Fatty Acid Distribution in Polar and Nonpolar Lipid Classes of Hazelnut Oil (*Corylus avellana* L.)

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The lipid extract from hazelnuts was classified as polar (glucolipids = 1.4% and phospholipids = <1%) or nonpolar (triacylglycerides = 98.4%) by column and thin-layer chromatography. Fatty acids derivatized as methyl esters were analyzed by gas chromatography with flame ionization detector and mass spectrometer. Oleic acid was predominant in all lipid classes, followed by linoleic, palmitic, and stearic. Myristic, palmitoleic, hexadecadienoic, linolenic, eicosanoic, and eicosenoic acids were also present in minor proportions. Monounsaturated fatty acids predominated in nonpolar lipids, whereas saturated and polyunsaturated acids were more prominent in polar lipids. Fatty acid contents were compared by ANOVA. Significant differences were found for fatty acid contents in the different lipid classes ($p < 0.05$). A negative correlation was found between monounsaturated−polyunsaturated and monounsaturated−saturated fatty acids.

Keywords: *Hazelnut oil; nonpolar and polar lipids; fatty acid distribution*

INTRODUCTION

Hazelnuts (*Corylus avellana* L.) are cultivated in several Mediterranean countries (Turkey, Italy, and Spain) and in some areas of Oregon and Washington (U.S.A.). Hazelnuts are a source of energy in the Mediterranean diet due to the 60% of oil content. Some authors have reported beneficial effects of nut consumption in the human diet (Abbey et al., 1994; Fraser et al., 1992; Sabaté, 1993; Sabaté et al., 1993). These effects could be related to the fatty acid profile of nut lipids, which are rich in mono- and polyunsaturated fatty acids. Moreover, other authors have reported that dietary oleic acid reduces the risk of coronary heart disease (Berry et al., 1991; Nydahl et al., 1994; Wahrburg et al., 1992).

Hazelnuts are high in mono- and polyunsaturated fatty acids, especially oleic and linoleic (Garcia et al., 1979; Gargano et al., 1981; Hadorn et al., 1977; Hadorn and Zurcher, 1967; Zurcher and Hadorn, 1975). However, no report was found on the lipid classes in hazelnuts or their fatty acid composition. The results presented here may provide more data on the effects of hazelnut consumption in the human diet (Bracco, 1994; Katan et al., 1994; Kritchevsky, 1994).

MATERIALS AND METHODS

Oil Extraction (Kates, 1986). Ten grams of fresh ground hazelnut kernels (variety Casina, 30 days after harvesting) were extracted using a Polytron homogenizer (Kinematica AG, Littau, Switzerland) at high speed for 2 min using a mixture of 50 mL of chloroform/methanol (2:1). The homogenate was filtered with suction, and the filtered residue was rehomogenized with the same volume of the chloroform/methanol mixture. A second homogenate was suctioned, and the two filtrates were combined; 20 mL of a 0.6% (w/v) sodium chloride solution was added, and the phases were vigorously shaken. After phase separation, the chloroform layer was withdrawn, dried with sodium sulfate, and filtered. The organic phase was concentrated under vacuum and transferred to a screw-capped glass tube. Finally, the chloroform was completely removed under a stream of nitrogen.

Column Chromatography (CC) (Gellerman et al., 1975). Thirty-five grams of silicic acid (100−200 mesh, Unisil, Clarkson Chemical Co., Williamsport, PA) was activated overnight at 110 °C for 12 h. The absorbent was stirred in petroleum ether, bp 60−70 °C, and then the mixture was poured into a 50 cm chromatographic column (2.5 cm i.d.). The column was loaded with a solution of 0.5 g of freshly extracted oil dissolved in 6 mL of chloroform. Finally, a layer of glass beads was placed on top of the absorbent. The elution scheme was as follows: (1) 250 mL of hexane/diethyl ether (99:1), (2) 150 mL of hexane/diethyl ether (96:4), (3) 450 mL of hexane/diethyl ether (96:4), (4) 280 mL of chloroform, (5) 100 mL of acetone/chloroform (1:1), (6) 70 mL of acetone, (8) 100 mL of acetone/methanol (7:3), (9) 50 mL of methanol, and (10) 225 mL of methanol.

Identification of Lipid Classes. The fractions eluting from the column were identified on silica gel G plate (Merck, Gibbstown, NJ) by thin-layer chromatography (TLC) (20 cm × 20 cm × 0.5 mm).

Nonpolar lipids were run on a plate using a solvent system composed of hexane/diethyl ether/concentrated acetic acid (80:20:1). After development, the mobile phase was allowed to evaporate. Triacylglycerides were visualized as yellow-brown spots by exposing the plate to iodine vapor ($R_f = 0.8$) (Kates, 1986). Standard triolein was run on the same plate as a control.

Glucolipids were separated by TLC using a solvent system composed of chloroform/acetone/water (60:30:2). After development, the plate was sprayed with a chromic−sulfuric acid mixture (Kates, 1986), and glucolipids were then detected as pink spots ($R_f = 0.8$). Pure monogalactosyldiacylglycerol (MGDG) was used as a standard; this glucolipid was isolated and identified from plant material following the procedure proposed by Gardner (1968).

Phospholipids were detected by TLC using a solvent system composed of chloroform/methanol/concentrated ammonia (65:30:4). Phospholipids were detected as blue spots by spraying

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Table 1. Percentages of Fatty Acids, SFA, MUFA, and PUFA of Lipid Fractions Extracted from Hazelnuts[a]

lipid class	fatty acids												
	14:0	16:0	16:1	16:2	18:0	18:1	18:2	18:3	20:0	20:1	SFA[2]	MUFA	PUFA
TG[c]	0.03[a]	5.75[a]	0.22[a]	0.04[a]	3.03[a]	80.02[c]	10.58[a]	0.05[a]	0.13[a]	0.14[a]	8.94[a]	80.39[c]	10.89[a]
SE	0.00	0.00	0.00	0.00	0.003	0.03	0.03	0.00	0.00	0.008	0.003	0.02	0.03
MGDG	0.46[b]	11.45[b]	0.32[b]	0.47[a]	4.51[c]	55.26[b]	22.41[b]	0.68[c]	1.27[b]	3.17[b]	17.69[b]	58.75[b]	32.88[b]
SE	0.01	0.06	0.01	0.00	0.015	0.04	0.05	0.00	0.17	0.011	0.09	0.04	0.05
PI	0.43[b]	16.06[d]	0.24[a]	6.38[b]	4.23[b]	46.99[a]	23.03[c]	1.47[d]	0.00[a]	1.18[a]	20.72[c]	48.41[a]	31.12[c]
SE	0.03	0.03	0.003	0.26	0.014	0.16	0.05	0.01	0.00	0.012	0.06	0.17	0.22
PC	0.43[b]	13.12[c]	0.34[b]	0.45[a]	4.03[b]	46.45[a]	32.83[d]	0.38[b]	0.75	1.18[a]	18.33[d]	48.03[a]	34.00[b]
SE	0.01	0.89	0.017	0.01	0.22	4.36	5.25	0.15	0.43	0.98	0.23	5.32	5.10

[a] Data are means of triplicate determinations. [b] a−d denote statistically significant differences for fatty acid contents between lipid classes ($p < 0.05$). SFA (14:0 + 16:0 + 18:0 + 20:0), MUFA (16:1 + 18:1 + 20:1), PUFA (16:2 + 18:2 + 18:3). [c] TG, triacylglicerols; MGDG, monogalactosyldiacylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine, SE, standard error.

the plate with a phosphomolybdic acid solution (Kates, 1986). Pure phosphatidylinositol and phosphatidylcholine were spotted on the same plate as standards.

Analysis of Fatty Acids. Fatty acid methyl esters (FAME) were prepared from nonpolar and polar lipid classes according to a modified method of Slover and Lanza (1979). Pure nonpolar and polar lipids isolated by column chromatography were saponified with a solution of sodium methoxide in methanol [0.5 M] at 100 °C in a water bath for 10 min; the solution was cooled, and 2 mL of 12% (w/w) boron trichloride in methanol was added. The solution was heated for a further 10 min in a boiling-water bath. After cooling, 1 mL of hexane was added, the mixture was shaken vigorously, and 1 mL of 0.6% (w/v) sodium chloride was poured over. The organic layer was transferred to a screw-capped test tube with a Pasteur pipet. The organic solution was dried with anhydrous sodium sulfate and filtered. Finally the filtrate was concentrated under a stream of nitrogen.

FAME were analyzed by gas chromatography (GC) coupled to mass spectrometry (MS). The sample (1 μL) was injected into the GC, a Hewlett-Packard 5890 Series II (Little Falls, Willmington, DE) with a flame ionization detector (FID). The GC was equipped with two columns: column A, which was connected to the FID, and column B, which was connected to the MS detector; both were 60 m Supelcowax 10 columns (Supelco, Bellefonte, PA) coated with poly(ethylene glycol) (0.25 mm i.d., 0.25 μm film thickness). The oven temperature was programmed as follows: 180 °C for 2 min, raised to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, and then raised to 215 °C at 2 °C/min. The final oven temperature was maintained for 10 min. The injector and detector temperatures were 200 and 250 °C, respectively. Helium was used as carrier gas at a flow rate of 1.5 mL/min (47.5 psi). FAME eluting from column B passed into a mass spectrometer, a Hewlett-Packard 5971 using electron impact ionization with an ion source temperature of 200 °C.

Samples were injected into the column inlet using a Hewlett-Packard 7673 automatic injector.

FAME were identified by comparison of their retention time, equivalent chain length (Hofstetter et al., 1965), and mass spectrum (Murphy, 1993; Hallgren et al., 1959) with those of standard FAME. FAME were quantified according to their percentage area obtained by integration of the peak as a semiquantitative method (Parcerisa et al., 1995).

Reagents and Standards. The solvents used (acetone, diethyl ether, chloroform, methanol, petroleum ether, and hexane) were all reagent grade from Fisher (Pittsburgh, PA). Anhydrous sodium sulfate, concentrated ammonia, and concentrated acetic acid were all analytical grade from Fisher. Sodium methoxide in methanol [0.5 M] was purchased from Aldrich (Milwaukee, WI); 12% (w/w) boron trichloride in methanol was purchased from Supelco. Pure triolein (99%) and standard FAME, which included caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, behenic, erucid, and lingnoceric fatty acid methyl esters, were purchased from Sigma (St. Louis, MO). Pure phosphatidylinositol and phosphatidylcholine were obtained from Supelco.

Statistical Analysis. The statistical analysis was performed by the one-way analysis of variance (one-way ANOVA). All analyses were carried out using the Statistical Package for Social Sciences (SPSS/PC+) (Hispanoportuguesa SPSS, S.L., Madrid, Spain) v 6.0 for Microsoft Windows 3.11 (Microsoft Ibérica, S.R.L., Madrid, Spain).

RESULTS

Lipid Composition. Three main lipid classes were isolated by column chromatography, i.e., triacylglycerols (TG) as nonpolar lipids and glucolipids and phospholipids as polar lipids. The total amount of TG eluted by CC with hexane/diethyl ether (96:4) was 492.24 mg. Glucolipids, 7.1 mg, were eluted with acetone/chloroform (1:1). The main polar compound was MGDG. Traces of other glucolipids were revealed by TLC (see Materials and Methods), although they were very unstable. Phospholipids were eluted with pure methanol; however, the total amount of phospholipids was <1 mg. Two compounds were identified as phospholipids: phosphatidylinositol (PI) and phosphatidylcholine (PC). No traces of other lipid classes were found by column chromatography.

Fatty Acid Composition. Table 1 shows the fatty acid composition of polar and nonpolar lipid classes (three replicates). Fatty acids were myristic (14:0), palmitic (16:0), palmitoleic (16:1), hexadecadienoic (16:2), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), eicosanoic (20:0), and eicosenoic (C20:1). Lauric acid (12:0) was detected in trace amounts (not included in Table 1). Table 1 also shows percentages of saturated (myristic + palmitic + stearic + eicosanoic), monounsaturated (palmitoleic + oleic + eicosenoic), and polyunsaturated (hexadecadienoic + linoleic + linolenic) fatty acids of lipid classes.

Oleic acid was the main fatty acid in all lipid classes; TG showed the highest percentage for oleic acid (80.02%), whereas polar lipids presented lower percentage of oleic acid: MGDG showed the highest percentage for oleic acid (55.26%), whereas PI and PC presented lower percentages (46.99 and 46.45%, respectively). Linoleic acid was most predominant in polar lipids; according to this, PC and PI showed the highest percentages (32.83 and 23.03%, respectively), whereas MGDG showed 22.42% for linoleic acid; the lowest percentage was found in TG for linoleic acid (10.58%). Palmitic acid showed the highest percentage in polar lipids (PI = 16.06%, PC = 13.12%, and MGDG = 11.45%, respectively), while TG had the lowest percentage (5.75%). Stearic acid also showed the highest percentages in polar lipid fraction (MGDG = 4.51%, PI = 4.23%, and PC = 4.03%, respectively), whereas TG showed 3.03% of stearic acid. Hexadecadienoic acid showed the highest percentage in PI (6.38%); eicosenoic acid showed the highest percentage in MGDG (3.17%). Myristic, palmitoleic, linolenic, and eicosanoic fatty acids were found in small percentages.

Table 2. Correlation Coefficients (*r*) and Significance Levels (*p*) for Fatty Acid Correlation Analysis

	16:0	16:1	16:2	18:0	18:1	18:2	18:3	20:1
14:0	−0.577	−0.577	−0.333	−0.510	−0.587	0.574	0.577	0.133
	NS[a]	NS	NS	NS	NS	NS	NS	NS
16:0		0.999	0.577	0.994	0.999	−0.999	−0.999	−0.688
		<0.001	NS	0.006	<0.001	<0.001	<0.001	NS
16:1			0.577	0.994	0.999	−0.999	−0.999	−0.688
			NS	0.006	<0.001	<0.001	<0.001	NS
16:2				0.638	0.608	−0.603	−0.577	−0.927
				NS	NS	NS	NS	NS
18:0					0.995	−0.996	−0.994	−0.760
					0.005	0.004	0.006	NS
18:1						−0.999	−0.999	−0.708
						<0.001	<0.001	0.292
18:2							0.999	0.710
							<0.001	NS
18:3								0.688
								NS

[a] NS, not significant.

Monounsaturated fatty acids (MUFA) presented the highest percentage in TG (80.39%). Regarding polar lipids MGDG showed the highest content for MUFA (58.75%), whereas the phospholipid fraction showed the lowest percentages (PI = 48.41% and PC = 48.03%, respectively). The saturated fatty acid (SFA) fraction was predominant in polar lipids; phospholipids showed higher percentages (PI = 20.72% and PC = 18.33%, respectively) than MGDG (17.69%); TG presented the lowest percentage for SFA fraction (8.94%). Polyunsaturated fatty acids (PUFA) were predominant in polar lipids (PC = 34.00%, MGDG = 32.88%, and PI = 31.12%, respectively), whereas TG showed the lowest percentage for PUFA fraction (10.89%).

Fatty acid percentages were compared between lipid classes using the one-way ANOVA (Table 1). Palmitic, linoleic, and linolenic fatty acid contents revealed significant differences ($p < 0.05$) between lipid classes.

SFA and PUFA fractions showed significant differences between TG, MGDG, PI, and PC lipid classes. MUFA revealed significant differences between TG, MGDG, and phospholipids; nevertheless, no significant differences were found between PI and PC for monounsaturated fatty acids (Table 1).

A correlation analysis between fatty acid contents was performed; results for the correlation coefficient (r) and significance level (p) are presented in Table 2. Only the main fatty acids in each lipid class have been pointed out. Oleic acid showed a negative correlation coefficient with linoleic acid, whereas oleic acid showed a positive correlation to stearic and palmitic. Linoleic acid showed a positive correlation to stearic and palmitic acid. Myristic, hexadecadienoic, and eicosenoic fatty acids did not reveal any significant correlation.

SFA fraction revealed a positive correlation coefficient between PUFA ($r = 0.9019$, $p < 0.0001$), whereas there was a negative correlation coefficient between MUFA and SFA ($r = -0.9562$, $p < 0.0001$). MUFA and PUFA revealed a negative correlation coefficient ($r = -0.9888$, $p < 0.0001$).

DISCUSSION

TG, the most important lipid class in hazelnut oil, shows a high percentage for oleic acid, whereas linoleic, palmitic, and stearic acids show smaller percentages. This is consistent with the results published by Bazan (Bazan et al., 1975), who studied fatty acid distribution in the hazelnut triacylglycerol fraction. Oleic acid is also predominant in polar lipids (MGDG, PI, and PC); however, the proportions decrease according to the following trend: TG > MGDG > PI > PC. Linoleic acid percentages are higher in MGDG, PI, and PC than in TG; therefore, it follows the opposite trend to oleic acid (PC > PI > MGDG > TG). Palmitic and stearic percentages are higher in polar lipids than in nonpolar lipids, following the same tendency as linoleic acid.

SFA and MUFA percentages are higher in polar lipids, while MUFA percentages are higher in TG. SFA and PUFA follow the opposite trend to MUFA. Although there is no evident explanation for this, some authors have suggested that polar lipids such as glucolipids and phospholipids are substrates for fatty acid desaturases in oilseeds, which are responsible for MUFA and PUFA biosynthesis in oilseeds (Andrews and Heinz, 1987; Schmidt and Heinz, 1990, 1993; Slack et al., 1979; Stymme and Appleqvist, 1978; Wilson et al., 1980). On this basis we could expect the negative correlation coefficient found between mono- and polyunsaturated fatty acid contents. It has been suggested that PC provides all the diacylglycerol from which TG is synthesized; therefore, it might be expected in the developing cotyledons from plants grown at constant temperature that the acyl composition at positions sn-1 and sn-2 of TG should resemble the fatty acid composition at positions sn-1 and sn-2 of phospholipids (Slack, 1983). It would thus be interesting to characterize the positional distribution of fatty acids in hazelnut lipid classes. However, this research has not yet been carried out on hazelnut oil.

Our results, however, do not show the fatty acid distribution within TG, MGDG, PI, and PC molecules, which remains for further investigation.

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