Characterization of Chilean Hazelnut (*Gevuina avellana* Mol) Seed Oil

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ABSTRACT: The fatty acid composition, tocopherol and tocotrienol content, and oxidative stability of petroleum benzene-extracted *Gevuina avellana* Mol (Proteaceae) seed oil were determined. Positional isomers of monounsaturated fatty acids were elucidated by gas chromatography–electron impact mass spectrometry after 2-alkenyl-4,4-dimethyloxazoline derivatization. This stable oil (Rancimat induction period at 110°C: 20 h) is composed of more than 85% monounsaturated fatty acids and about equal amounts (6%) of saturated and polyunsaturated (principally linoleic) fatty acids. Unusual positional isomers of monounsaturated fatty acids, i.e., $C_{16:1}\ \Delta^{11}$, $C_{18:1}\ \Delta^{12}$, $C_{20:1}\ \Delta^{11}$, $C_{20:1}\ \Delta^{15}$, $C_{22:1}\ \Delta^{17}$, and presumably $C_{22:1}\ \Delta^{19}$ were identified. The $C_{18:1}\ \Delta^{12}$ and $C_{22:1}\ \Delta^{19}$ fatty acids are described for the first time in *G. avellana* seed oil. While only minute quantities of α-, γ-tocopherols and β-, γ- and δ-tocotrienols were found, the oil contained a substantial amount of α-tocotrienol (130 mg/kg). The potential nutritional value of *G. avellana* seed oil is discussed on the basis of its composition.
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KEY WORDS: 2-Alkenyl-4,4-dimethyloxazoline, electron impact mass spectrometry, FAME, gas chromatography, *Gevuina avellana*, oxidative stability, tocotrienol.

Chilean hazelnuts (*Gevuina avellana* Mol, Proteaceae) are a native variety that grows preferentially in the southern part of Chile and Argentina. They produce nuts with edible kernels, similar to macadamia. Attempts are underway to develop this nut commercially in Chile and New Zealand (1).

The fatty acid composition of *G. avellana* seed oil has been reported several times (1–6). In a study of the fatty acid composition of seed oils from Proteaceae, Vickery (2) reported that these oils possess high amounts of monounsaturated fatty acids with an unusually large number of positional isomers. Cattáneo *et al.* (4) studied the structure of fatty acids present in *G. avellana* more than 30 yr ago. They fractionated the methyl esters by distillation under vacuum and then chemically transformed the isolated methyl esters into substances typical of the fatty acids. These secondary compounds were identified either by gas–liquid chromatography or by their melting points.

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Revised data on *G. avellana* seed oil, obtained by modern analytical methods, are presented here. The fatty acid composition, including the positional isomers, and tocopherols and tocotrienols of *G. avellana* seed oil are discussed in terms of the oil's oxidative stability and nutritional value.

EXPERIMENTAL PROCEDURES

Materials. Gevuina avellana nuts were obtained from Italchile (Varese, Italy). Dichloromethane (analytical grade), the solvents used for high-performance liquid chromatography (HPLC) measurements (solvents for chromatography), α-, β-, γ-, δ-tocopherols, and α-, β-, γ-, δ-tocotrienols were obtained from E. Merck (Darmstadt, Germany). 2-Amino-2-methyl-1-propanol (analytical grade) was purchased from Fluka Chemie AG (Buchs, Switzerland).

Oil extraction. The husk, which represents more than 50% of the nut's weight, was separated by hand prior to oil extraction. The oil was obtained by Soxhlet extraction of the chopped kernels with (40–60°C) petroleum benzene (7). The solvent was removed under vacuum in a rotary evaporator.

Fatty acid composition. Fatty acid methyl esters (FAME) were prepared by reacting the oil in *n*-hexane with a methanolic solution of KOH (2 N) at room temperature and centrifuging at 2000 rpm for 5 min. The upper layer, containing the FAME, was then diluted with *n*-hexane and injected into the gas chromatograph. FAME were analyzed in a Carlo Erba (Rodano/MI, Italy) gas chromatograph of the HRGC 5300 mega series, equipped with a flame-ionization detector (FID), kept at 280°C and an automatic sampler (Carlo Erba AS550). Separation was achieved on a WCOT fused-silica capillary column, coated with CP-Sil-88, 50 m × 0.32 mm i.d., film thickness 0.20 μm (Chrompack, Middelburg, The Netherlands). Injection was on-column. The carrier gas was hydrogen (purity > 99.997 vol%) at 80 kPa. The oven program was as follows: 70°C 2 min iso, 30°C/min to 135°C, 1 min iso, 3°C/min to 180°C, 15°C/min to 220°C, 5 min iso. A Spectra Physics integrator (Spectra Physics, Allschwil, Switzerland) was used for data acquisition and integration.

The locations of the double bonds in the polyunsaturated fatty acids were determined by gas chromatography–mass spectroscopy (GC–MS) after 2-alkenyl-4,4-dimethyloxazoline derivatization (9). The FAME were reacted overnight at

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180°C with 2-amino-2-methyl-1-propanol. The 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives formed were extracted with dichloromethane and analyzed by GC–MS. The GC–MS experiments were carried out on a Finnigan MAT 8430 double focusing mass spectrometer, connected to an HP-5890 gas chromatograph (Finnigan MAT, Bremen, Germany), equipped with a J&W Scientific capillary column DB™-WAX (MSP Friedli, Koeniz, Switzerland), 30 m × 0.32 mm i.d., film thickness 0.25 μm. Helium was used as carrier gas at a pressure of 10 psi. The samples were injected with an on-column injector. The oven program was 50°C for 2 min, then 4°C/min up to 240°C, hold for 5 min. The transfer line was held at 220°C, and the source at 180°C. Electron impact mass spectra were acquired at 70 eV from 20 to 600 Da.

Determination of tocopherols and tocotrienols. Tocopherols and tocotrienols were measured by HPLC with a LaChrom acquisition system (degasser L-7612, pump L-7100, autosampler L-7200, fluorimetric detector L-7480, interface module D-7000) from Merck-Hitachi (E. Merck; Hitachi Ltd., Tokyo, Japan), controlled by D-7000 HPLC system manager software from Merck-Hitachi. Conditions for HPLC measurements were as follows: column: Lichrospher Si 60 (5 μm), 250 mm × 4 mm (direct phase); precolumn: Si 60 (5 μm), 4 mm × 4 mm (both columns were purchased from E. Merck); mobile phase: mixture of *n*-hexane and dioxane; program: *n*-hexane/dioxane (95:5, vol/vol) for 23 min, *n*-hexane/dioxane (50:50, vol/vol) for 5 min, and finally *n*-hexane/dioxane (95:5, vol/vol) for 10 min; flow rate: 1.6 mL/min; fluorescence excitation: 295 nm; fluorescence emission: 330 nm.

Peaks were identified by comparison with a standard (each tocopherol and tocotrienol homolog diluted to 0.005 mg/mL in *n*-hexane). The presence of α-tocotrienol was confirmed by GC–MS with an apolar capillary column J&W Scientific DB™-1701: 30 m × 0.32 mm i.d., film thickness 0.25 μm. Helium was used as carrier gas at a pressure of 10 psi. The samples were injected with an on-column injector. The oven program was 50°C for 2 min, then 40°C/min up to 200°C, then 5°C/min up to 320°C, hold for 105 min. The transfer line was held at 280°C, and the source at 180°C. Electron impact mass spectra were acquired at 70 eV from 20 to 600 Da.

Oxidative stability index. Oxidative stability of the oil was determined at 110°C according to the AOCS official method with the Rancimat apparatus (9).

RESULTS AND DISCUSSION

The crude oil recovered by petroleum benzene extraction of *G. avellana* kernels amounts to 46% (w/w). Previous papers reported extraction yields in the range 40–48% (1,3–5). The FID trace of the FAME mixture is reported in Figure 1. The fatty acids have carbon chainlengths of 14–24. The major components are monounsaturated fatty acids, namely, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$, which represent more than 85% of the total fatty acids. The remainder are saturated fatty acids, i.e., $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, $C_{24:0}$, and polyunsaturated fatty acids,

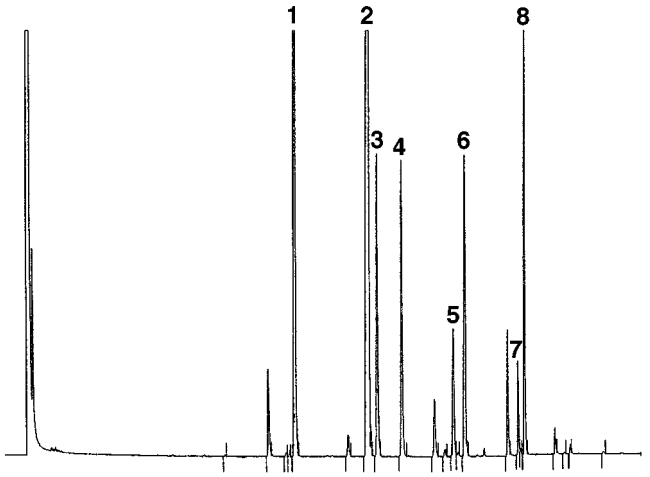

FIG. 1. Flame-ionization detection (FID) gas chromatogram for the fatty acid methyl ester (FAME) mixture of *Gevuina avellana* seed oil. **1**, $C_{16:1}$ Δ^{11}; **2**, $C_{18:1}$ Δ^{9}; **3**, $C_{18:1}$ Δ^{12}; **4**, $C_{18:2}$ $\Delta^{9,12}$; **5**, $C_{20:1}$ Δ^{11}; **6**, $C_{20:1}$ Δ^{15}; **7**, $C_{22:1}$ Δ^{19}; **8**, $C_{22:1}$ Δ^{19}.

mainly $C_{18:2}$, which each account for around 6% of the total fatty acids. Except for the slightly higher level of $C_{18:1}$ and lower level of $C_{18:2}$, this fatty acid pattern is in good agreement with previously published data on *G. avellana* seed oil (1–6).

Determination of positional isomers in the mono- and polyunsaturated fatty acids of *G. avellana* seed oil was carried out by GC–MS after DMOX derivatization. GC–MS analysis of DMOX derivatives of fatty acids allows determination of the position of the double bonds in the fatty acid carbon chain. The point of unsaturation is indicated in the mass spectrum by an interruption of the 14-mass spaced homologous ion series (8). A mass separation of 12 Da, instead of 14 Da, between two neighboring homologous fragments, containing $n - 1$ and n carbon atoms of the original fatty acid moiety, signals a double bond between carbons n and $n + 1$. Table 1 summarizes the results obtained for all identified fatty acids. Only the diagnostic fragments, locating the double bond positions, are presented.

Only one isomer was found for $C_{16:1}$: the Δ^{11} isomer. The mass spectrum of this fatty acid (Fig. 2) shows the double bond located in the Δ^{11} position according to the fragmentation rules of DMOX derivatives (8). Moreover, in addition to the general rule, stated above for Δ^{n}-monoenoic acids, the double bond located between carbons n and $n - 1$ (ions at *m/z* 236 and 224) also gives abundant ions with $n + 2$ and $n - 2$ carbon atoms (ions at *m/z* 264 and 210, respectively). These data are in agreement with the spectrum of the reference compound $C_{16:1}$ Δ^{11}, published by Zhang *et al.* (10).

Two positional isomers were found for each of the other monounsaturated fatty acids. These fatty acids were $C_{18:1}$ Δ^{9} and Δ^{12}, $C_{20:1}$ Δ^{11} and Δ^{15}, and $C_{22:1}$ Δ^{17} and presumably Δ^{19} isomers. For the polyunsaturated fatty acids, only one isomer, $\Delta^{9,12}$, was observed for $C_{18:2}$.

TABLE 1
Characteristic Ions in Electron Impact Mass Spectra of DMOX[a] Derivatives of the Fatty Acids of the Seed Oil Extracted from *Gevuina avellana*[b]

Fatty acid DMOX	Molecular ion *m/z* (intensity, %)	$[M - CH_3]^+$ *m/z* (intensity, %)	Diagnostic fragments *m/z* (intensity, %)
$C_{16:1}\ \Delta^{11}$	307(65)	292(57)	210(41), **224**(6), **236**(11), 250(13), 264(71)
$C_{18:1}\ \Delta^{9}$	335(61)	320(41)	182(55), **196**(9), **208**(15), 222(20), 236(57)
$C_{18:1}\ \Delta^{12}$	335(60)	320(57)	224(21), **238**(40), **250**(38), 264(20), 278(35)
$C_{20:1}\ \Delta^{11}$	363(24)	348(24)	210(20), **224**(9), **236**(16), 250(9), 264(26)
$C_{20:1}\ \Delta^{15}$	363(51)	348(27)	266(16), **280**(3), **292**(4), 306(6), 320(31)
$C_{22:1}\ \Delta^{17}$	391(64)	376(26)	294(20), **308**(3), **320**(5), 334(8), 348(34)
$C_{22:1}\ \Delta^{19}$	391(57)	376(16)	322(12), **336**(5), **348**(11), 362(12), 376(16)
$C_{18:2}\ \Delta^{9,12}$	333(34)	318(14)	182(37), **196**(10), **208**(6), 222(46), **236**(24), **248**(6), 262(11), 276(39)

[a]DMOX: 2-alkenyl-4,4-dimethyloxazoline.
[b]All spectra are characterized by a base peak at *m/z* 113 and an abundant ion at *m/z* 126 (relative intensity higher than 70%). The double bonds are located between carbons *n* and *n* + 1, and the corresponding ions are written in bold characters.

The percentage fatty acid composition of *G. avellana* seed oil was determined by GC–FID. Results are summarized in Table 2. These results are in good agreement with those reported by Cattáneo *et al.* (4), except for some monounsaturated fatty acids. Although they also reported the presence of $C_{16:1}\ \Delta^{11}$, $C_{18:1}\ \Delta^{9}$, and $C_{20:1}\ \Delta^{11}$ fatty acids, we did not observe the other fatty acids mentioned in their study. Instead, we identified $C_{18:1}\ \Delta^{12}$, $C_{20:1}\ \Delta^{15}$, $C_{22:1}\ \Delta^{17}$, and presumably $C_{22:1}\ \Delta^{19}$ fatty acids. Neither the $C_{18:1}\ \Delta^{12}$ nor the $C_{22:1}\ \Delta^{19}$ isomer has previously been identified in Proteaceae seed oils (1–6).

Traces of α- (0.4 mg/kg) and γ-tocopherol (0.6 mg/kg) were found in *G. avellana* seed oil; concentrations of other tocopherols were below the detection limit (0.05 mg/kg). In the same way, only tiny amounts of β-, γ-, and δ-tocotrienols were found: 1.3, 0.9, and 0.1 mg/kg for the β-, γ-, and δ-homologs, respectively. In contrast, a rather large amount of α-tocotrienol (130 mg/kg) was observed and confirmed by GC–MS. Similar amounts of antioxidants have already been reported in *G. avellana* seed oil (3). However, we identified α-tocotrienol as the major antioxidant in this oil. A higher level of tocotrienols in respect to tocopherols is not commonly found in vegetable oils (11). It has, however, been observed in some specialty lipids (12).

Petroleum benzene-extracted *G. avellana* seed oil has good oxidative stability. The induction period for this oil is 20 h at 110°C. This high stability originates from its low content of polyunsaturated fatty acids and from the presence of a substantial amount of tocotrienol.

The husk of *G. avellana* fruit might render industrial production of the oil difficult. As previously stated (1) and confirmed by our evaluation, producing *G. avellana* seed oil requires special shelling methods. Nonetheless, the oil could be interesting from a nutritional point of view (13). Indeed, apart from having high oxidative stability, *G. avellana* seed oil contains few saturated fatty acids (~6%), while the amounts of oleic acid (~40%) and α-tocotrienol (130 mg/kg) are high. It could thus be a dietary source for reducing pathological risk factors, e.g., for reducing plasma low-density lipoprotein cholesterol. Besides, it has been reported that incorporating *Gevuina* nuts in chick rations increases their body weight (14). *Gevuina avellana* seed oil, however, contains substantial amounts of unusual positional isomers of monounsaturated fatty acids, principally of the n-5 family. These fatty acids, although produced by several microorganisms, are not widely found in plants (15). Apart from $C_{16:1}\ \Delta^{11}$, which has been detected in a few Proteaceae seed oils (2–4), a number of n-5

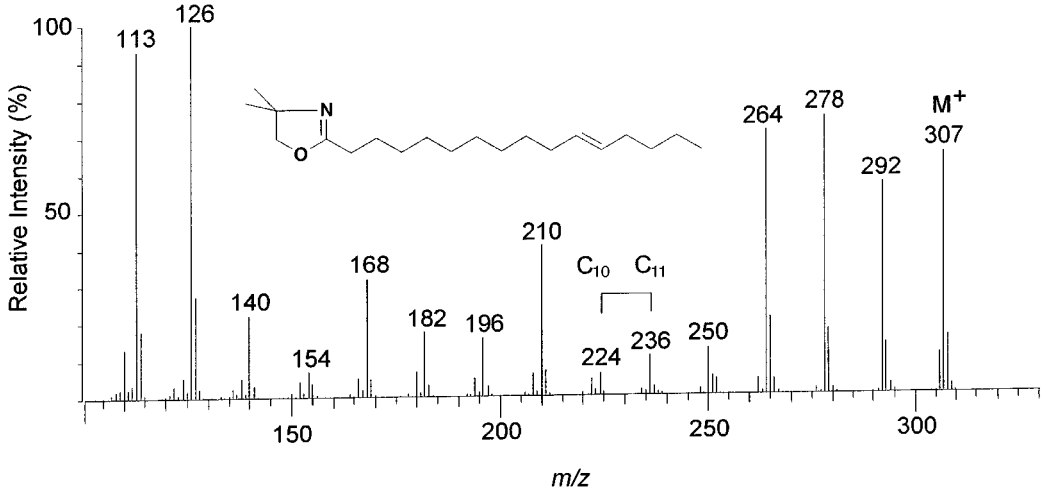

FIG. 2. Electron impact mass spectrum of the 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivative of $C_{16:1}\ \Delta^{11}$.

TABLE 2
Percentage Fatty Acid Composition of Crude Petroleum Benzene-Extracted *Gevuina avellana* Seed Oil[a]

Fatty acids	Amount (%)	Fatty acids	Amount (%)
$C_{16:0}$	1.9	$C_{20:0}$	1.4
$C_{16:1}\ \Delta^{11}$	22.7	$C_{20:1}\ \Delta^{11}$	3.1
$C_{18:0}$	0.5	$C_{20:1}\ \Delta^{15}$	6.6
$C_{18:1}\ \Delta^{9}$	39.4	$C_{22:0}$	2.2
$C_{18:1}\ \Delta^{12}$	6.2	$C_{22:1}\ \Delta^{17}$	7.9
$C_{18:2}\ \Delta^{9,12}$	5.6	$C_{22:1}\ \Delta^{19}$	1.6
$C_{18:3}$	0.1	$C_{24:0}$	0.5
Others	0.3		

[a]Fatty acids were quantitated from their flame-ionization detector (FID response.

monounsaturated fatty acids have been mentioned in *Grevillea robusta* seed oil (16). Little is known, however, about the effect of dietary fats that contain these unusual fatty acids. The position of the double bond in monounsaturated fatty acids influences their metabolic behavior (17). Although *G. avellana* nuts have long been eaten in South America, it would be interesting to investigate *in-vivo* properties of the uncommon monounsaturated fatty acids found in *G. avellana* seed oil.

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