3	3.1±1.3
β-sitosterol	70.6±3.5	9.0±2.5	83.8±2.9	63.2±0.8	69.9±0.1	79.7±0.1	55.4±8.2	85.5±0.7	82.5±5.1	83.0±2.0	79.9±0.2	78.1±3.1	80.6±1.8	74.3±6.7	85.9±4.6	73.0±2.4	73.0±6.9	81.2±1.7
Stigmaterol	5.4±0.8	5.7±2.5	3.7±0.9	11.6±0.9	16.1±0.0	3.4±0.9	6.8±1.5	1.8±0.4	5.4±2.9	5.8±1.0	0.2±0.1	1.4±0.3	7.2±0.8	7.5±2.0	4.0±1.1	8.0±1.6	3.1±1.0	4.4±1.6
Fucosterols ^a	1.4±1.1	11.2±13.0	0.3±0.6		0.3±0.4		1.0±1.8	<0.2	1.2±1.1	2.5±0.6	<0.2	3.9±0.1	0.9±0.3	1.5±2.6	0.7±1.3	0.9±1.5	<0.2	0.9±0.9
Avenasterols ^a	8.1±1.6	9.3±10.4	<0.2	<0.2	0.5±0.7	2.0±0.3	4.5±2.2	0.6±0.1	<0.2	0.6±0.8				0.7±0.2	0.5±0.8	4.3±0.7	6.2±1.1	<0.2
Δ7-stigmaterol ^b		16.2±18.8	<0.2															
Δ7Δ25-stigmasta dienol ^b		28.9±3.7	<0.2		<0.2		<0.2			0.3±0.2			<0.2		0.5±0.7		1.2±1.5	
Δ7-stigmastenol ^b		5.9±8.4			<0.2			0.5±0.7		<0.2		0.3±0.5		0.6±1.0			3.3±4.6	
Campesterol	7.4±2.0	0.9±0.5	2.9±1.3	5.0±1.7	5.8±0.3	7.3±0.6	11.0±3.3	2.5±0.4	2.5±0.8	1.5±0.4	2.3±0.0	0.8±0.2	2.1±0.4	5.0±2.3	1.8±0.5	8.1±0.8	4.1±1.0	2.1±0.9
Δ7-campesterol ^b		1.1±1.3															0.3±0.6	
Brassicasterol +crinosterol	0.3±0.2	0.6±0.7	<0.2	<0.2	<0.2	<0.2	1.2±0.4				<0.2		<0.2	<0.2		0.7±0.5	<0.2	<0.2
Other sterols ^c	2.9±1.8	6.6±2.2	5.0±2.7	6.7±1.2	4.2±0.6	2.1±1.0	12.4±15.7	3.1±2.0	4.8±3.7	1.5±1.1	14.3±0.3	3.8±1.5	2.6±1.3	5.9±3.3	1.4±0.8	1.4±0.5	2.3±1.0	6.1±5.4
mg/100 g sample																		
β-sitosterol	72.7±3.6	5.7±1.7	81.4±2.8	9.9±0.1	7.0±0.1	99.8±0.1	76.3±11.2	110±1	132±8	153±4	151±0.3	86.0±3.4	106±2	177±16	254±14	242±8	131±13	107±2.2
Campesterol	7.7±2.0	0.6±0.3	2.8±1.2	0.8±0.3	0.6±0.1	9.1±0.7	15.2±4.6	3.2±0.5	4.1±1.3	2.7±0.7	4.3±0.0	0.9±0.2	2.7±0.5	11.9±5.4	5.2±1.4	26.9±2.6	7.4±1.8	2.7±1.2
Δ7-sterols		32.8±20.1			0.3±0.1			0.8±0.9	0.2±0.0	0.7±0.4		0.3±0.6	0.1±0.0	1.4±2.4	1.5±2.1		8.4±11.6	

The data are presented as mean in weight%±SD format. Only relevant sterols are listed

^aThese peaks contain Δ5, Δ7, and Δ8 isomers due to acidic hydrolysis

^bIdentification of these sterols was not verified by standards

^cOther minor components analyzed include C28 sterols as campestanol, C27 sterols as cholesterol, lathosterol, desmosterol, etc.; Blank space—only trace amounts of sterol (0–0.05 wt%), < 0.2—negligible content of sterol (group average 0.05–0.20 wt%)

Table 2 Fatty acids content in analyzed samples

	Peanut	Pump- kin/ melon	Cashew	Coconut	Chestnut	Hemp- seed	Linseed	Hazelnut	Macada- mia	Almond	Apricot	Brazil nut	Pecan	Pine nuts	Pistachio	Sesame	Sunflower	Walnut
8:0				6.0±0.1														
10:0				5.2±0.2														
12:0				41.5±1.4														
14:0		<0.2		155±0.7	12±0.1		<0.2		0.6±0.3	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2		<0.2	
16:0	10.4±3.5	12.2±1.6	9.8±2.3	19.7±0.5	14.6±0.3	5.6±0.5	5.6±1.5	5.9±0.4	8.4±0.8	6.6±0.3	3.8±0.1	14.9±1.9	6.7±1.0	6.3±0.9	10.4±2.2	9.3±1.5	6.1±1.5	7.6±1.3
16:1n-7	<0.2	<0.2	0.4±0.1	3.5±0.1	0.4±0.0	<0.2	<0.2	0.4±0.5	15.9±4.5	0.5±0.0	0.5±0.1	0.4±0.2	<0.2	<0.2	1.4±0.6	<0.2	<0.2	<0.2
18:0	3.9±1.2	7.2±1.4	8.8±1.6	3.1±0.1	2.3±0.0	3.9±0.4	4.3±0.9	2.6±0.8	3.5±0.9	1.9±0.5	0.8±0.1	9.9±1.3	3.0±0.2	3.4±0.7	1.3±0.2	6.4±0.5	4.1±0.7	2.7±0.5
18:1trans	0.7±0.3	0.5±0.1	0.7±0.2	0.5±0.2	0.4±0.1	<0.2	0.4±0.1	0.8±0.3	0.6±0.1	0.8±0.3	<0.2	0.5±0.1	0.7±0.2	0.6±0.1	0.7±0.3	0.6±0.1	0.4±0.3	0.3±0.1
18:1n-9	58.8±12.8	35.8±9.0	62.4±6.7	4.2±0.3	17.9±0.0	16.2±6.2	20.1±5.3	77.9±8.1	59.0±5.7	73.6±2.6	67.6±0.2	39.0±7.6	61.0±7.4	33.1±5.6	55.9±5.8	44.2±4.7	29.5±17.9	18.0±3.2
18:1n-7	0.6±0.0	0.8±0.1	0.4±0.1	<0.2	1.7±0.1	0.8±0.1	0.8±0.2	1.2±0.2	3.6±1.3	1.4±0.2		1.1±0.3	1.2±0.1	0.7±0.1	2.3±0.0	1.1±0.1	0.7±0.1	1.0±0.3
18:2n-6	17.9±8.6	42.2±9.4	16.3±4.2	0.3±0.1	49.7±0.2	54.7±4.1	16.7±1.2	10.7±8.6	2.5±1.5	14.7±2.4	26.8±0.1	32.7±3.9	25.7±7.8	45.3±2.2	26.7±8.4	36.7±6.0	57.2±17.4	57.1±1.7
18:3n-6							<0.2		<0.2					4.9±8.5 ^a				
20:0	1.7±0.4	0.6±0.1	0.7±0.1	<0.2	0.3±0.0	0.9±0.1	<0.2	<0.2	2.6±0.9	<0.2		0.3±0.1	<0.2	0.4±0.1	<0.2	0.7±0.1	0.3±0.1	<0.2
18:3n-3	<0.2	<0.2	<0.2		9.8±0.1	16.2±4.0	51.2±7.4	<0.2	<0.2		<0.2	0.7±0.5	0.9±0.3	2.9±2.9	0.3±0.1	0.4±0.2	0.4±0.6	12.8±2.8
18:4n-3						0.5±0.5												
20:1n-9	1.7±0.4	<0.2	<0.2		0.8±0.0	0.4±0.1	<0.2	<0.2	2.0±0.4	<0.2	<0.2	<0.2	0.3±0.1	1.3±0.3	0.6±0.1	<0.2	<0.2	<0.2
20:2n-6		<0.2	<0.2		<0.2	<0.2	<0.2	<0.2	<0.2			<0.2	<0.2	0.8±0.1	<0.2	<0.2	<0.2	<0.2
22:0	3.0±0.6	<0.2	<0.2		0.7±0.1	0.4±0.1	<0.2	<0.2	0.7±0.2	<0.2		<0.2	<0.2	<0.2	<0.2	<0.2	0.8±0.1	<0.2
24:0	1.7±0.4	<0.2	<0.2		<0.2	<0.2	<0.2	<0.2	<0.2									
ΣSFA	20.7±5.7	20.3±2.5	19.5±3.4	91.2±0.4	19.3±0.1	10.9±0.7	10.4±2.3	8.7±1.1	15.9±1.8	8.9±0.5	4.7±0.1	25.4±3.2	9.9±1.1	10.3±1.7	12.0±2.4	16.6±1.9	11.5±2.4	10.5±1.0
ΣMUFA	60.6±13.2	36.8±8.9	63.3±6.7	7.9±0.4	20.8±0.1	17.5±6.3	21.1±5.4	79.6±7.8	80.6±0.7	75.6±2.5	68.2±0.1	40.6±7.1	62.6±7.5	35.2±5.6	60.1±6.3	45.5±4.8	30.4±17.8	19.2±3.4
ΣPUFA-n-6	17.9±8.6	42.3±9.4	16.4±4.2	0.4±0.1	49.8±0.3	54.8±4.2	16.9±1.1	10.8±8.7	2.8±0.5	14.7±2.4	26.8±0.1	32.8±3.9	25.8±7.8	51.0±10.0	26.8±8.5	36.9±6.1	57.3±17.3	57.2±1.7
ΣPUFA-n-3	<0.2	<0.2	<0.2		9.8±0.1	16.7±4.4	51.2±7.3	<0.2	<0.2		<0.2	0.7±0.5	0.9±0.3	2.9±2.9	0.3±0.1	0.4±0.2	0.4±0.6	12.8±2.8
TFA	0.7±0.3	0.5±0.1	0.7±0.2	0.4±0.2	0.4±0.1	<0.2	0.4±0.1	0.8±0.3	0.6±0.1	0.8±0.3	<0.2	0.5±0.1	0.7±0.2	0.6±0.1	0.7±0.3	0.6±0.1	0.4±0.3	0.3±0.1
g/100 g sample																		
ΣSFA	9.5±2.9	8.6±1.5	8.3±1.6	47.2±0.4	0.5±0.3	5.0±0.9	4.1±0.8	5.2±1.4	10.6±1.8	4.6±0.2	2.1±0.1	16.1±2.3	7.2±0.9	5.9±1.3	6.5±1.8	8.7±1.2	5.7±1.6	7.1±0.5
ΣMUFA	27.5±5.3	14.9±4.6	26.2±5.6	3.4±0.2	0.5±0.3	7.9±3.9	8.0±1.7	44.9±11.3	52.2±3.8	36.6±1.4	27.7±0.3	24.5±3.8	42.2±4.9	19.0±3.9	30.1±5.4	22.7±2.9	15.3±12.4	12.5±2.5
ΣPUFA-n-6	8.4±4.1	16.7±2.1	6.6±1.4		1.1±0.8	24.1±0.9	6.4±0.7	5.4±2.9	1.7±1.0	7.2±1.4	11.0±0.1	19.9±2.8	17.5±5.3	27.4±4.9	13.2±3.5	18.4±2.7	27.1±7.1	37.1±2.9
ΣPUFA-n-3	<0.2	<0.2	<0.2	<0.2	<0.2	7.3±1.2	19.7±3.4	<0.2				0.4±0.3	0.6±0.2	1.7±1.8	<0.2	<0.2	0.2±0.3	8.4±1.9
TFA	0.3±0.1	<0.2	<0.2					0.4±0.2	0.4±0.1	0.4±0.1		0.3±0.1	0.5±0.1	0.3±0.1	0.4±0.1	0.3±0.1	<0.2	<0.2

The data are presented as mean in weight%±SD format. The shorthand notation for fatty acids: number of carbon atoms; number of double bonds, n—number of carbon atoms from methyl end to the nearest double bond; Σ, sum; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA n-6, polyunsaturated fatty acids of n-6 family; PUFA n-3, polyunsaturated fatty acids of n-3 family; TFA, trans unsaturated fatty acids. Blank space—only traces of fatty acids (0–0.05 wt%), <0.2—negligible content of fatty acids (group average 0.05–0.20 wt%)

^aContains pinolenic acid

Fig. 1 Heat map evaluating sterol content in samples of nuts and seeds (classes). The dendrogram above represents the results of the hierarchical clustering based on transformed data (indicated with cell color). Brassic, brassicasterol; Crin, crinosterol

seeds and nuts. The total analyzed sterol content in pumpkin/melon seeds was around 160 mg/100 g oil, which is below under reported content (190–250 mg/100 g oil) by other authors [21, 34]. Probable sources of decreased observed content include SRM approach for LC MS/MS analyses not monitoring the transitions for C29 trienols (i.e., $\Delta 7,22,25$ -stigmastatrienol comprising up to 25 wt% of total sterols [21]) and possible decomposition/isomerization of $\Delta 7$ -sterols under acidic hydrolysis [41]. Moreover, the isomers of avenasterols or fucosterols at C22(24) bonds can be formed during acid catalysis [42]. Notably, it is possible that the same isomerization (to some extent) could take place in the acidic environment of gastric juice. Khanavi revealed that $\Delta 5$ -fucosterol, the most abundant phytosterol in brown algae, is responsible for cytotoxic effect of this extract against breast and colon carcinoma cell lines [43]. Furthermore, fucosterols were suggested as activators of the insulin signaling pathway in insulin-resistant cells and potential antidiabetic agents [44]. In peanuts and sunflower seeds, we also found high amounts

of avenasterols and fucosterols (ranged from 6 to 9 wt% of total sterols).

The content of analyzed amyryns (C30 sterols) in linseeds reached 4 wt% of total sterols. Another C30 sterol, cycloartenol (a precursor of cholesterol biosynthesis in plants), was found in linseeds in amounts comparable to campesterol content or even higher [45]. It is known that the sterol composition varies during maturation of the linseed [46]. Sitostanols comprise a special group of sterols. In our study, Brazil nuts were found as the richest sources of sitostanols. Apart from coconut, which contained less than 1 wt%, the content of sitostanols ranged from 3 to 5 wt% of sterols. Other sterols, including C27 sterols, were only minor components. The cholesterol content in plant oils usually reaches the order of percent in wild peanuts is circa 1.5 wt% [47] and in linseed and other seeds is 0.2–1.5 wt% [48]. The presence of cholesterol is believed to be a result of the same biosynthetic pathway as that of plant sterols, i.e., via cycloartenol as a key intermediate,

but with downstream metabolites including C27 sterols as lathosterol and 7-dehydrocholesterol.

3.2 The fatty acid content of analyzed samples

Seed oils contain over 95% of neutral lipids, particularly triacylglycerols (TAG) [49]. We checked the TAG content of extracted oils with thin-layer chromatography (TLC) and densitometric scans proved the integrated density of TAG spots over 90% in all cases (see Supplementary Figs. 2a–2c for TLC of samples). Therefore, it is sufficient to estimate the FA content of oils in samples based only on FAME analyses of molecules transesterified under basic methanolate conditions, such as TAG. Analyses of FA content are presented in Table 2. The coconut was the richest source of saturated FA (91 wt%). Peanut, cashew, hazelnut, macadamia, almond, apricot, pecan, and pistachio contain high amounts of monounsaturated FA (more than 60 wt% of total FA). Important sources of polyunsaturated FA (PUFA) n-3 were linseed (51 wt%), hempseed (17 wt%), walnut (13 wt%), and chestnut (10 wt%). On the other hand, the PUFA n-6 content of more than 37 wt% of total FA was observed in pumpkin/melon seed, chestnut, hempseed, sunflower, walnut, and pine nuts. The ratio of PUFA n-3/PUFA n-6 is of high health relevance. The most optimal range was observed in linseed [50].

The macadamia nuts exhibited the highest content of palmitoleic acid (16:1n-7), which is around one-fifth of total FA [51]. Macadamia oil is typical for very high content of monounsaturated FA [33] with health claims for TC lowering as well as for their antioxidant and anti-inflammatory action. In our samples, we also observed very high content of this FA which was not country specific. These nuts are a poor source for linoleic acid (18:2n-6), probably due to high specificity of thioesterases for palmitoleic acid (16:1n-7) thus preventing biosynthesis of other FA [52].

Peanuts are known to have a high content of very long-chain FA ($C > 20$), especially those of 22:0 and 24:0. Occasional reports on high content of docosahexaenoic acid (DHA, 22:6n-3) in these nuts [51], probably due to misidentification of closely eluting peaks on 30 m DB-WAX column, are not supported by other authors both in wild peanuts [47] and cultivars [53]. Nevertheless, genetically modified novel cultivars of some seeds can contain significant amounts of DHA [54]. Different contents of oleic acid (18:1n-9) ranging from 50 to 78 mol % can be attributed

to normal vs. high oleic acid cultivars, which are accompanied by highly variable content of linoleic acid, 18:2n-6 [53].

We also monitored the content of PUFA n-3, especially α -linolenic (18:3n-3) and stearidonic (SDA, 18:4n-3) acids. From the analyzed samples, the highest content of α -linolenic acid was observed in linseed, which contained around 50 wt% of total FA; the second richest sources were walnut, edible chestnut, and hempseed samples with the content of α -linolenic acid around 10 wt%. The availability of walnut in the central Europe region diet seems to be an interesting source of PUFA n-3, although this acid is not readily transformed into the distal intermediates of the eicosapentaenoic (EPA, 20:5n-3)/DHA forming pathway [55]. The populations without fish as a rich source for EPA/DHA have probably increased the conversion of α -linolenic acid or SDA, which is a better source for the biosynthesis of longer PUFA n-3 [56]. Nevertheless, the content of SDA was negligible in the analyzed samples but for the hemp seeds, which contained 0.1–1.0 wt% of SDA.

In all samples, the area before 18:1n-9 (corresponding to *trans*- 18:1 region) contributed to 0.5–1.0 wt%. The *trans*- isomers of 18:1 can be detected in the samples due to the excessive drying during sample preparation [57]. However, the only roasted sample, peanut, has lower *trans*-FA content vs. other peanut samples. Possible artifacts formed during sample processing might include the preparation of methyl esters, but the sodium methanolate method does not isomerize FA to a considerable extent. The injector temperature used in the oven program was 250 °C, commonly used for FAME analyses including *cis*/*trans*-isomers separations. Other sources for increased area could arise from the possible appearance of *cis*-more distal 18:1 isomers (18:1n-12) which can be present in some seeds [58] and we were not able to distinguish them from *trans*-isomers of FA in used chromatographic separation system. Figure 2 presents the heat map of fatty acids in samples.

Figure 3 presents the correlations between FA and sterols. The most pronounced correlation was observed between the content of 16:1n-9 and stigmaterol (p value < 0.001 , $R = 0.5984$), 18:1n-9 and stigmaterol (p value < 0.001 , $R = 0.5579$), 20:0 and stigmaterol (p value = 0.0184, $R = 0.5642$), 20:1n-9 and stigmaterol (p value < 0.001 , $R = 0.5586$), 16:1n-9 and sitostanols (p value < 0.001 , $R = -0.4292$), 18:1n-9 and sitostanols (p value < 0.001 ,

Fig. 2 Heat map evaluating fatty acid content in samples of nuts and seeds (classes). The dendrogram above represents the results of the hierarchical clustering based on transformed data (indi-

cated with cell color). MCFA—medium chain fatty acids (sum of 8:0 + 10:0 + 12:0 + 14:0 fatty acids)

$R = -0.3979$). C18 fatty acids strongly correlate with each other. This points at their mutual conversion in plant samples. On the other hand, we did not observe these trends for C16 fatty acids. Correlations of Δ^7 -stigmasterol, $\Delta^7,25$ -stigmastadienol, and Δ^7 -campesterol are caused by their high amounts only in pumpkin seed. Negative correlations of β -sitosterol with other sterols are caused by its increased level in samples at the expense of other sterols.

From the data for per 100 g of matrix, it could be concluded that the presented samples are good sources of β -sitosterol except for coconut, edible chestnut, and pumpkin/melon seeds, the last being rich in unusual Δ^7 -sterols. Generally, nuts are not a good source of PUFA

n-3, unless considerable amounts of walnuts are consumed. The consumption of the richest sources of PUFA n-3 (hempseed and linseed) is usually low. From the practical point of view, walnuts are promising natural source of both PUFA n-3 and phytosterols, the compounds reducing both the levels of LDL-cholesterol and TAG, thus promoting cardiovascular health. As the beneficial effects of nut/seed consumption could not be claimed only due to their FA and/or sterol content, but also thanks to many other components (vitamins, trace elements), walnuts again emerge as one of the best choices because of their high antioxidant potential [59].

Fig. 3 Correlation between fatty acid and sterol content in samples. The cell colors reflect values of Pearson's coefficient of correlation. MCFA—medium chain fatty acids (sum of 8:0 + 10:0 + 12:0 + 14:0 fatty acids), Brassic, brassicasterol; Crin, crinosterol

4 Conclusions

In this study, we analyzed 19 samples of seeds, nuts, and kernel for wide range of fatty acids and sterols. Wide variation in the fatty acids and sterols profile was observed and some of them have a unique composition. Hemp seed, walnut, and edible chestnut were characterized by

high content of n-3 polyunsaturated fatty acids. On the other hand, coconut oil had high content of short saturated fatty acids. The sterol content in pumpkin/melon seeds differs from others by high content of unusual $\Delta 7$ sterols, which had to be separated from their $\Delta 5$ isobaric isomers. The consumption of nuts and seeds with required composition can contribute to the favorable

n-3/n-6 polyunsaturated fatty acids ratio of our diet. Moreover, high content of oleic acid as well as phytosterols can have beneficial effects on cholesterol levels and dietary recommendations for cholesterol lowering must be taken into account when consuming higher amount of the nutraceuticals.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Bullo M, Juanola-Falgarona M, Henández-Alonso P, Salas-Salvadó J (2015) Nutrition attributes and health effects of pistachio nuts. *Br J Nutr* 113(Suppl):S79–S93
- Wang M, Huang W, Hu Y, Zhang L, Shao Y, Wang M, Zhang F, Zhao Z, Mei X, Li T, Wang D, Liang Y, Li J, Huang Y, Zhang L, Xu T, Song H, Zhong Y, Lu B (2018) Phytosterol profiles of common foods and estimated natural intake of different structures and forms in China. *J Agric Food Chem* 66:2669–2676
- Stuchlík M, Zák S (2002) Vegetable lipids as components of functional foods. *Biomedic Papers (Czechoslovakia)* 146(2):3–10
- Chen C-YO, Blumberg JB (2008) Phytochemical composition of nuts. *Asia Pacific J Clin Nutr* 17(Suppl 1):329–332
- Kornsteiner-Krenn M, Wagner K-H, Elmadfa I (2013) Phytosterol content and fatty acid pattern of ten different nut types. *Int J Vitam Nutr Res* 83(5):263–270
- Moreau RA, Whitaker BD, Hicks KB (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progr Lipid Res* 41(6):457–500
- Moreau RA, Nyström L, Whitaker BD, Winkler-Moser JK, Baer DJ, Gebauer SK, Hicks KB (2018) Phytosterols and their derivatives: structural diversity, distribution, metabolism, analysis, and health promoting uses. *Prog Lipid Res* 70:35–61
- Bolling BW, Oliver Chen C-Y, McKay DL, Blumberg JB (2011) Tree nut phytochemicals: composition, antioxidant capacity, bioactivity, impact factors. A systematic review of almonds, Brazils, cashews, hazelnuts, macadamias, pecans, pine nuts, pistachios and walnuts. *Nutr Res Rev* 24(2):244–275
- Hartmann M (1998) Plant sterols and the membrane environment. *Trends Plant Sci* 3(5):170–175
- Zullo MAT, Adam G (2002) Brassinosteroid phytohormones—structure, bioactivity and applications. *Braz J Plant Physiol* 14(3):143–181
- Gylling H, Simonen P (2015) Phytosterols, phytostanols, and lipoprotein metabolism. *Nutrients* 7(9):7965–7977
- Raicht RF, Cohen BI, Fazzini EP, Sarwal AN, Takahashi M (1980) Protective effect of plant sterols against chemically induced colon tumors in rats. *Cancer Res* 40:403–405
- Awad B, Fink CS (2000) Phytosterols as anticancer dietary components: evidence and mechanism of action. *J Nutr* 130(9):2127–2130
- Harper CR, Jacobson TA (2005) Usefulness of omega-3 fatty acids and the prevention of coronary heart disease. *Am J Cardiol* 96(11):1521–1529
- Fumeron F, Bard JM, Lecerf JM (2017) Interindividual variability in the cholesterol-lowering effect of supplementation with plant sterols or stanols. *Nutr Rev* 75(2):134–145
- Benetti A, Del Puppo M, Crosignani A, Veronelli A, Masci E, Frige F, Micheletto G, Panizzo V, Pontiroli AE (2013) Cholesterol metabolism after bariatric surgery in grade 3 obesity: differences between malabsorptive and restrictive procedures. *Diab Care* 36(6):1443–1447
- Ros E (2015) Nuts and CVD. *Brit J Nutr* 113(Suppl):S111–S120
- Millar AA, Smith MA, Kunst L (2000) All fatty acids are not equal: discrimination in plant membrane lipids. *Trends Plant Sci* 5(3):95–101
- Calder PC (2015) Functional roles of fatty acids and their effect on human health. *JPEN* 39(15):185–325
- Saini RK, Keum Y-S (2018) Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance—a review. *Life Sci* 203:255–267
- Hrabovski N, Sinadinović-Fišer S, Nikolovski B, Sovilj M, Borota O (2012) Phytosterols in pumpkin seed oil extracted by organic solvents and supercritical CO₂. *Eur J Lipid Sci Technol* 114:1204–1211
- Mo S, Dong L, Hurst WJ, van Breemen RB (2013) Quantitative analysis of phytosterols in edible oils using APCI liquid chromatography–tandem mass spectrometry. *Lipids* 48:949–956
- Lagarda MJ, García-Llatas G, Farré R (2006) analysis of phytosterols in foods. *J Pharm Biomed Anal* 41:1486–1496
- Staňková B, Kremmyda L-S, Tvřizická E, Žák A (2013) Fatty acid composition of commercially available nutrition supplements. *Czech J Food Sci* 31(3):241–248
- Esche R, Muller L, Engel KH (2013) Online LC-GC-based analysis of minor lipids in various tree nuts and peanuts. *J Agric Food Chem* 61:11636–11644
- Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226(1):497–509
- Jonker D, van der Hoek GD, Glatz JFC, Homan C, Posthumus M, Katan MB (1985) Combined determination of free, esterified, and glycosylated plant sterols in foods. *Nutr Rep Int* 32:943–951
- Aycirix S, Regazzetti A, Gaudin M, Prost E, Dargere D, Massicot Auzeil N, Laprévote O (2012) Development of a novel method for quantification of sterols and oxysterols by UPLC-ESI-HRMS: application to a neuroinflammation rat model. *Anal Bioanal Chem* 404:3049–3059
- Tvřizická E, Vecka M, Staňková B, Žák A (2002) Analysis of fatty acids in plasma lipoproteins by gas chromatography–flame ionization detection: quantitative aspects. *Anal Chim Acta* 465(1–2):337–350
- Appaiah P, Sunil L, Prasanth Kumat PK, Gopala Krishna AG (2014) Composition of coconut testa, coconut kernel and its oil. *J Am Oil Chem Soc* 91:917–924
- Amaral JS, Casal S, Pereira JA, Seabra RM, Oliveira BPP (2003) Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. *J Agric Food Chem* 51(26):7698–7702
- Savage GP, McNeil DL, Dutta PC (1997) Lipid composition and oxidative stability of oils in hazelnuts (*Corylus avellana* L.) grown in New Zealand. *JAOCs* 74(6):755–759
- Kajiser A, Dutta A, Savage G (2000) Oxidative stability and lipid composition of macadamia nuts grown in New Zealand. *Food Chem* 71:67–70
- Phillips KM, Ruggio DM, Ashraf-Khorassani M (2005) Phytosterol composition of nuts and seeds commonly consumed in the United States. *J Agric Food Chem* 53(24):9436–9445

35. Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi A-M (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. *J Sci Food Agric* 80:939–966
36. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissa G, Wolf C, Jacques C, Berenbaum F (2010) Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthritis Cartil* 18(1):106–116
37. Normén L, Ellegard L, Brants H, Dutta P, Andersson H (2007) A phytosterol database: fatty foods consumed in Sweden and in the Netherlands. *J Food Comp Anal* 20:193–201
38. Itoh T, Tamura T, Matsumoto T (1973) Sterol composition of 19 vegetable oils. *Lipids* 50:122–125
39. Normén AL, Brants HAM, Voorrips LE, Andersson HA, van den Brandt PA, Goldbohm RA (2001) Plant sterol intakes and colorectal cancer risk in the Netherlands Cohort Study on Diet and Cancer. *Am J Clin Nutr* 74:141–148
40. McDonald JG, Thompson BM, McCrum EC, Russell DW (2007) Extraction and analysis of sterols in biological matrices by high performance liquid chromatography electrospray ionization mass spectrometry. *Methods Enzymol* 432:145–170
41. Piironen V, Toivo J, Puupponen-Pimia R, Lampi A-M (2003) Plant sterols in vegetables, fruits and berries. *J Sci Food Agric* 83:330–337
42. Kamla-Eldin AK, Määttä K, Toivo J, Lampi A-M, Piironen V (1998) Acid-catalyzed Isomerization of Fucosterol and D5-avenasterol. *Lipids* 33:1073–1077
43. Khanavi M, Gheidarloo R, Sadati N, Ardekani MRS, Nabavi SMB, Tavajohi S, Ostad SN (2012) Cytotoxicity of fucosterol containing fraction of marine algae against breast and colon carcinoma cell line. *Pharmacogn Mag* 8(29):60–64
44. Jung HA, Bhakte HK, Min B-S, Choi JS (2016) Fucosterol activates the insulin signaling pathway in insulin resistant HepG2 cells via inhibiting PTP1B. *Arch Pharmac Res* 39(10):1454–1464
45. Ciftci ON, Przybylski R, Rudzinska M (2012) Lipid components of flax perilla, and chia seeds. *Eur J Lip Sci Technol* 114(7):794–800
46. Herchi W, Harrabi S, Sebei K, Rochut S, Boukkchina S, Pepe C, Kallel H (2009) Phytosterols accumulation in the seeds of *Linum usitatissimum* L. *Plant Phys Biochem* 47:880–885
47. Grosso NR, Zygodlo JA, Burrioni LV, Guzmán CA (1997) Fatty acid, sterol and proximate compositions of peanut species (*Arachis* L.) seeds from Bolivia and Argentina. *Grasas y Aceites* 48(4):219–225
48. Teneva OT, Zlatanov MD, Antova GA, Angelova-Romova MY, Marcheva MP (2014) Lipid composition of flaxseeds. *Bulgar Chem Commun* 46(3):465–472
49. Tzen JTC, Cao Y, Laurent P, Ratnayake C, Huang AHC (1993) Lipids, proteins and structure of seed oil bodies from diverse species. *Plant Physiol* 101:267–276
50. Hodson L, Crowe FL, KJ McLachlan, Skeaff CM (2018) Effect of supplementation with flaxseed oil and different doses of fish oil for 2 weeks on plasma phosphatidylcholine fatty acids in young women. *Eur J Clin Nutr* 72:832–840
51. Maguire LS, O'Sullivan SM, Galvin K, O'Connor TP, O'Brien NM (2004) Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *Int J Food Sci Nutr* 55(3):171–178
52. Moreno-Pérez AJ, Sánchez-García A, Salas JJ, Garcés R, Martínez-Force E (2011) Acyl-ACP thioesterases from macadamia (*Macadamia tetraphylla*) nuts: cloning, characterization and their impact on oil composition. *Plant Phys Biochem* 49(1):82–87
53. Jonnalala RS, Dunford NT, Dashiell KE (2005) New high-oleic peanut cultivars grown in the Southwestern United States. *JAOS* 82(2):125–128
54. Kitesa SM, Abeywardena M, Wijesundera C, Nichols PD (2014) DHA-containing oilseed: a timely solution for the sustainability issues surrounding fish oil sources of the health-benefiting long-chain omega-3 oils. *Nutrients* 6:2035–2058
55. Burdge GC, Calder PC (2005) Conversion of α -linolenic acid to longer-chain polyunsaturated fatty acid in human adults. *Reprod Nutr Dev* 45:581–597
56. Welch AA, Shakyia-Shrestha S, Lentjes MAH, Wareham NJ, Khaw KT (2010) Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non fish-eating meat-eaters, vegetarians, and vegans and the precursor-product ratio of α -linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. *Am J Clin Nutr* 92:1040–1051
57. Brühl L (1996) Determination of trans fatty acids in cold pressed oils and in dried seeds. *Fett/Lipid* 89(11):S380–S383
58. Baylin A, Siles X, Donovan-Palmer A, Fernandez X, Campos H (2007) Fatty acid composition of Costa Rican foods including trans fatty acid content. *J Food Compos Anal* 20:182–192
59. Vinson JA, Cai Y (2012) Nuts, especially walnuts, have both antioxidant quantity and efficacy and exhibit significant potential health benefits. *Food Funct* 3:134–140

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