CHEMICAL PROFILE OF COLD PRESSED BEECH NUT (Fagus Sylvatica L.) OIL

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Abstract

The objective of the study was to evaluate the chemical characteristics of cold-pressed beech nut oil. The nuts collected from the forest consisted of 25.35% water, 13.19% oil, and 19.40% protein. Dominant fatty acid was linoleic (40.5%) followed by oleic (35.0%) and gondoic acid (7.7%). All four tocopherols were present in the oil with dominant γ- tocopherol 99.38 mg 100g-1 oil. Total sterol content was 2708.73 mg kg-1 oil with β-sitosterol accounting for 80.5% of all sterols. The oil's main characteristics were the relatively high content of tocopherols and gondoic acid, dominant oleic-linoleic fatty acid profile, and higher levels of carotenoids.

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Keywords: *Fagus Sylvatica* L., cold-pressed oil, fatty acids, tocopherols, sterols

Introduction

Tree nuts present interesting and cheap sources of different macronutrients and bioactive compounds. There are numerous data on tree nut composition from a majority of popular nuts like hazelnut, almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut but information on wild-grown nuts is much scarcer . European beech (*Fagus Sylvatica* L.) is the most widespread forest tree in Europa. It spreads from southern parts of Scandinavia to Spain, Sicily, and northwest Turkey. With a usual life span of 150-300 years, it is characterized by irregular seed production and fruiting which occurs every 5 to 8 years . It is estimated that beech covers around 14 Mha of Europa (excluding Caucasian mountains) while

a maximum yield of beech nuts per 1 ha reaches 4 tons . Oil yield in beech nuts varies between 15-20 %, which significantly depends on moisture content (up to 30 %) . The theoretical production potential for beechnut oil in Europa can be estimated in the range of 8.4 and 11.2 million tons per year. Based on the latest FAO data this equals around 4.7 % of global oil production . Scientific data on beech nut composition is extremely limited covering mostly information on oil and moister quantity with one recent paper from Siger et al. on physicochemical characteristics of beech nut oil and paper from Dandik et al. on closely related *Fagus orientalis*fatty acids and technological characteristics . This paper aims to give more detailed information on European beech nut cold-pressed oil, primarily fatty acid composition and unsaponifiable fraction.

Materials and experimental methods

Samples

Nuts from European beech (*Fagus Sylvatica* L.) were collected in early autumn in the northwest part of Croatia in the area of Nature Park Žumberak. After the reception, the moister, oil, and protein yield was measured, and nuts were dried at 60 ºC until under 10 % of moister. Nuts were kept in dry and dark storage until the oil extraction.

Reagents

All chemicals and solvents were analytical grade, obtained from Carlo Erba Réactifs-SdS (Chaussée du Vexin, France). Fatty acid methyl ester (FAME) standards (C8-C22) were obtained from Supelco (Bellefonte, PA, USA). Sterol standards (β-sitosterol, campesterol, and stigmasterol) were all purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Tocopherols (α-, β-, γ- and δ-) were acquired from Merck KGaA (Darmstadt, Germany).

Oil Extraction

Oil was produced within two weeks after collecting and drying by cold pressing 600 g of nuts. The temperature did not exceed 50 °C. Oil was pressed by using a laboratory screw press Komet CA/53 (Monforts & Reiners, Rheydt, Germany). Oil was filtered through a sintered glass filter (10-16 μm pore size) and stored at room temperature in a dark bottle under nitrogen until further analysis.

Oil analysis

Quality parameters of oil

Peroxide value (PV), free fatty acids (FFA), and UV extinction coefficients (K_{232} and K_{270}) were determined according to ISO 3960:2007, ISO 660:2009, and ISO 3656:2011 methods .

Pigments

Pigments were determined spectrophotometrically. Total chlorophylls, expressed as pheophytin a, were determined by using the method of Pokorny et al. [16] and by measuring the absorbance of the oils against the air at 630, 670, and 710 nm. Total carotenoids were determined by measuring the absorbance of oil solution in cyclohexane at 445 nm using the BSI method [17]. Equations 1 and 2 were used to calculate total chlorophylls and carotenoid content, respectively.

$$\text{Total chlorophylls} = 34.53\ \frac{A_{670} - 0.5\ (A_{630} + A_{710})}{L} \quad (1)$$

$$\text{Total carotenoids} = \frac{383 \times A_{445}}{L \times c} \quad (2)$$

Where A_i was absorbance at the specified wavelength, L was the thickness of the glass cell (cm) and c was the concentration (g/100 mL) of oil solution in cyclohexane.

Fatty Acid Composition

The fatty acid composition was determined by using gas chromatography. Fatty acid methyl esters (1 μL), prepared by ISO method 5509 [23], were injected into a GC equipped with an FID detector according to ISO

method 5508 [24]. Fatty acid methyl esters were separated on a TRACE TR-FAME capillary column (30 m × 0.22 mm × 0.25 μm) using a stationary phase of 70% cyanopropyl polysilphenylene-siloxane (Thermo Scientific, Waltham, MA, USA). Helium was used as the carrier gas at a 0.7 mL/min flow rate. The temperature of the injector was set at 250 °C and of the detector at 280 °C. The temperature of the oven was programmed to increase 4 °C/min from an initial value of 120 to 160 °C, and then at 10 °C/min to 190 °C, where it was held for 10 min. The split ratio was 75:1. Fatty acid methyl ester peaks were identified by comparing their retention times with those of FAME standards (C8-C22).

Tocopherols

Tocopherol content was determined according to ISO method 9936 [15] by using normal-phase HPLC analysis. Samples were prepared by dissolving 0.1 g of oil in 10 mL n-hexane and then analyzed by HPLC equipped with a fluorescence detector and LiChroCART Silica 60 column (250 mm 9 4.6 mm, 5 l; Merck, Darmstadt, Germany). Tocopherols were detected at 295 nm and 330 nm excitation-emission wavelengths and separated by isocratic chromatography by mobile phase of 0.7 % propan-2-ol in n-hexane at 0.9 mL/min flow rate. Analyses were performed at room temperature. Tocopherols were quantified by standard calibration curves for α-, β-, γ- and δ-tocopherols.

Sterol Content

For sterol content and composition ISO 12228:2014 method was employed using GC-FID and GC-MS analysis. Analised unsaponifiable fraction was isolated by column chromatography using aluminum oxide (neutral, particle size 0,063 to 0,200 nm, activity grade I) with ethanol and diethyl ether as solvents. Separated unsaponifiables were collected in a round bottom flask, and solvents were removed in a rotary evaporator and again dissolved in a small amount of diethyl ether. The solution was further applied for separation by silica thin-layer chromatography (20x20 cm plates, thickness of layer 0.25 mm) in a diethyl ether: hexane (50:50) solvent system. Zones with sterols were determined with methanol spraying, scratched with a spatula from the plates, dissolved in ethanol and diethyl ether, and reduced till dry in a rotary evaporator. Again, dissolved in 1 ml of diethyl ether for transfer in a test tube after the solvent was blown off with a stream of nitrogen. Sterols were derivatized by pyridine: hexamethyldisilazane: trimethylchlorosilane (5:2:1, v/v/v). The chromatographic conditions were set as follows: volume of injection of prepared sterol fraction was 1 μL, split mode 13.3:1, and helium was used as a carrier gas at a flow rate of 1.5 mL min-1 and inlet pressure of 0.51 bar. The temperature of the injector was set to 290 °C and the temperature of the detector to 250 °C. The temperature regime of the column was programmed to increase for 6 °C min-1 from 180 to 270 °C and then remained at 270 °C for 30 min. The mass-selective detector was operated in the electron impact-selected ion-monitoring (EI-SIM) mode with the ionizing voltage set at 70 eV. Peaks were identified by comparing the retention times of sterols with those of the standards and by comparing the mass spectra with available literature (Li et al., 2007) and the NIST 2017 library. Quantification of all sterols was based on an internal standard method using α-cholestanol (˜95%).

Total sterols were expressed as g kg^{-1} while concentrations of individual sterols represent % in a total fraction of sterols. Results are presented as mean values±standard error (SE) (n=3).

Statistical Analysis

When experimental values were issued from three independent extractions, data are expressed as mean values ± standard deviation (SD).

Results and discussion

Beech nut properties

Beech nuts consist of around 33 % of husk and 67 % of kernels . Freshly collected beech nuts had a very high moister content of 25.4 % which presents one of the main problems regarding storage and oil quality. According to some previous studies, moister content can be as high as 30.0 % which can significantly influence the quality of oil, due to possible hydrolyzation of triglycerides . After preliminary research on moister, oil,

and protein content the nuts were dried at 60º C until the moister value was under 10.0 % based on literature recommendations for storage conditions and by work by Siger et al. .

Oil content was 13.2 % (17.7 % for the dry matter) which was lower than in previously published research of 15-20 % for *F. orientalis* and 27.25% for *F. Sylvatica* . Cesarettin and Shahidi reported an oil content of 50.0 % but with a moister value of 6.6 %. The same authors published the values on similarly widespread oak tree nuts – acorns with 27.9 % of moister and 23.9 % of oil. Contrary to just 10 *Fagus* species, oaks belong to the *Quercus* genera of trees with 350-400 species where the oil content of acorns from white oaks does not exceed 12 % while some black and red oaks contain up to 31 % of oil . Here we can comment on problems resulting from a limited number of research and differences between laboratory measurements and practical circumstances during the real possible production of oil. Beech nuts consist of a hull and kernel but the previous research makes a difference between oil determination and oil production . For oil determination, they used just kernels while for oil production they used the whole nut. Higher oil content in the kernel is to be expected but in real-life conditions demands more equipment and could be misleading for private investors. Our results are based on whole nut oil content, with the hull.

Protein yield was 19.4 %, similar to the protein content in cashew, hazelnut, and pistachio and much higher than previously reported by Cesarettin and Shahidi at 6.2 % and under 25 % by Kaliniewicz et al. . These papers did not give any detail about the methods of determination. When we look deeper into their cited data Cesarettin and Shahidi quoted results from the U.S. Department of Agriculture (USDA) while Kaliniewicz et al. cited Reyes et al. and Pukacka and Ratajczak which have no data on the protein content of beech nut.

Physicochemical characteristics of oil

Oil was extracted by cold pressing in laboratory conditions, using a screw press after which sedimentation and filtering were carried out to obtain pure virgin oil. Oil had light yellow color with a distinctive nutty aroma resembling the initial raw material.

Free fatty acids, which are indicators of oil hydrolysis and seed quality were relatively high at 1.74 % but still under 2 % which is recommended for cold pressed and virgin oils in Codex Standard for Named Vegetable Oils . This borderline value could be expected because of the much higher moister content than in usual oilseeds and nuts. During the process of forest nut collecting and storage, moisture control is one of the most critical points for oil quality.

The peroxide value, an indicator of primary oxidation, of freshly cold-pressed oil, was 1.86 meq O_2 kg oil^{-1} which is also by Codex and far below the upper limit . A similar value of 1.11 meq O_2 kg oil^{-1} was reported in research by Siger et al. . Extinction parameters for primary oxidation products (conjugated peroxides) at 232 nm had an absorption of 1.88. In the extensive research on the oxidative stability of cold-pressed oil Dedebas et al. reported K_{232} values for fresh oils (sesame, black cumin, grape seed, flaxseed, coriander) between 1.01-2.96. Looking deeper into their data, fresh sesame oil with a similar fatty acid profile as beech nut (oleic and linoleic fatty acids account for around 80 % of the total) had K_{232} at 1.70. Absorption for secondary products of oxidation (aldehydes and ketones) at 270 nm was 0.34. Compared with Dedebas et al. findings, K_{270} was in the range of 0.17-0.64, with sesame oil at 0.44.

Pigment content

Total chlorophyll and carotenoids in oil were determined spectrophotometrically and presented in Table 2. They are extracted into oil through oil seed pressing. Values for chlorophyll (1.47 mg kg^{-1}) were relatively low and under 2.56 mg kg^{-1} published by Siger et al. . Chlorophyll levels decrease during plant maturation and are not desirable in oils because of their photooxidative properties under UV light influence. The upper limits of chlorophyll are not strictly regulated, especially for cold-pressed oils. For large-scale industrial crude oils like rapeseed, there are some maximum levels set for chlorophyll content before refination, for example in Canada, at 30 mg kg^{-1}, . Carotenoids are desirable pigments in oils and increase their nutritional value while protecting them against oxidation. The total carotenoids detected were 7.16 mg kg^{-1} which is a little less but comparable with Siger et al. at 10.68 mg kg^{-1}.

When looking at other tree nut oils, only pistachio oil has relatively higher levels of carotenoids (6.70 mg kg^{-1}) while others are much lower (hazelnut oil at 2 mg kg^{-1}) or not detected with almond, walnuts, pecan, macadamia, cashew, and Brazilian nuts .

Fatty acid profile

Results for fatty acids of analyzed oil are presented in Table 3. Composition is characterized by low levels of saturated fatty acids (SFA) at 11.4% and relatively equal values for monounsaturated fatty acids (MUFA) at 42.9% and PUFA polyunsaturated fatty acids (PUFA) at 45.7%. The most dominant fatty acid was essential omega-6 linoleic acid 40.5%, followed by oleic at 35.0%. Similar fatty acid profile was reported in some other beech nut oil research: 38% oleic and 38.6% linoleic , 30.3 % oleic and 48.7% linoleic , 37.5% and 42.3% . Results reported by Dandik et al. had much bigger variations of 40-76% oleic and 9.2-38.0% linolenic. Significant differences in results are expected since the fatty acid profile depends on the influence of climate, soil, and time of harvesting . Beech nut oil from similar species of *Fagus orientalis* Lipsky harvested in Turkey had 30.4% oleic and 48.9% linoleic .

The third most represented fatty acid in oil was gondoic (C20:1 n-9) at 7.7%. It is a relatively rare fatty acid that is found in high quantities in jojoba oil , and less in rapeseed, mustard seed and camelina oils. Research on the nutritional value and health effect of gondoic acid is still in its early stages and ranges from neutral in mortality and cardiovascular health study , positive with inhibiting inflammation to possibly negative with glucose metabolism .

Similar fatty acid profiles of SFA, MUFA, and PUFA with a dominant oleic-linoleic profile in mostly equal share and around 80% of total fatty acids were reported with sesame, peanut, and pumpkin seed oil . Apart from gondoic acid, the main difference between these oils is the higher content of essential omega-3 α-linolenic fatty acid (ALA) at 5.2% compared with under 1% at mentioned oils. Other researchers published similar or smaller values: 4.3% , 0.2% , 0.4-2.8% . Siger et al. , did not detect ALA but γ-linolenic fatty acid at 4.2% which was not present in the current study or other previously published papers.

Dominant SFA were C16:0 palmitic (7.3%) and C18:0 stearic (2.8%) with small quantities of C14:0 (0.2%), C17:0 (0.1%) and C21:0 (0.3%). Palmitic and stearic fatty acids had similar values in other previously published research .

Tocopherols

All four tocopherols alpha (α-T), beta (β-T), gamma (γ-T), and delta (δ-T) were detected in the sample (Table 4) with a total of 117.93 mg $100g^{-1}$ oil. There was just one previous published research on beech nut oil tocopherols from Siger et al. with a similar total of tocopherols at 110.23 mg $100g^{-1}$ oil and three tocopherols detected (excluding β-T). Siger et al. collected their samples from Poland in a much colder climate than our samples and had more than 20x higher content of δ-T.

When compared with other tree nut oils this is 2 to 7 times higher value than for almond, hazelnut, pistachio, pecan, pine nut, and walnut published in Miraliakbari and Shahid research . The dominant was γ-T with 99.38 mg $100g^{-1}$ oil or 84.27% of the total. α-T was detected at 15.32 mg $100g^{-1}$ oil or 12.99% while β-T was 1.65 and δ-T 1.58 mg $100g^{-1}$ oil. Other tree nuts with similar tocopherol profile – dominant γ-T with smaller quantities of α-T are Brazilian nuts, pine nuts, cashew, and peanuts . Almonds and hazelnut have dominant α-T, while pecans, pistachio, and walnuts are dominant in γ-T with no or significantly low levels of α-T. Moderate intake of biologically most active α-T has a cardioprotective effect through induced inhibition of LDL oxidation while γ-T is the most potent antioxidant in oils . Variations in tocopherols compared to Siger et al. could be explained by plants' adjustment to environmental conditions and stages of seed/nut maturity. In research on soyabean oil and tocopherol content at different stages of maturity, it was found that α-T, β-T, and γ-T increase during maturation while δ-T is relatively static from early stages to full maturation. Also, δ-T is an important antioxidant during earlier stages of seed development or lower temperatures while its biosynthetic pathway is as not active during later phases .

Daily consumption of selected α-T rich nuts and nuts oils is one of the easiest ways of reaching Recommended

Dietary Allowances (RDAs) for Vitamin E for children and adults of 6-13 mg day^{-1}which could be easily met with the addition of beech nut oil into the diet.

Sterols

Results for individual and total sterols are presented in Table 5. There were four sterols detected: campesterol, stigmasterol, β-sitosterol and Δ5-avenasterol. Total sterol content was 2708.73 mg kg^{-1} oil which is 2 times higher than reported in the only previous research by Siger et al. . Compared to other tree nut oils it had similar value as pecan, almond, and walnut (2620-2990 mg kg^{-1} oil) while higher than Brazilian nut, pine nut, pistachio, and hazelnut (1290-2060 mg kg^{-1} oil) . The predominant membrane sterols of the higher plants are β-sitosterol, stigmasterol, and campesterol. Sterols differ from each other in the presence of a methyl or ethyl group in the side chain at the 24th carbon atom and, accordingly, are called 24-methyl sterols (campesterol) or 24-ethyl sterols (β-sitosterol and stigmasterol) . The dominant sterol in the sample was β-sitosterol (2181.13 mg kg^{-1} oil), accounting for 80.5% of all sterols. β-sitosterol is a natural micronutrient that is present in higher plants, while humans ingest it through a balanced diet. In the serum and tissues of healthy individuals, its concentration is 800-to-1000-fold lower in comparison with endogenous cholesterol . Second most abundant sterol was Δ5-avenasterol (246.62 mg kg^{-1} oil or 9.1%). There could be a possible correlation between [?]5-avenasterol and β-sitosterol based on the previous olive oil findings. It has been reported that β-sitosterol is present in minimal and [?]5-avenasterol in maximal amounts when olives are harvested at their optimum . This observation could be a useful tool for the selection of the optimal time for beech nut harvesting. Campesterol was present at 222.79 mg kg^{-1}oil or 8.2% while sitosterol was 58.19 mg kg^{-1} oil or 2.1% of total sterols.

Differences in sterol composition from Siger et al. findings (9 different sterols – 4 the same and 5 different - cholesterol, sitostanol, cycloartenol, 24-methylenecycloartanol, and citrostadienol) can be explained by climate differences and plant adaptation to climate. The role of sterols in plant adaptation to environmental conditions is well documented. It has been suggested that the ability of plants to synthesize the 24-ethyl sterols, β-sitosterol, and stigmasterol, maybe a part of an evolutionary adaptation to stresses and maintenance of important membrane-associated metabolic processes . The closest profile of total and individual sterols to beech nut, from global industrial oils, is to cottonseed oil (2700-6400 mg kg^{-1} oil, β-sitosterol 76.0-87.1%) [15].

Conclusions

New trends in nutrition emphasize the return to traditional foods and foods that remain neglected due to the dominant influence of the food industry. Given the growing interest of consumers in cold-pressed oils and oils with a specific taste and aroma, there is a potential for the production and sale of beech nut oil. The common beech tree (*Fagus Sylvatica*) is widespread throughout continental Europe and most of Great Britain. It grows in a very wide altitude range, from lowland areas up to 2000 m above sea level, and considering the total share and the share in the amount of wood, it is considered the most important deciduous tree in Europe. Significant differences in its habitat have a strong influence on beech nut oil composition. Current data on its chemical profile is extremely limited which emphasizes the need for further research. Relatively high content of tocopherols and gondoic acid, a dominant oleic-linoleic fatty acid profile, and higher levels of carotenoids are the main characteristics of this oil. Further research is necessary for the chemical profiling of oil from different habitats as well as proper application in the cosmetic industry or as a part of a health-conscious diet.

Acknowledgments

References

Table 1. Physical and chemical properties of *Fagus sylvatica* L. nut

Component	
Water content (%)	25.35±0.07

Component	
Oil content (%)	13.19±0.01
Protein content (%)	19.40±0.27

Table 2. Physicochemical properties of *Fagus sylvatica* L. nut oil

Component	
Free fatty acids (%)	1.74±0.11
Peroxide value (mEq O_2 kg^{-1})	1.86±0.18
K_{232}	1.88±0.06
K_{270}	0.32±0.02
Chlorophyll (mg kg^{-1})	1.47±0.09
Total carotenes (mg kg^{-1})	7.16±0.39

Table 3. Fatty acid profile of *Fagus sylvatica* L. nut oil

Fatty acid			(%)
Saturated			
	Myristic acid	$C_{14:0}$	0.2±0.00
	Palmitic acid	$C_{16:0}$	7.3±0.01
	Margaric acid	$C_{17:0}$	0.1±0.00
	Stearic acid	$C_{18:0}$	2.8±0.00
	Arachidic acid	$C_{20:0}$	0.6±0.02
	Heneicosylic acid	$C_{21:0}$	0.3±0.01
Monounsaturated			
	Palmitoleic acid	$C_{16:1}$	0.2±0.00
	Oleic acid (n-9)	$C_{18:1}$	35.0±0.01
	Gondoic acid (n-9)	$C_{20:1}$	7.7±0.01
Polyunsaturated			
	Linoleic acid (n-6)	$C_{18:2}$	40.5±0.01
	α-Linolenic acid	$C_{18:3}$	5.2±0.00
	SFA		11.4±0.03
	MUFA		42.9±0.02
	PUFA		45.7±0.01

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 4. Tocopherol profile and pigments of *Fagus sylvatica* L. nut oil.

	mg $100g^{-1}$ oil	(%)
α-Tocopherol	15.32±0.63	12.99%
β-Tocopherol	1.65±0.18	1.40%
γ- Tocopherol	99.38±3.41	84.27%
δ-Tocopherol	1.58±0.06	1.34%
Total	117.93±3.92	

Table 5. Sterol profile of *Fagus sylvatica* L. nut oil.

	mg kg^{-1} oil	(%)
Campesterol	222.79±12.39	8.2%
Stigmasterol	58.19±19.91	2.1%
β-sitosterol	2181.13±103.49	80.5%
Δ5-avenasterol	246.62±23.01	9.1%
Total	2708.73±140.53	