Lipid composition and DPPH activities of the seed oil of five Turkish hazelnut genotypes (*Corylus colurna* L.)

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Five genotypes of Turkish hazelnuts (*Corylus colurna* L.) were analyzed for chemical composition, including total oil content, fatty acid and sterol composition. The oil yields from these kernels varied from 36.5% to 60.8% and the main fatty acids were oleic acid (79.34–83.0%) and linoleic acid (7.52–10.81%). The polyunsaturated/saturated fatty acid ratio was low, ranging from 0.87 to 1.25. Total phytosterol content ranged from 4.52 to 6.50 mg g^{-1} of oil. Among the eleven sterols identified and quantified, β-sitosterol was the major one with a mean percentage of 65.09% while fucosterol and campesterol were the second and the third components of the group with mean values of 10.91% and 4.36%, respectively. The radical scavenging activity was evaluated using 2.2-diphenyl-1 picrylhydrazyl (DPPH) assay.

Key words: *Turkish hazel; oil content; fatty acids; sterols*

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1. INTRODUCTION

Genus *Corylus* is part of the Betulaceae family. It contains about 15 species of shrubs and trees that are native to the northern temperate regions of Europe, Asia, and North America. Commonly known as hazels, most of these plants feature clusters of yellow flowers and edible nuts. The commercial hazelnut, one of the world's major tree nut crops, is the European hazelnut (*C. avellana* L.). *C. colurna*, commonly known as the Turkish hazel, is native to southeast Europe and southwest Asia, through to the Balkans, Northern Turkey and Northern Iran (Thompson et al., 1996).

Due to their organoleptic characteristics, hazelnuts are consumed all over the world, not only as a fruit but also in a diversity of manufactured food products, such as snacks, chocolates, cereals, bakery, dairy, salad, entree, sauce, ice creams, and other dessert formulations (Ozdemir and Akinci, 2004; Amaral et al., 2006; Oliveira et al., 2008). Although Turkish tree hazelnuts are consumed, there are limited reports about their chemical composition. In a recent study, Erdogan and Aygun (2005) reported that the content of oil obtained from nuts of some Turkish tree hazel types ranged between 64.48% and 71.92%. Oleic and linoleic acids were the predominant fatty acids, together representing 91.70% of the total. The amount of palmitic and stearic acids was low while palmitoleic, margaric, margaroleic, linolenic, arachidic, and gadoleic acids were present in trace amounts. Similar oil content (48.60–69.90%) and fatty acid composition was reported from Serbian samples of *C. colurna* nuts (Miletić et al., 2007; Ninić-Todorović, 1990).

The chemical composition of European hazelnuts has been the subject of numerous investigations. Examination of 24 hazel cultivars from Italy and other climates revealed that the fruit quality depends on the interaction between the genotype and environmental conditions (Cristofori et al., 2008). The authors found that high content of palmitic acid is present in the dry period. Also, the fatty acid content analysis was performed on Nebraska hybrid hazel nuts by Xu and Hanna (2010). Their findings indicated that oleic and linoleic acids were most abundant, contributing by over 90% to the total fatty acid content.

Fruit analysis of three hazel genotypes sourced from natural populations reveal presence of 16 fatty acids (Seyhan et al., 2007). The authors found no differences in the saturated fatty acid content at various stages of fruit maturity. Similarly, the samples of *C. avellana* oil contained the highest proportion

of monounsaturated fatty acids (up to 79.5%), followed by polyunsaturated fatty acids (12.6%) and saturated fatty acids (8%) (Parcerisa et al., 1998). In the resent study, Alasalvar et al. (2006) indicated that, in the hazelnut oil, oleic acid was the most common fatty acid with 82.78%, followed by linoleic (8.85%), palmitic (4.81%) and stearic acid (2.69%).

After analyzing the composition of authentic hazelnut oils obtained from nuts collected from five countries, Crews et al. (2005) found that the oleic acid content varied between 75.3 and 83.8%, linoleic was in the 6.2–15.9% range, palmitic ranged from 5.0 to 6.6% and stearic varied between 1.6 to 2.9%. Owing to the high oil content, hazel kernels could be used as raw material in biodiesel production (Xu and Hanna, 2009). Three decades ago, the Turkish hazel genotype selection program, using natural populations and trees of secondary origin, started in Serbia. The selection included the Turkish hazel population in the Danube Park, Futoški Park and the plant nursery in Novi Sad, from which 45 specimens were chosen. Although lipid characteristics of the Turkish hazelnut have been reported (Erdogan and Aygun, 2005; Miletić et al., 2007), there is no information on phytosterol compositions of hazelnut oil extracted from the Turkish hazelnut.

The aim of this study was to determine the oil content as well as the fatty acid and sterol composition of seeds from five Turkish hazelnut genotypes, to evaluate their potential for nutritional and medicinal applications.

2. MATERIALS AND METHODS

2.1. Plant material collection

During August 2016, seeds from five Turkish hazel genotypes were collected from a plant nursery in Novi Sad. Kernels of these genotypes were labelled as A8, A9, A11, B6 and B10, and sown in October in the Faculty of Agriculture plant nursery located in Rimski Šančevi, near Novi Sad.

2.2. Oil content

The oil contents of hazelnuts were determined according to AACC (1991). The nuts from different genotypes were first grounded separately and dried thoroughly in oven at 105 ºC. The dried ground samples (approximately 3 g) were placed in a thimble and the oil was extracted using 50 mL of *n*-hexane in a Soxhlet extractor for 8h. After the extraction, the *n*-hexane was evaporated and the collection cups containing the extracted oil were placed in a vacuum oven at 95 ºC for 1 h in order to remove all traces of hexane. The oil content was expressed as the following:

$$\%\ \text{oil content} = \frac{weight\ of\ oil}{weight\ of\ sample} \times 100$$

2.3. Chemical analysis of methyl esters

Fatty acid methyl esters were prepared following IUPAC methodology without heating (IUPAC, 1987). A 100 mg of raw seed oil was accurately weighed into a 20 mL centrifuge tube and dissolved in 5 mL *n*-hexane. Then 0.2 mL of 2 M solution of KOH in methanol was added. The tube was sealed and mixed vigorously for 30 s in a vortex shaker. Saturated NaCl solution (2.0 mL) was added and the organic phase was separated. The resulting methyl esters of fatty acids were analyzed by GC and GC-MS analysis.

The GC and GC-MS analyses were performed on an Agilent 7890A GC system equipped with 5975C inert XL EI/CI MSD and a FID detector connected by capillary flow technology 2-way splitter with make-up gas. A DB-23 capillary column (60 m × 0.25 mm × 0.25 µm) was used. Samples were injected in split mode (30:1). The injection volume was 1 µL and the injector temperature was 220 °C. The carrier gas (He) flow rate was 3.3 mL/min at 60 °C (constant pressure mode). The column temperature was initially set at 50 °C (1 min), after which it was programmed to increment linearly in the 50-175 °C range (25 °C/min) and 175-235 °C (4 °C/min), with final 5-min hold. The transfer line was heated at 235 °C. The FID detector temperature was 300 °C. EI mass spectra (70 eV) were obtained in *m/z* range of 35-550 atomic mass units (AMU), with 3 min solvent delay. The ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. Retention times of the fatty acid components of seeds were compared with the corresponding original fatty acids from GC, and mass spectra of individual components were identified by comparison with Wiley 07 and NIST 05 databases.

2.4. Chemical analysis of unsaponifiables

Sterols were determined by GC-MS of the complete unsaponifiable fraction. Internal standard solution of 10.0 mg of cholesterol in methylene chloride (0.1%) was added to 2.3 g of oil sample and the methylene chloride was evaporated. Prepared sample was saponified with 10 mL of 6 M solution of KOH in water and 15 mL of EtOH (ethanol with up to 5% diethyl ether). The solution was refluxed for 90 min at 70 °C (water bath at 85-90 °C).

Following saponification, 15 mL of water was added and non-saponifiable compounds were extracted, first with 22.5 mL of petroleum ether, and then with 22.5 mL of diethyl ether. The two extracts were combined and washed twice with 20 mL of 0.5 M solution of KOH in water and with 20 mL of 5% NaCl solution in water until the pH of the washing water was neutral.

The organic fraction was dried with anhydrous Na_2SO_4 and filtrated over folded filter paper into a conical flask. The residue obtained after evaporation was derivatized by adding 1.5 mL of dry pyridine as solvent, 0.2 mL hexamethyldisilazane and 0.1 mL trimethylsilane as silylation agents. The flask was placed in an oven at 70 °C for 30 min for completion of the silylation. The derivatized sample was transferred into a vial and was ready for GC analysis. All samples were analyzed within 6 hours after derivatization. GC and GC-MS analysis were performed on same instrument (Agilent 7890A GC). An HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used. The GC oven temperature was programmed to increase from 60 °C to 300 °C with 3 °C/min increments, each held for 10 min. The carrier gas (He) flow rate was 3.3 mL/min at 60 °C (constant pressure mode). The sample was analyzed in the splitless mode. The injection volume was 1 µL, GC detector temperature was 300 °C. MS data was acquired in EI mode, with scan range 30-550 *m/z*, source temperature of 230 °C and quadrupole temperature of 150 °C. Solvent delay was set at 13 min. Identification was confirmed by retention time lock (RTL) method and Wiley 07 and NIST 05 databases.

2.5. DPPH radical scavenging activity

The antioxidant activity of toluene solutions of the seed oils were tested against DPPH radicals using method described by Ramadan and Moersel (2006). Briefly, a volume of 1600 µL of 0.1 mM DPPH solution prepared also in toluene was added to 400 µL of the toluene solutions of the oils in concentrations of 0.1, 1.0, 10, and 100 µg/mL, vortexed for 10 s and left in the dark for 1 h at room temperature. Absorbance of the samples at 515 nm was then recorded using a CINTRA 40 GBC spectrophotometer. DPPH scavenging activity was determined from the equation:

$$\text{DPPH}\ (\%) = 1 - \frac{A_x}{A_0} \times 100$$

The same procedure was carried out in toluene, instead of oil, in order to measure A_0. The value A was the absorbance

Table 1. Total oil and fatty acid content in the cotyledons of Turkish hazel genotypes (%)

Compound/Genotype		A8	A9	A11	B6	B10	min	max	avg	SD
oil yild (%)		50.90	39.20	60.80	39.60	36.50	36.50	60.80	45.40	10.20
palmitic	C16:0	7.40	5.95	5.82	6.10	6.30	5.82	7.40	6.31	0.63
margaric	C17:0	0.44	0.04	0.06	0.07	0.04	0.04	0.44	0.13	0.17
stearic	C18:0	2.21	3.09	3.39	2.41	2.25	2.21	3.39	2.67	0.54
arachidic	C20:0	0.11	0.16	0.15	0.13	0.11	0.11	0.16	0.13	0.02
ΣSAFAs		10.16	9.24	9.42	8.71	8.70	8.18	10.16	9.24	0.74
palmitoleic	C16:1*E*	0.04	0.02	0.03	0.02	0.02	0.02	0.04	0.03	0.01
palmitoleic	C16:1*Z*	0.46	0.24	0.26	0.26	0.32	0.24	0.46	0.31	0.09
cis-10-heptadecenoic acid	C17:1	0.82	0.06	0.09	0.08	0.07	0.06	0.82	0.22	0.33
elaidic	C18:1	0.41	0.03	0.33	0.24	0.067	0.03	0.41	0.21	0.17
oleic	C18:1	80.14	81.34	79.34	79.57	83.00	79.34	83.00	80.68	1.51
gadoleic	C20:1	80.14	81.34	79.34	79.57	83.00	79.34	83.00	80.68	1.51
ΣMUFAs		82.03	81.88	80.22	80.34	83.62	80.22	83.62	81.62	1.40
linoleic	C18:2	8.81	8.78	10.20	10.81	7.52	7.52	10.81	9.22	1.30
linolenic	C18:3	0.09	0.07	0.11	0.09	0.09	0.07	0.11	0.09	0.01
ΣPUFAs		8.90	8.85	10.31	10.90	7.61	7.61	10.90	9.31	1.30
ΣPUFAs/ΣSAFAs/		0.87	0.96	1.09	1.25	0.87	0.87	1.25	1.00	0.16

SAFAs – saturated fatty acids (C16:0, C17:0, C18:0, C20:0); MUFAs – unsaturated fatty acids (C16:1, C17:1, C18:1, C20:1); PUFAs – polyunsaturated fatty acids (C18:2, C18:3).

Table 2. Sterol contents of five genotypes of Turkish hazelnut (mg/g oil)

Compound/Genotype	RI	A8	A9	A11	B6	B10	Mean	Mean (%)	Cv (%)
cholesta-3,5-dien-7-on	3227	0.11	0.15	0.13	0.10	0.20	0.14	2.55	29.77
campesterol	3284	0.30	0.28	0.20	0.19	0.22	0.24	4.36	20.03
stigmasterol	3314	0.08	0.10	0.12	0.07	0.11	0.10	1.80	22.78
n.i. (MW=490)	3352	0.21	0.15	0.16	0.13	0.14	0.16	2.90	19.96
β-sitosterol	3377	3.93	4.25	3.18	2.96	3.57	3.58	65.09	14.74
n.i. (MW=488)	3382	0.08	0.07	-	0.06	0.09	0.06	1.09	58.81
fucosterol	3395	0.73	0.71	0.60	0.45	0.49	0.60	10.91	21.60
gramisterol	3415	0.03	0.03	0.02	0.03	-	0.02	0.36	57.04
(3*β*)-lanosta-8,24-dien-3-ol	3432	0.05	0.04	0.02	0.04	0.13	0.06	1.09	74.94
cycloartenol acetate	3435	0.12	0.09	0.04	0.06	-	0.06	1.09	74.02
(3*β*)-cholest-5-ene-3,25-diol	3453	0.18	0.17	0.19	0.14	0.22	0.18	3.27	16.41
n.i. (MW=512)	3476	0.02	0.02	0.02	0.02	-	0.02	0.36	59.60
(9,19)-ciclolanostan-3-ol	3489	0.15	0.12	0.06	0.06	0.08	0.09	1.63	43.84
n.i. (MW=500)	3514	0.02	0.02	0.02	0.02	-	0.02	0.36	58.00
n.i. (MW=498)	3520	0.13	0.22	0.14	0.15	0.14	0.16	2.90	24.36
(3*β*)-acetoxyurs-12-en-28-al	3630	0.02	0.08	0.01	0.02	-	0.02	0.36	143.45
Total	-	6.16	6.50	5.10	4.50	5.39	5.50	-	-

n.i. indicates that the compound is not identified.

Table 3. Antioxidant activity of the Turkish hazelnut oils against DPPH radical

	EC_{50} (mL/mL)	EC_{50} (mg/mL)
A8	0.1189	107.01
A9	0.0867	78.03
A11	0.0999	89.91
B6	0.1228	110.52
B10	0.0879	79.11
BHT	/	1.10

of the remaining DPPH after a reaction with the oil solution. Each sample was measured in four different dilutions and every measurement was performed in triplicate and subsequently averaged. The EC_{50} value for each oil sample was determined from the graph DPPH (%) = f (c) constructed from four dilutions (concentrations). Butylated hydroxytoluene (BHT), a synthetic antioxidant, was used as reference.

2.6. Statistical analysis

Statistical analysis was performed using "Statistica 10" software (StatSoft. Inc., Tulsa, OK. USA).

3. RESULTS AND DISCUSSION

Fatty acid compositions of hazelnut oils are summarized in Table 1. The analyses of the Turkish hazel established a high total oil content, which ranged from 36.50% to 60.80%. The greatest amount of total oil was extracted from the A11 (60.80%) and A8 (50.90%) genotypes. The average oil content of our samples (45.40%) was lower than the 56.2–56.4% and 64.48–71.92% previously reported for Serbian and Turkish cultivar, respectively (Erdogan and Aygun, 2005; Miletić et al., 2007).

Oleic (79.34–83.00%) and linoleic acids (7.52–10.81%) were the major unsaturated fatty acids found in oil seeds of Turkish hazel, and comprised nearly more than 90% of the fatty acid composition in all nuts. Palmitic acid was the main saturated fatty acid (5.82–7.40%) followed by stearic acid (2.21–3.39%). Other saturated fatty acids detected were margaric and arachidic acids, but only in very small quantities (<0.5%). Saturated fatty acids comprised about 9.24% of the total in average.

In addition to fatty acid composition, their ratio is also important, in particular the ratio of monounsaturated and saturated, and polyunsaturated and saturated fatty acids (Table 1). Polyunsaturated fatty acids in the cotyledons participated by 7.61% to 10.90%, and saturated fatty acids by 8.18% to 10.16%. Ratio of polyunsaturated and saturated fatty acids in the samples was low and ranged from 0.87 to 1.24. The monounsaturated fatty acid content in Turkish hazel kernel was about ten times higher than the content of polyunsaturated and saturated acids (Table 1). The high nutritional value of Turkish hazel was partly due to the high ratio of monounsaturated and saturated fatty acids. Unsaturated fatty acids favorably affect blood cholesterol, regulate blood pressure, and enable the intake of Vitamin E (Alasalvar et al., 2003). The fatty acid profiles of our samples *C. colurna* were similar to literature data for *C. colurna* from Turkey (Erdogan and Aygun, 2005). The most common phytosterols, namely, β-sitosterol, campesterol, and fucosterol, were found in Turkish hazelnut oil. Among them, β-sitosterol comprised in average 65% (2.96–4.25 mg/g) of the total phytosterlols followed by fucosterol (0.45–0.73 mg g^{-1}) and campesterol (0.19–0.30 mg/g). β-sitosterol contents found in this study were three to four times higher than those reported in the oils extracted from different *C. avelana* cultivars (Alasalvar et al., 2009). Significant content of phytosterols is important knowing their role in reducing total and LDL cholesterol and decreasing the risk of certain forms of cancer (Alasalvar et al., 2003; Moreau et al., 2002). Sterol content of Turkish hazelnut are presented in Table 2.

The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. The decrease in absorbance at 515 nm is used as a measure of the scavenging effect of a particular extract for DPPH radicals. The DPPH values for the five Turkish hazel oil against the control (BHT) expressed as EC_{50} are shown in Table 3. The antioxidant activity of Turkish hazelnut oil was tested against DPPH radical at 0.1, 1.0, 10, and 100 mg/mL concentrations. The highest antioxidant activity was displayed by the oil from genotype A9 (EC_{50} = 78.03 mg/mL). The synthetic antioxidant BHT had EC_{50} of 1.10 mg/mL under the same conditions. The results showed that all of the oils exerted a similar scavenging effect towards DPPH.

CONCLUSION

In this work, composition of fatty acids and phytosterols in the oil obtained from cotyledons of Turkish hazel genotypes (*C. colurna* L.) was determined. High oil content was established, ranging from 36.50% to 60.80%. The predominant fatty acid in the analyzed Turkish hazel kernels was monounsaturated oleic acid, contributing by 79.34% to 83.00%. Among polyunsaturated acids, linoleic was predominant with content ranged from 7.52% to 10.81%. Phytosterol content analysis revealed β-sitosterol as dominant, with concentrations ranging from 2.96% to 4.25%. High content of monounsaturated fatty acids (oleic) favorable ratios of mono- and polyunsaturated to saturated fatty acids. High content of phytosterols recommend the oil of Turkish hazel kernels as high quality nutritional and medicinal food.

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