Lipid Composition and Oxidative Stability of Oils in Hazelnuts (*Corylus avellana* L.) Grown in New Zealand[1]

G.P. Savage[a,*], D.L. McNeil[a], and P.C. Dutta[b]

[a]Food Group, Lincoln University, Canterbury, New Zealand, and [b]Department of Food Science, Swedish University of Agricultural Sciences, S-750 07, Uppsala, Sweden

ABSTRACT: Hazelnut (*Corylus avellana* L.) samples were collected from six different cultivars of trees grown in an experimental orchard at Lincoln University. Three U.S. commercial cultivars (Butler, Ennis, and Barcelona), two European commercial cultivars (Tonda di Giffoni and Campanica), and one New Zealand selection (Whiteheart) were evaluated. The total oil, stability to oxidation of the oil, and fatty acid, tocopherol, and sterol composition were determined on samples of freshly extracted hazelnut oil. The total oil content of the seeds ranged from 54.6 to 63.2% while the stability of the oil, as measured by the Rancimat test ranged from 15.6 to 25.3 h. The content of the monounsaturated oleic acid in the oils ranged from 73.8 to 80.1% of the total fatty acids, while the tocopherol content ranged from 225.8 to 552.0 mg/g freshly extracted oil. The major desmethylsterols were sitosterol, ranging from 1416 to 1693 μg/g, campesterol, ranging from 78 to 114 μg/g, and Δ5-avenasterol, ranging from 110 to 170 μg/g. The oil extracted from the cultivar Whiteheart was more stable (measured by Rancimat) than the oil from all other cultivars grown at the same location and under the same conditions. Whiteheart contained higher levels of total and γ-tocopherol when compared to the other cultivars. The higher levels of tocopherol in Whiteheart help to explain the greater stability of the oil during the oxidative stress test. These results suggest that nuts from the cultivar Whiteheart could be stored longer than the other nuts tested. *JAOCS 74*, 755–759 (1997).

KEY WORDS: *Corylus avellana* L., fatty acids, hazelnut, lipids, oxidative stability, sterols, tocopherol.

The major storage reserve in hazelnut (*Corylus avellana* L.) is triacylglycerols, which can account for up to 60% of the dry weight of seed. Previous studies have reported on the influence of geographical origin and environmental conditions on the lipid composition (1–3) of some hazelnut varieties cultivated in Spain. Bonvehi and Coll (3) suggested that the high linoleic acid content of hazelnut oil was the main cause of auto-oxidation and that the levels found in nut oils were modified by environmental growing conditions. They also highlighted the variation in oil stability that can be observed in different cultivars of hazelnuts but only briefly mentioned the possible effects of natural antioxidants, such as tocopherols, found in hazelnuts. In an earlier report (4) on the tocopherol content of hazelnuts (*C. avellana*) grown in France, it was shown that the levels were relatively low (ranging from 335 to 420 mg/kg oil) but significantly higher in hybrids of *C. avellana* (range 602 to 690 mg/kg). The stability of hazelnuts in various storage conditions was considered by Hadorn *et al.* (5). The most important factors during storage were temperature and humidity, while the composition of the nuts had some effect on how the nuts responded to more adverse conditions (5).

The major cause of reduction in palatability of stored hazelnuts is oxidation of their oils, and an investigation of this phenomenon forms part of an ongoing series of experiments. The present study is a preliminary investigation of the fat and fatty acid compositions and fat stability of six of the potentially most useful cultivars of hazelnuts that can be grown in the Canterbury region of New Zealand. These data may help in the selection of the most useful cultivars for future commercial production in the region.

MATERIALS AND METHODS

Nut samples. A replicated hazelnut variety trial was planted at Lincoln University in 1985. The experimental planting consisted of several different hazelnut cultivars, which had been imported from a number of different growing areas of the world or selected locally. Three U.S. commercial cultivars (Butler, Ennis, and Barcelona), two European commercial cultivars (Tonda di Giffoni and Campanica), and one New Zealand selection (Whiteheart) were evaluated. The trees were planted as a completely randomized design with six replications. The trial was planted in Templeton Silt Loam, and the entire block was surrounded by guard trees to act as pollenizers. Details of the management practices and climate conditions are given elsewhere (6). The harvesting and drying conditions followed the standard methods used at Oregon State University (7). The hazelnuts analyzed in this

[1]Presented as a poster at the 87th AOCS Annual Meeting, Indianapolis, Indiana, April 28–May 1, 1996. E-mail: savage@lincoln.ac.nz.

*To whom correspondence should be addressed at Lincoln University, PO Box 84, Canterbury, New Zealand.

experiment were harvested in April and May 1995 with harvests at weekly intervals; chemical analysis was carried out on bulked harvest samples.

Total fat content. The total fat content was determined in accordance with AOAC method 7.061 (8).

Lipid extraction. Finely chopped nuts (10 g) were extracted with 30 mL hexane/isopropanol (3:2, vol/vol) at room temperature under vigorous horizontal shaking for 1 h in steel tubes, which contained four steel balls to facilitate homogenization of the seeds (9). The homogenates were filtered through defatted filter papers on a Buchner funnel under vacuum, and the residues were washed twice with 20 mL of the same solvent; thereafter 35 mL of 6.7% sodium sulfate was added, and the upper layer was rotary-evaporated under reduced pressure at 40°C. The pure oil was stored at 4°C until analysis commenced the following day.

Oxidative stability of hazelnut oil by Rancimat. Extracted oil (2.5 g) was weighed into a 25 × 150 mm test tube and connected to a Rancimat 679 (Metrohm, Herisau, Switzerland). Air was passed through the samples at 15 L/min while the oil was heated to 110°C. The gases released during oxidation of the oil sample were carried into a cell that contained 60 mL water. The change in conductivity of the cell was plotted on a graph for 28 h. The oxidative stability was taken as the time that corresponded to the point of intersection of the two parts of the graph (the linear part at the beginning of the analysis and the exponential part at the end of the analysis). All analyses were carried out in triplicate.

Preparation of fatty acid methyl esters (FAME). Approximately 20 mg of extracted oil was treated with 2 mL 0.01 M NaOH in dry methanol at 60°C for 30 min under continuous shaking, essentially as described by Appelqvist (10). The tubes were cooled under running water, and 2 mL of 10% NaHSO4/25% NaCl in water (1:1) and 1.183 mg $C_{21:0}$ methyl ester (Nu-Chek-Prep, Elysian, MN) as internal standard were added. Thereafter, 3 mL water and 1 mL hexane were added. The tubes were shaken vigorously and left to stand to allow the layers to separate. The upper hexane layer with the FAME was transferred to a small tube and stored at −20°C for later analysis by capillary–column gas–liquid chromatography (GC).

FAME analysis by GC. For this purpose, a 50 m × 0.22 mm, 0.25-μm film thickness fused-silica capillary column BPX70 (SGE, Austin, TX) was connected to a Varian 3700 gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector and split/splitless injector. Helium was used as the carrier gas at a velocity of 23 cm/s, and as the makeup gas at a rate of 30 mL/min. A temperature program of 158°C for 5 min, rising to 220°C at a rate of 2°C/min, was used. The FAME, dissolved in hexane, was injected (1 μL) in a split mode of injection at a split ratio of 40:1. The injector and detector temperatures were 230 and 250°C, respectively. A Varian 4270 integrator was used for recording the peak areas. No response factors were applied in calculating fatty acid composition because the GC temperature program showed almost equal responses for different FAME standard mixtures. Known amounts of $C_{21:0}$ methyl ester were used as internal standard to calculate the absolute weight of the fatty acids in the oil samples. All analyses were carried out in duplicate.

Analyses of tocopherol by high-pressure liquid chromatography (HPLC). Oil samples weighing 0.5 g were dissolved in 5.0 mL *n*-heptane, and 20 μL was injected into a 10 × 0.46 cm Spherisorb 3-mm NH_2 column, fitted onto an LDC III liquid chromatograph (Shimadzu, Kyoto, Japan), equipped with a Shimadzu RF 535 fluorescence detector set at 290/330 nm. The mobile phase was 0.3% isopropanol and 5% *tert*-butylmethyl ether in *n*-heptane at a flow rate of 1.6 mL/min. The relative amounts of each tocopherol were calculated by using standard samples, and a Shimadzu integrator was used to calculate the peak areas.

Saponification for sterol analysis. For this purpose, *ca.* 20 mg of total lipids and 20 mg 5α-cholestane (Sigma Chemical Co., St. Louis, MO) as internal standard were mixed thoroughly with 1 mL 2 M KOH in 95% ethanol in ground-glass stoppered tubes. The tubes were kept for 45 min at 60°C in a glycerol bath under shaking. The reaction was stopped by cooling the tubes under running cold water, and 1 mL water, 2 mL hexane, and 0.1 mL ethanol were added. The tubes were shaken vigorously and then centrifuged briefly in a Hettich centrifuge EBA 12 (Hettich, Tuttlingen, Germany). The hexane layer was transferred to small glass tubes, dried under nitrogen, and derivatized to trimethylsilyl (TMS) ethers for subsequent analyses by GC as described below.

Preparation of TMS ether derivatives of sterols. Total unsaponifiables were derivatized to TMS ethers by adding 100 mL of Tri-Sil reagent (Pierce Chemical Co., Rockford, IL) and incubating the tubes at 60°C for 45 min. Thereafter, the solvent was evaporated under a stream of nitrogen, and the TMS ether derivatives were dissolved in 1 mL hexane. The tubes were sonicated in an ultrasonic bath for 1 min and centrifuged for 3 min. The hexane layer was transferred to another tube, avoiding any solid particles, evaporated to dryness, and dissolved in 0.5 mL hexane for further analyses by GC.

Analyses of sterols by GC. For this purpose, a fused-silica capillary column CP-Sil-5 CB (Chrompack, Middelburg, The Netherlands), 30 m × 0.25 mm, 0.25-mm film thickness, was used. The column was connected to a Varian 3700 gas chromatograph equipped with a flame-ionization detector and a falling needle injector. Helium was used as the carrier gas at an inlet pressure of 17 psi and as makeup gas at a flow rate of 30 mL per min. The GC oven was run with a temperature program of 258°C for 31 min, rising to 270°C at a rate of 3°C, and held for an additional 15 min. The detector temperature was at 300°C. The peaks were computed by an HP 3396A integrator (Hewlett-Packard, Avondale, PA). The desmethylsterols were identified by comparing the relative retention times of 5α-cholestane with those of standard cholesterol, campesterol, stigmasterol, and sitosterol. The other desmethylsterols were identified by comparing the elution pattern of the peaks from the GC, along with samples rich in

Δ7-stigmasterol and Δ5-avenasterol, *viz.* safflower oil and oat lipids. No response factors were used. All samples were analyzed in triplicate, and mean results are reported.

Analytical thin-layer chromatography (TLC). For this purpose, precoated TLC plates, silica gel 60, 20 × 20 cm, 0.25-mm thickness, were used (Merck, Darmstadt, Germany). The pattern of lipid composition and the completeness of FAME preparation and saponification were checked by analytical TLC. The FAME and the unsaponifiables, along with TLC reference standard 18-4 A (Nu-Chek-Prep) were spotted on a TLC plate and developed in the solvent system hexane/diethyl ether/acetic acid (85:15:1, vol/vol/vol). After the solvent front had reached the top, the plate was taken out of the TLC chamber and dried in air for a short time and then sprayed with phosphomolybdate reagent. Color development was effected by heating the plates at 120°C for 15 min.

Solvents and reagents. Ethanol (Kemetyl, Stockholm, Sweden) and other solvents and reagents were of analytical grade (Merck) unless otherwise stated.

RESULTS AND DISCUSSION

The total oil content of the kernels ranged from 54.6 to 63.2%, while the stability of the freshly extracted oil ranged from 15.6 to 25.3 h (Table 1). The results of oil content and oxidative stability, presented as induction time in this study, concur with previous reports on different hazelnut cultivars (2,5,11–14).

The levels of tocopherol in New Zealand-grown nuts (Table 2) were generally higher than those found in hazelnuts grown in France (4) or Oregon, United States (14), but some allowance has to be made for the improved techniques of extraction and analysis used in this study. The α-tocopherol contents of the nuts were similar to values reported earlier (4,13). In the report by Pershern *et al.* (14), γ-tocopherol was shown to be present in trace amounts in domestic cultivars, whereas a wild hazelnut *C. cornuta* contained 85.5 μg/g of γ-tocopherol. The six cultivars analyzed in this study had 19 to 149 μg/g of γ-tocopherol in the oils, with Whiteheart being 428% higher than Barcelona, the next highest cultivar (Table 2). This higher level of γ-tocopherol may be the main factor contributing to the higher oxidative stability index in the cultivar Whiteheart because it has been shown that γ-tocopherol has a higher antioxidant capacity in model systems than α-tocopherol (15). Analysis of these results showed that no significant regression between total or α-tocopherol and oxidative stability exists. There was, however, a significant regression correlation ($r^2 = 0.85$; $P < 0.05$) between γ-tocopherol and oxidative stability. Most of this result was due to the substantially higher γ-tocopherol value of Whiteheart.

TABLE 1
Total Oil and Oxidative Stability, Measured by the Rancimat System, of Oil Extracted from Hazelnut Cultivars Grown in New Zealand[a]

Cultivar	Total oil (g/100 g DM[b])	Oxidative stability (h)
Whiteheart	61.0	25.3
Barcelona	58.3	15.9
Butler	57.1	15.6
Ennis	54.6	18.6
Tonda di Giffoni	63.2	17.9
Campanica	56.1	18.7

[a]Means of triplicate analyses.
[b]Dry matter.

The major fatty acids in hazelnuts, as determined by capillary–column gas chromatography (Table 3), were palmitic, stearic, oleic, and linoleic acids; oleic acid (18:1) accounted for 73.8 to 80.0% of the total fatty acids. The total saturated fatty acids made up a small proportion (<8.0%) of the total fatty acid content of the oil, while the polyunsaturated fatty acid (PUFA) content (18:2 + 18:3) of the oil was relatively low (Table 4). These results are comparable to data previously reported in the literature (1–3,5,11,12,14,16,17), except that this is the first study to report the presence of *cis*-vaccenic acid ($18:1^{\Delta 11}$) in hazelnut oil (Table 3). The presence of short-chain and medium-chain fatty acids (from 4:0 to 12:0) was reported in hazelnut oil (14). No efforts were made to analyze these fatty acids in this study because analyses of short-chain fatty acids require different techniques. The pattern of total lipids, checked by preparative TLC, showed that triacylglycerols were the major lipid component in hazelnut oil examined in this study. Analytical TLC was done after methylation and saponification and confirmed that the reactions were complete and that the samples were not contaminated.

The composition of fatty acids did not appear to vary substantially between different cultivars. Within the limits of the slightly different analytical methods used around the world, the composition of hazelnut oil is uniform. In the present study, more individual fatty acids were observed as the result of using improved separation and detection methodology. A significant relationship between the stability of the oil and linoleic acid composition was demonstrated previously with the Rancimat method for a short time with the test temperature set at 120°C (3). In this study, no clear relationship existed between the oxidative stability of the oils, measured by the Rancimat method with the temperature set at 110°C over an extended evaluation time, and the linoleic acid content (Tables 1 and 4). However, if Whiteheart was excluded, there was a significant regression relationship ($r^2 = 0.41$; $P < 0.05$) between oxidative stability and PUFA. The oil extracted from Whiteheart proved to be significantly more stable in an oxidation test than any of the other hazelnuts. The improved stability was not due to any change in the fatty acid content of the hazelnut oil and is more likely to be due to changes in the total and individual contents of tocopherols in hazelnut oil.

The major sterols were sitosterol, ranging from 1416 to 1693 μg/g; campesterol, ranging from 78 to 114 μg/g; and Δ5-avenasterol, ranging from 110 to 170 μg/g. Other minor components were Δ7-avenasterol, at 37 to 60 μg/g; Δ7-stigmasterol, at 21 to 58 μg/g; and a small amount of cholesterol, at 7

TABLE 2
The Tocopherol Content of Oil Extracted From Hazelnut Cultivars Grown in New Zealand

	μg/g Oil (% composition)[a]				
Cultivar	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Whiteheart	379.8	16.7	148.9	6.6	552.0
	(68.8)	(3.0)	(27.0)	(1.2)	
Barcelona	409.0	11.0	28.2	1.7	449.9
	(90.9)	(2.4)	(6.3)	(0.4)	
Butler	337.0	12.9	18.6	2.6	371.1
	(90.8)	(3.5)	(5.0)	(1.0)	
Ennis	199.1	6.2	18.7	1.8	225.8
	(88.2)	(2.7)	(8.3)	(0.8)	
Tonda di Giffoni	403.9	13.0	27.8	2.5	447.2
	(90.3)	(2.9)	(6.2)	(0.6)	
Campanica	310.3	7.2	27.8	1.0	346.3
	(89.6)	(2.1)	(8.0)	(0.3)	

[a]Mean of duplicate analyses.

TABLE 3
Fatty Acid Composition (% of total) of Oil Extracted from Hazelnut Cultivars Grown in New Zealand[a]

Cultivar	16:0	16:1	17:0	17:1	18:0	$18:1^{\Delta 9}$	$18:1^{\Delta 11}$	18:2	18:3	20:0	20:1
Whiteheart	4.08	0.12	0.04	0.08	2.03	80.07	0.87	12.13	0.12	0.11	0.15
Barcelona	4.72	0.15	0.04	0.08	1.89	77.17	1.05	14.50	0.14	0.09	0.15
Butler	4.61	0.15	trace	0.08	1.86	73.80	1.08	16.53	0.16	0.09	0.14
Ennis	5.94	0.24	0.24	0.10	1.62	75.42	1.18	14.46	0.14	0.08	0.12
Tonda di Giffoni	4.83	0.16	0.04	0.09	1.95	78.97	1.02	12.53	0.12	0.10	0.14
Campanica	4.99	0.15	0.04	0.07	1.92	79.57	0.89	11.96	0.15	0.08	0.13

[a]Means of duplicate analyses. Trace amounts of 14:0 and 22:0 were present in all cultivars; these fatty acids made up <0.04% of the total fatty acids.

TABLE 4
Summary of the Important Fatty Acid Parameters of Oil Extracted from Hazelnut Cultivars Grown in New Zealand

		Total saturated acids	Total unsaturated acids	Ratio
Cultivar	PUFA[a]	(g/100 g)		unsaturated/saturated
Whiteheart	12.3	6.3	93.7	14.8
Barcelona	14.6	6.7	93.1	13.9
Butler	16.7	6.6	91.8	14.0
Ennis	14.6	7.6	91.5	12.0
Tonda di Giffoni	12.7	6.9	92.9	13.5
Campanica	12.1	7.0	92.8	13.2

[a]Polyunsaturated fatty acids.

to 12 μg/g (Table 5). Sterol levels found in this study concur with other published results (18,19). The phytosterols, particularly Δ5-avenasterol, are known to act as antioxidants and as antipolymerization agents in frying oils (20,21). These authors also explain that sterols with an ethylidene group in the side chain are most effective as antioxidants and suggested that a synergistic effect of sterols may occur. However, in this study, Δ5-avenasterol content in Whiteheart was not considerably different from other cultivars.

The results obtained in this experiment suggest that the oil of a locally selected hazelnut, Whiteheart, is more stable in the Rancimat test than oil extracted from other locally grown nuts. Long-term storage trials and oil stability tests at lower temperatures need to be carried out to confirm that the cultivar Whiteheart has improved storage characteristics and to demonstrate that the extracted oil stabilities reflect the actual shelf life and market quality of the intact and ground hazelnuts, which are the normal products of commerce.

TABLE 5
Quantitative Composition of Desmethylsterols of Oil Extracted from Hazelnut Cultivars Grown in New Zealand[a]

	µg/g Lipids (% composition)								
Cultivar	Cholesterol	Campesterol	Stigmasterol	Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Δ7-Stigmasterol	Total desmethylsterols	Total unsaponifiables
Whiteheart	12	114	23	1528	128	49	21	1875	2488
	(0.7)	(6.2)	(1.2)	(81.5)	(6.8)	(2.6)	(1.1)		
Barcelona	8	91	16	1416	111	37	43	1722	2323
	(0.5)	(5.3)	(0.9)	(82.3)	(6.4)	(2.2)	(2.5)		
Butler	8	78	15	1518	110	39	3.1	1800	2345
	(0.5)	(4.4)	(0.8)	(84.4)	(6.1)	(2.2)	(1.7)		
Ennis	11	83	17	1481	120	60	40	1812	2577
	(0.6)	(4.5)	(0.9)	(81.9)	(6.6)	(3.3)	(2.2)		
Tonda di Giffoni	7	80	15	1453	33	42	33	1760	2484
	(0.4)	(4.6)	(0.8)	(82.5)	(7.4)	(1.9)	(2.4)		
Campanica	9	101	18	1693	170	43	58	2001	2695
	(0.4)	(5.0)	(0.9)	(82.1)	(8.5)	(2.1)	(2.9)		

[a]Means of triplicate analyses.

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