Varietal and Crop Year Effects on Lipid Composition of Walnut (*Juglans regia*) Genotypes

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ABSTRACT: The FA, unsaponifiable, and volatile constituents of oil from three walnut varieties from two consecutive crop years were studied. The walnut oils (WO) were rich in PUFA and low in saturated FA. The tocopherol fraction consisted mainly of γ-tocopherol. High contents of β-sitosterol were found, together with campesterol and Δ^5-avenasterol in similar amounts. Methylsterols present in WO were identified as cycloartenol, cyclolaudenol, cycloeucalenol, and 24-methylenecycloartanol. The hydrocarbon fraction was characterized by the predominance of C_{14}–C_{20} *n*-alkanes. The major volatiles were aldehydes produced through the linoleic acid oxidative pathway. FA, methylsterols, and some hydrocarbons presented statistically significant differences among varieties. Most of this variation was due to the genotype. The Franquette variety was noteworthy by its higher oil and oleic acid contents. In contrast, tocopherols and volatile compounds showed minor differences among varieties; they were strongly influenced by the crop year. Chemical data were subjected to principal component analysis. The parameters that gave the greatest discrimination between the walnut varieties were oleic and linolenic acids, tetradecane, eicosane, tetracosane, cycloartenol, and 24-methylenecycloartanol. These components presented the major varietal influences and could be useful to determine the identity of walnut genotypes.

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KEY WORDS: Chemical markers, Juglandaceae, *Juglans regia*, oil composition, walnut genotypes.

Walnut (*Juglans regia* L.) fruits have been used in human nutrition since ancient times. The walnut seed contains high levels of oil (52–70%) (1–3). The major constituents of walnut oil (WO) are TG, in which monounsaturated FA (mainly oleic acid) and PUFA (linoleic and α-linolenic acids) are present in high amounts (1,4–8). The proportions of these FA are important to the economic and nutritional value of the nut. Higher linoleic and linolenic acids contents may result in a poorer oxidative stability and a shorter shelf life of the oils. Higher oleic acid levels are desirable because of their potential health benefits. Tocopherols occur in WO (3,7–10), and they are important in providing some protection against oxidation. Walnuts also contain several phytosterols that have been used as nutraceuticals, as it appears that they can inhibit intestinal absorption of cholesterol. The presence of other minor unsaponifiable lipid constituents, such as hydrocarbons, has been reported by McGill *et al*. (11).

Although WO is not described by the current Committee on Fats and Oils of the Codex Alimentarius, the knowledge of their chemical composition is necessary not only to assess their commercial and nutritional quality but also to encourage walnut consumption. There are no published data for the composition of WO from Argentina. This work describes the FA, unsaponifiable, and volatile compositions of oils from the most common walnut varieties grown in this country. The data obtained are useful in screening cultivars that may be used for future commercial production of WO. Moreover, the feasibility of using oil components as chemical markers to determine the identity of walnut genotypes is discussed.

EXPERIMENTAL PROCEDURES

Plant material. Walnut fruits (*J. regia* L.) of the varieties Franquette, Chandler, and Criolla were collected from commercial plantations at Belén location, Catamarca Province, Argentina.

Walnut plants were grown under natural rainfall (averaging 320 mm per year), plus supplemental irrigation of 280 mm per year. The study was carried out during two successive crop years, 2004 and 2005. In each crop year, three samples (10 kg each) of fruits at full maturity from each variety were picked by hand from the trees. After cleaning, the fruits were dried at 30 ± 2°C for 24 h and then were shelled manually. Whole kernels were used to obtain oil samples, using a pilot-plant hydraulic press as described previously (12). The WO obtained were dried over Na_2SO_4, filtered through Whatman no. 1 paper and stored at –10°C under nitrogen, without further treatment. Extraction and chemical analyses of oils were performed after collection of walnut fruits in each crop year.

Oil content. Samples of dry, finely chopped walnuts (10 g) were extracted with *n*-hexane using a Soxhlet apparatus, and oil content was determined in accordance with AOCS method Am 2-93 (13).

Oil analyses. All chemical analyses were performed using the pressure-extracted oils. For the determination of FA composition,

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each oil sample (1 g) was subjected to alkaline saponification by reflux (45 min) using 20 mL 1 N KOH in methanol. Unsaponifiable matter was extracted with *n*-hexane (3 × 30 mL). The FA were converted to methyl esters (FAME) by reflux (45 min) using 40 mL 1 N H_2SO_4 in methanol and analyzed by GC using a fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) CP Wax 52 CB (Varian, Walnut Creek, CA); carrier gas N_2 at 1 mL min^{-1}; column temperature programmed from 180°C (5 min) to 240°C at 4°C min^{-1}; injector and detector temperatures 250°C, with an FID. The identification of FAME was carried out by GC–MS (14) and by comparison of their retention times with those of reference compounds.

Unsaponifiable materials were fractionated on preparative TLC (0.5 mm silica gel; Merck, Darmstadt, Germany) by development with toluene/acetone (95:5, vol/vol). After developing, the left half of the plate was sprayed with a 2,7-dichlorofluorescein solution in ethanol (3%) and observed under UV light. Three separated zones containing sterols, methylsterols, and hydrocarbons were removed from the plate and extracted with chloroform. Each fraction was purified three times by repeated preparative silica gel TLC for subsequent GC analysis.

Sterols and methylsterols were run without further treatment using a VF-5ms (Varian, Walnut Creek, CA) capillary column (30 m × 0.25 mm i.d.) coated with a 0.25 μm layer of 5% phenyl, 95% polydimethylsiloxane; carrier gas N_2 at 1 mL min^{-1}; column temperature programmed from 240°C (1 min) to 290°C at 2°C min^{-1}; injector and detector temperatures 300°C; FID. GC–MS used an HP 5 (Hewlett-Packard, Palo Alto, CA) fused-silica capillary column (30 m × 0.25 mm i.d.) coated with a 0.25 μm layer of 5% phenyl methyl siloxane, and helium (flow rate 1 mL min^{-1}) as carrier gas. The column, injector, and detector temperatures were as for GC analysis. Sterols and methylsterols were identified by comparison of the mass spectral data with those of authentic reference compounds. When standards were not available, the components were identified by mass spectrum matching using the Wiley mass spectra search library, and published data (15,16). For methylsterols, the following trivial names were used: cycloartenol (9β,19-cyclo-5α-lanost-24-en-3β-ol), cyclolaudenol (24-methyl-9β, 19-cyclo-5α-lanost-25-en-3β-ol), 24-methylenecycloartanol (24-methylene-9β,19-cyclo-5α-lanostan-3β-ol), and cycloeucalenol (4β-demethyl-24-methylenecycloartanol).

Hydrocarbons were analyzed by GC and GC–MS according to Maestri and Guzmán (14). Briefly, hydrocarbons purified by TLC as described above were analyzed by GC using a VF-5ms capillary column. The column temperature was programmed from 70 to 300°C at 4°C min^{-1}, injector and detector temperatures 320°C, carrier gas N_2 at 1 mL min^{-1}, FID. GC–MS used an HP 5 capillary column and helium (flow rate 1 mL min^{-1}) as carrier gas. The column, injector, and detector temperatures were as for GC analysis. Hydrocarbons were identified by their retention times and comparison of the mass spectral data with those of authentic reference compounds.

Tocopherols were analyzed by HPLC according to the procedure of Pocklington and Dieffenbacher (17). In brief, samples of 1 g oil were placed into 25-mL volumetric flasks. A quantity of *n*-hexane was added, the flask was swirled to dissolve the sample and then made up to volume with the same solvent. An aliquot of 20 μL of this solution was injected onto a Lichrosorb Si 60 (Varian) column. The mobile phase was *n*-hexane/2-propanol (99.5:0.5 vol/vol) with a flow rate of 1 mL min^{-1}. Detection was at 292 nm. Individual tocopherols were identified by comparison of their retention times with those of authentic standards and published data (3,7).

Volatile compounds analysis was carried out by solid-phase microextraction followed by GC–MS. Briefly, 5-mL oil samples were put into 15-mL headspace vials that were fitted with silicon septa. Volatiles were sampled for 30 min at 50°C from the headspace of the vial, with a 100 μm fiber coated with divinylbenzene/carboxene on polydimethylsiloxane, conditioned prior to use as recommended by the producer. After sampling, the fiber was immediately inserted into the injection port of a gas chromatograph coupled to a mass-selective detector. The GC separations were performed using an HP 5 capillary column and helium (flow rate 1 mL min^{-1}) as carrier gas. The injector temperature was kept at 250°C, and the GC oven temperature was initially maintained at 50°C (2 min) and then increased at 5°C min^{-1} to 250°C. Volatile compounds were identified by comparison of the mass spectral data with those of authentic reference compounds. When standards were not available, the components were identified by mass spectrum-matching using the Wiley mass spectra search library.

Method validation. For chemical analyses, all samples (variety × crop year) were run in three repetitions. Reproducibility was evaluated with standard solutions of reference compounds. Acceptance criteria were that results for the triplicate analyses had to fall within 2% of the mean values.

All chemicals and solvents used were either analytical or HPLC grade. *n*-Hexane, methanol, chloroform, and 2-propanol were obtained from Merck. Standards for GC were purchased from Sigma-Aldrich (St. Louis, MO). Tocopherol standards were from ICN Biomedicals (Aurora, OH). All GC analyses were run on a Shimadzu (Kyoto, Japan) gas chromatograph. GC–MS analyses were run on a Hewlett-Packard 5890 gas chromatograph coupled to a HP 5972 A mass selective detector.

Statistical analyses. Statistical differences were estimated by ANOVA test at the 5% level ($P \leq 0.05$) of significance for all parameters evaluated. Whenever the ANOVA test indicated a significant difference, a pairwise comparison of means by least significant difference (LSD) was carried out. A multivariate statistical analysis of the chemical data from three walnut varieties analyzed in two consecutive years was performed using principal component analysis (PCA).

RESULTS AND DISCUSSION

Table 1 shows the oil content and FA and tocopherol compositions of the walnut varieties analyzed in this study. The oil content (Soxhlet, *n*-hexane) ranged from 67.61 (var. Criolla) to 72.41% (var. Franquette). These values are higher than the mean value (69%) reported by Prasad (2) in WO from different varieties and geographic origins. Linoleic (*cis* 9,*cis* 12-oc-

TABLE 1
Oil Content (% dry basis) and FA and Tocopherol Composition of Walnut Oil Varieties from Two Crop Years[a]

	Walnut varieties					
	Criolla		Chandler		Franquette	
	2004	2005	2004	2005	2004	2005
Oil content (%)	$68.88^{a,B} \pm 0.24$	$67.61^{a,A} \pm 0.10$	$70.80^{a,b,A} \pm 1.07$	$69.47^{a,A} \pm 0.74$	$71.77^{b,A} \pm 0.82$	$72.41^{b,A} \pm 2.17$
Fatty acids (%)						
Palmitic	$7.68^{b,A} \pm 0.14$	$7.81^{b,A} \pm 0.06$	$6.95^{a,A} \pm 0.15$	$6.61^{a,A} \pm 0.28$	$7.54^{b,B} \pm 0.09$	$6.59^{a,A} \pm 0.10$
Stearic	$1.71^{b,A} \pm 0.02$	$1.88^{a,B} \pm 0.08$	$1.50^{a,A} \pm 0.01$	$1.69^{a,A} \pm 0.15$	$2.12^{c,A} \pm 0.01$	$2.16^{b,A} \pm 0.14$
Oleic	$17.31^{b,A} \pm 0.09$	$21.16^{b,B} \pm 0.76$	$16.52^{a,A} \pm 0.22$	$17.84^{a,A} \pm 1.22$	$26.28^{c,A} \pm 0.06$	$28.44^{c,A} \pm 2.42$
Linoleic	$57.82^{c,B} \pm 0.03$	$57.27^{b,A} \pm 0.23$	$56.46^{b,A} \pm 0.23$	$56.71^{b,A} \pm 0.45$	$52.12^{a,A} \pm 0.04$	$50.23^{a,A} \pm 1.21$
Linolenic	$15.61^{b,B} \pm 0.21$	$11.88^{a,A} \pm 0.63$	$18.58^{c,A} \pm 0.06$	$16.99^{b,A} \pm 1.14$	$11.94^{a,A} \pm 0.01$	$12.52^{a,A} \pm 1.17$
PUFA/MUFA	$4.24^{b,B} \pm 0.04$	$3.27^{b,A} \pm 0.16$	$4.54^{c,A} \pm 0.08$	$4.15^{c,A} \pm 0.37$	$2.43^{a,A} \pm 0.01$	$2.22^{a,A} \pm 0.29$
Iodine value	$163.17^{b,B} \pm 0.55$	$155.42^{a,A} \pm 1.46$	$168.11^{c,A} \pm 0.31$	$165.42^{b,A} \pm 2.80$	$150.85^{a,A} \pm 0.14$	$150.96^{a,A} \pm 3.23$
Tocopherols (%)						
α-Tocopherol	Tr^{a}	Tr^{a}	$Tr^{a,A}$	$1.50^{a,b,B} \pm 0.47$	$Tr^{b,A}$	$2.55^{b,B} \pm 0.16$
γ-Tocopherol	$88.28^{a,B} \pm 0.73$	$86.81^{b,A} \pm 0.27$	$86.44^{a,A} \pm 0.57$	$83.44^{a,A} \pm 1.77$	$86.89^{a,B} \pm 0.55$	$83.72^{a,A} \pm 0.33$
δ-Tocopherol	$11.73^{a,A} \pm 0.73$	$13.19^{a,B} \pm 0.27$	$13.56^{a,A} \pm 0.57$	$15.06^{b,A} \pm 0.97$	$13.11^{a,A} \pm 0.55$	$13.74^{a,A} \pm 0.43$

[a]Mean values of each variety in each crop year were the averages of three independent measurements. MUFA, monounsaturated FA; Tr, trace (<0.3%). Values in each row for the same crop year with different superscript small letters represent significant differences ($P \leq 0.05$) among walnut varieties. Values in each row for the same walnut variety with different superscript capital letters represent significant differences ($P \leq 0.05$) among crop years.

tadecadienoic) acid was the predominant FA, followed by oleic (*cis* 9-octadecenoic), linolenic (*cis* 9, *cis* 12, *cis* 15-octadecatrienoic), palmitic (hexadecanoic), and stearic (octadecanoic) acids in a decreasing order. Palmitoleic (*cis* 9-hexadecenoic), arachidic (eicosanoic), and *cis* 11-eicosenoic acids were detected in small proportions (<0.1%). These data are in general agreement with those of earlier reports (1,3–5,7,8). As expected, WO were exceptionally rich in PUFA, whereas saturated FA represented only 8.30–9.69% of the total FA. All of them showed significant variations among walnut varieties. The Franquette variety presented the highest oleic acid content and, consequently, the lowest linoleic and linolenic acid concentrations. To establish the sources of this variability, a two-way ANOVA test was applied to the data set of variety × crop year. The variability observed for FA composition could be explained, mainly, by the genotypic variation (62.3 to 94.1% of the total variability) and, then, the crop year (Table 2). The interaction among variety and crop year was significant for palmitic and linolenic acids.

Among the natural antioxidants present in WO, tocopherols stand out because of their antioxidant activity and important nutritional properties (18). Three tocopherol isomers were found, and their contents were in general agreement with those of WO from different varieties and geographic origins (3,7–9). γ-Tocopherol represented between 83.44 and 88.28% of the total tocopherol content, followed by δ-tocopherol. α-Tocopherol was at very low concentration. There were few significant differences among varieties in individual tocopherol isomers, and the crop year was the main variability source (Table 2).

The sterol profile from walnut varieties exhibited a similar chemical composition, with β-sitosterol (85.21–91.78%) being the major component. In addition to this compound, campesterol and Δ^5-avenasterol were found in similar amounts (Table 3). The results were in reasonable agreement with the data of earlier reports for WO from different origins (5,7,8) except for cholesterol, stigmasterol, and clerosterol, which were not found in this study.

Itoh *et al.* (15) and Gaydou *et al.* (19) studied methylsterols from a number of vegetable oils and found that the most common

TABLE 2
Variability Expressed as Percentage of the Total Sum of Squares for Chemical Parameters from Three Walnut Oil Varieties[a]

Compounds	Variety	Crop year	Variety × crop year
Palmitic acid	62.3*	17.5*	13.8*
Stearic acid	80.5*	5.6	2.8
Oleic acid	87.7*	5.7*	1.3
Linoleic acid	94.1*	1.3	1.6
Linolenic acid	74.5*	7.0*	12.2*
PUFA/MUFA	84.9*	7.4*	3.4
Iodine value	82.3*	4.8*	6.2
α-Tocopherol	18.95	31.6*	18.95
γ-Tocopherol	34.22	41.79*	3.81
δ-Tocopherol	17.88	28.86*	3.19
Campesterol	48.1*	35.7*	8.5
Cycloartenol	58.7*	25.7*	14.4*
Cycloeucalenol	91.9*	1.6	0.8
24-Methylenecycloartanol	46.2*	35.3*	17.2*
Tetradecane	41.9*	26.7*	28.6*
1-Pentadecene	41.2*	17.3*	41.2*
Eicosane	85.3*	17.9*	10.8*
Docosane	86.8*	8.8*	4.0*
Tetracosane	48.6*	0.01	18.9
Hexacosane	53.2*	3.2	33.8*
n-Pentane	17.32	46.65*	35.75*
Pentanal	8.31	75.89*	13.19*
Hexanal	0.007	80.38*	4.89*
2-Heptenal	1.26	94.16	1.27*
2-Octenal	3.13	71.45*	3.13
Nonanal	4.11	95.24*	0.37
2-Nonenal	7.66	79.19*	12.96
2-Decenal	4.98	90.25*	4.77
2, 4-Decadienal	0.12	98.57*	1.27*
2-Undecenal	9.99	76.64*	9.91

[a]An asterisk (*) indicates a significance level of $P \leq 0.05$.

TABLE 3
Sterol, Methylsterol, and Hydrocarbon Composition of Walnut Oil Varieties from Two Crop Years[a]

	Walnut varieties					
	Criolla		Chandler		Franquette	
Compounds	2004	2005	2004	2005	2004	2005
Sterols (%)						
Campesterol	$5.38^{a,A} \pm 0.26$	$5.11^{b,A} \pm 0.01$	$5.34^{a,A} \pm 0.06$	$5.25^{b,A} \pm 0.02$	$5.17^{a,B} \pm 0.12$	$4.51^{a,A} \pm 0.11$
β-Sitosterol	$91.78^{a,B} \pm 0.12$	$87.54^{b,A} \pm 0.10$	$91.66^{a,B} \pm 0.50$	$85.21^{a,A} \pm 0.32$	$91.49^{a,B} \pm 0.11$	$88.62^{c,A} \pm 0.17$
Δ5- Avenasterol	$2.84^{a,A} \pm 0.37$	$7.35^{a,B} \pm 0.12$	$3.01^{a,A} \pm 0.57$	$9.54^{b,B} \pm 0.34$	$3.34^{a,A} \pm 0.24$	$6.87^{a,B} \pm 0.06$
Methylsterols (%)						
Cycloartenol	$80.75^{b,A} \pm 1.72$	$85.94^{b,A} \pm 0.63$	$62.62^{a,A} \pm 0.93$	$78.59^{a,B} \pm 1.71$	$81.78^{b,A} \pm 0.09$	$84.13^{b,A} \pm 0.82$
Cyclolaudenol	Tr	Tr	Tr	Tr	Tr	Tr
Cycloeucalenol	$8.10^{a,A} \pm 0.46$	$8.77^{a,A} \pm 0.47$	$10.60^{b,A} \pm 0.40$	$10.67^{b,A} \pm 0.75$	$7.27^{a,A} \pm 0.23$	$7.59^{a,A} \pm 0.32$
24-Methylenecycloartanol	$11.15^{a,A} \pm 2.18$	$5.29^{a,A} \pm 0.17$	$26.78^{b,B} \pm 0.53$	$10.74^{c,A} \pm 0.97$	$10.94^{a,B} \pm 0.33$	$8.29^{b,A} \pm 0.51$
Hydrocarbons (%)						
Dodecane	Tr^{a}	Tr^{a}	$1.00^{a,A} \pm 0.41$	$2.00^{b,A} \pm 0.0001$	Tr^{a}	Tr^{a}
Tetradecane	$13.98^{b,B} \pm 1.10$	$6.70^{a,A} \pm 0.38$	$13.84^{c,B} \pm 0.40$	$11.77^{c,A} \pm 0.23$	$8.03^{a,A} \pm 1.06$	$8.27^{b,A} \pm 0.23$
Hexadecane	$22.98^{a,B} \pm 0.70$	$17.54^{aA} \pm 1.07$	$22.26^{a,A} \pm 0.43$	$20.97^{b,A} \pm 0.30$	$18.86^{a,A} \pm 2.69$	$20.33^{b,A} \pm 0.35$
Octadecane	$21.89^{a,B} \pm 0.13$	$34.66^{b,B} \pm 1.30$	$28.99^{a,B} \pm 0.24$	$23.05^{a,A} \pm 0.37$	$29.27^{a,A} \pm 5.02$	$24.55^{a,A} \pm 0.37$
1-Pentadecene	$2.25^{b,B} \pm 0.004$	$0.10^{a,A} \pm 0.0001$	$2.51^{c,A} \pm 0.12$	$2.39^{c,A} \pm 0.01$	$1.84^{a,A} \pm 0.04$	$2.07^{b,B} \pm 0.04$
2,4-Dimethyl eicosane	$1.42^{c,B} \pm 0.03$	$Tr^{a,A}$	$0.89^{b,A} \pm 0.01$	$1.27^{b,A} \pm 0.38$	Tr^{a}	Tr^{a}
Eicosane	$14.70^{b,A} \pm 0.33$	$18.40^{c,B} \pm 0.26$	$11.36^{a,A} \pm 0.10$	$13.10^{a,B} \pm 0.14$	$15.49^{c,A} \pm 0.23$	$15.64^{b,A} \pm 0.25$
1-Hexadecene	$2.16^{b,B} \pm 0.09$	$0.10^{a,A} \pm 0.0001$	$1.39^{a,A} \pm 0.02$	$1.50^{a,b,B} \pm 0.02$	$0.97^{a,A} \pm 0.21$	$1.95^{b,A} \pm 0.95$
5-Methyl heneicosane	Tr^{a}	Tr^{a}	$2.07^{b,A} \pm 0.04$	$1.90^{b,A} \pm 0.09$	$Tr^{a,A}$	$0.10^{a,B} \pm 0.0001$
Docosane	$2.29^{a,A} \pm 0.09$	$5.37^{a,B} \pm 0.47$	$7.00^{b,A} \pm 0.03$	$8.74^{b,B} \pm 0.13$	$10.14^{c,A} \pm 0.11$	$10.41^{c,A} \pm 0.16$
Tetracosane	$7.38^{b,A} \pm 0.63$	$5.93^{a,A} \pm 0.68$	$3.86^{a,A} \pm 0.08$	$5.44^{a,B} \pm 0.40$	$6.75^{b,A} \pm 0.27$	$6.94^{a,A} \pm 0.13$
Hexacosane	$4.48^{b,A} \pm 0.41$	$3.36^{aA} \pm 0.61$	$2.39^{a,A} \pm 0.08$	$3.49^{a,b,B} \pm 0.08$	$4.00^{b,A} \pm 0.14$	$4.98^{b,A} \pm 0.56$
1-Docosene	$1.40^{a,A} \pm 0.48$	$0.10^{a,A} \pm 0.0001$	$0.71^{a,A} \pm 0.02$	$1.37^{b,A} \pm 0.47$	$0.88^{a,B} \pm 0.04$	$0.10^{a,A} \pm 0.0001$
Octacosane	$2.90^{c,A} \pm 0.03$	$4.97^{c,B} \pm 0.12$	$1.36^{a,A} \pm 0.04$	$2.07^{a,B} \pm 0.01$	$2.47^{b,A} \pm 0.06$	$2.91^{b,B} \pm 0.01$
Triacontane	$2.00^{b,A} \pm 0.10$	$3.06^{c,B} \pm 0.18$	$0.80^{a,A} \pm 0.06$	$1.34^{a,B} \pm 0.01$	$1.79^{b,A} \pm 0.32$	$1.94^{b,A} \pm 0.01$

[a]Mean values of each variety in each crop year were the averages of three independent measurements. Tr, trace (<0.1%). Values in each row for the same crop year with different superscript small letters represent significant differences ($P \leq 0.05$) among walnut varieties. Values in each row for the same walnut variety with different superscript capital letters represent significant differences ($P \leq 0.05$) among crop years.

TABLE 4
Volatile Composition (%) of Walnut Oil Varieties from Two Crop Years[a]

	Walnut varieties					
	Criolla		Chandler		Franquette	
Compounds	2004	2005	2004	2005	2004	2005
n-Pentane	$13.60^{a,A} \pm 0.003$	$15.23^{b,B} \pm 0.002$	$26.84^{b,B} \pm 0.002$	$14.60^{b,A} \pm 0.91$	$31.05^{c,B} \pm 0.001$	$11.29^{a,A} \pm 1.00$
n-Octane	$0.15^{a,B} \pm 0.001$	$Tr^{a,A}$	$6.52^{b,B} \pm 0.003$	$0.14^{a,b,A} \pm 0.01$	$9.22^{cB} \pm 0.0003$	$0.23^{b,A} \pm 0.05$
n-Nonane	$0.59^{b,B} \pm 0.001$	Tr^{A}	Tr^{a}	Tr	Tr^{a}	
Ethanol	$3.26^{b,B} \pm 0.001$	$Tr^{a,A}$	$6.83^{c,B} \pm 0.003$	$0.12^{a,b,A} \pm 0.03$	$0.16^{a,A} \pm 0.001$	$0.21^{b,A} \pm 0.05$
Cyclobutanol	$3.42^{c,B} \pm 0.001$	$Tr^{a,A}$	$0.16^{a,A} \pm 0.001$	$0.14^{b,A} \pm 0.01$	$0.15^{b,A} \pm 0.001$	$1.43^{c,B} \pm 0.01$
1-Pentanol	$1.31^{b,A} \pm 0.001$	$1.91^{a,B} \pm 0.005$	$Tr^{a,A}$	$1.15^{a,A} \pm 0.43$	$Tr^{a,A}$	$0.45^{a,A} \pm 0.26$
1-Hexanol	$0.88^{b,B} \pm 0.001$	Tr^{A}	Tr^{a}	Tr	Tr^{a}	Tr
1-Heptanol	$2.03^{b,B} \pm 0.005$	Tr^{A}	Tr^{a}	Tr	Tr^{a}	Tr
1-Octanol	$1.74^{b,B} \pm 0.006$	Tr^{A}	Tr^{a}	Tr	Tr^{a}	Tr
Butanal	$0.15^{a,B} \pm 0.001$	$Tr^{a,A}$	$0.16^{b,A} \pm 0.001$	$2.86^{b,B} \pm 0.05$	$0.16^{c,A} \pm 0.001$	$0.23^{a,A} \pm 0.05$
Pentanal	$9.37^{b,B} \pm 0.003$	$5.57^{a,A} \pm 0.001$	$8.53^{a,A} \pm 0.002$	$4.11^{a,A} \pm 1.74$	$13.29^{c,B} \pm 0.001$	$4.06^{a,A} \pm 0.68$
Hexanal	$10.37^{c,A} \pm 0.003$	$16.40^{a,B} \pm 0.002$	$8.89^{b,A} \pm 0.001$	$17.86^{a,B} \pm 1.37$	$7.80^{a,A} \pm 0.006$	$19.11^{a,A} \pm 0.35$
2-Hexenal	$0.15^{a,B} \pm 0.001$	$Tr^{a,A}$	$0.14^{b,A} \pm 0.001$	$0.14^{a,b,A} \pm 0.01$	$0.18^{c,A} \pm 0.001$	$0.22^{b,A} \pm 0.05$
Heptanal	$6.97^{c,B} \pm 0.001$	$Tr^{a,A}$	$5.82^{b,B} \pm 0.005$	$0.14^{a,b,A} \pm 0.01$	$0.14^{a,A} \pm 0.001$	$0.23^{b,A} \pm 0.05$
2-Heptenal	$0.15^{a,A} \pm 0.001$	$7.18^{a,B} \pm 0.003$	$0.16^{b,A} \pm 0.001$	$5.44^{a,B} \pm 1.28$	$0.16^{c,A} \pm 0.001$	$6.25^{a,B} \pm 0.53$
2,4-Heptadienal	$1.37^{b,B} \pm 0.001$	$Tr^{a,A}$	$Tr^{a,A}$	$0.14^{a,B} \pm 0.01$	$Tr^{a,A}$	$1.88^{a,A} \pm 0.29$
Octanal	$8.62^{c,B} \pm 0.01$	$Tr^{a,A}$	$7.23^{b,B} \pm 0.003$	$0.14^{a,b,A} \pm 0.01$	$0.16^{a,A} \pm 0.001$	$0.23^{b,A} \pm 0.05$
2-Octenal	$0.15^{a,A} \pm 0.001$	$3.25^{a,B} \pm 0.003$	$0.15^{b,A} \pm 0.001$	$3.72^{a,B} \pm 0.15$	$0.15^{c,A} \pm 0.001$	$2.26^{a,A} \pm 0.82$
Nonanal	$9.80^{b,B} \pm 0.002$	$2.39^{a,A} \pm 0.004$	$9.59^{a,B} \pm 0.01$	$3.12^{a,b,A} \pm 0.03$	$11.47^{c,B} \pm 0.002$	$4.07^{b,A} \pm 0.67$
2-Nonenal	$2.12^{b,B} \pm 0.005$	$3.37^{a,B} \pm 0.003$	$Tr^{a,A}$	$3.41^{a,B} \pm 0.05$	$Tr^{a,A}$	$3.91^{b,B} \pm 0.26$
Decanal	$1.02^{b,B} \pm 0.008$	Tr^{A}	Tr^{a}	Tr	Tr^{a}	Tr
2-Decenal	$9.22^{b,B} \pm 0.002$	$Tr^{a,A}$	$5.66^{a,B} \pm 0.005$	$0.14^{a,b,A} \pm 0.01$	$10.00^{c,B} \pm 0.003$	$0.20^{b,A} \pm 0.05$
2, 4-Decadienal	$11.52^{a,A} \pm 0.003$	$39.84^{b,B} \pm 0.01$	$13.13^{b,A} \pm 0.003$	$37.65^{a,B} \pm 0.26$	$15.72^{c,A} \pm 0.001$	$37.15^{a,B} \pm 0.76$
2-Undecenal	$0.15^{a,A} \pm 0.001$	$1.75^{a,B} \pm 0.005$	$0.16^{b,A} \pm 0.001$	$2.47^{a,b,B} \pm 0.24$	$0.16^{c,A} \pm 0.001$	$3.98^{b,B} \pm 0.92$
2-Pentylfuran	$1.19^{b,A} \pm 0.007$	$1.45^{a,B} \pm 0.01$	$Tr^{a,A}$	$1.74^{a,B} \pm 0.11$	$Tr^{a,A}$	$2.56^{b,B} \pm 0.25$
2-Octylfuran	$0.69^{b,B} \pm 0.009$	Tr^{A}	Tr^{a}	Tr	Tr^{a}	Tr

[a]Mean values of each variety in each crop year were the averages of three independent measurements. Tr, trace (< 0.1%). Values in each row for the same crop year with different superscript small letters represent significant differences ($P \leq 0.05$) among walnut varieties. Values in each row for the same walnut variety with different superscript capital letters represent significant differences ($P \leq 0.05$) among crop years.

were cycloartenol and 24-methylenecycloartanol. There are no published studies on the presence of methylsterols in WO, even though the occurrence of these compounds in common vegetable oils is highly probable. In the present work, cycloartenol, cycloeucalenol, and 24-methylenecycloartanol were the major components of the methylsterol fraction of WO (Table 3). Minor amounts of cyclolaudenol were also detected. The fact that the WO contained high amounts of cycloartenol reaffirms the pathway model of sterol biosynthesis postulated by Guo *et al.* (20): the cycloartenol transformation to 24-alkyl-sterols (sitosterol). Unlike sterol composition, methylsterols had greater differences among WO samples. Most of this variability was due to the genotype, with some contribution as well from the crop year (Table 2).

Fifteen hydrocarbons were found in the WO studied. The hydrocarbon profile of all samples was characterized by the predominance of even carbon-numbered *n*-alkanes, among which the main components were those from C_{14} to C_{20} (Table 3). Branched and unsaturated hydrocarbons were detected in small amounts. These data differ from those for other WO in which odd-numbered *n*-alkanes predominated (11). Differences may be attributed to genotypical and/or environmental influences. In species other than walnut, such as olive, alkane composition is known to depend strongly on cultivar, but it may be affected by environmental conditions (21). The two-way ANOVA test showed that both variety and crop year had significant effects on the hydrocarbon components of WO, but the effect of the genotype was more remarkable (Table 2). The interaction of variety × crop year was also significant for most of the compounds analyzed, and their contribution to the total variability was considerable for tetradecane, 1-pentadecene, and hexacosane.

Twenty-six components that contribute to the composition of volatile compounds of WO were identified (Table 4). These included low-M.W. hydrocarbons, alcohols, aldehydes, and furan derivatives. Most of the identified compounds were previously reported in the literature as constituents of WO flavor (6,22,23). The major components in all oil samples were saturated and unsaturated aldehydes, which represented 54.9–83.8% of the total volatile compounds. Pentanal, hexanal and 2,4-decadienal were predominant. *n*-Pentane was the major component of the hydrocarbon fraction. Aliphatic alcohols and furan derivatives were found in small amounts. As expected, the major headspace volatile compounds in WO were breakdown products of lipid hydroperoxides. Linoleic was the major FA, and most of the fat-derived flavors derived from it. The C_5–C_6 compounds (*n*-pentane, pentanal, and hexanal) are typical linoleate 13-hydroperoxide derivatives, whereas 2,4-decadienal is formed exclusively from linoleate 9-hydroperoxide (24). Although linolenic acid was abundant in the WO studied, 2-hexenal and 2,4-heptadienal (two of the most important linolenate hydroperoxide derivatives) were found at very low concentrations.

There were statistically significant differences in volatile compounds among the varieties studied, but a two-way ANOVA of the whole data set of varieties × crop year found that all of them were strongly influenced by the crop year. This means that varieties had a specific response to the environmental conditions of different years. Hence, WO volatile components should have little discriminative power to separate the walnut varieties studied.

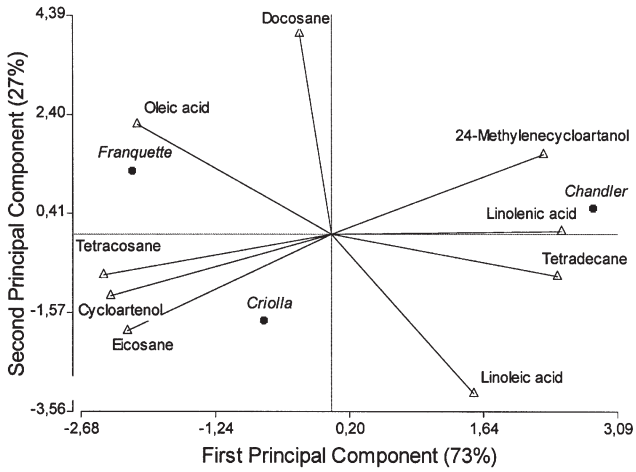

FIG. 1. Score plot of principal components 1 and 2 for chemical data (△) from three walnut oil varieties (●).

A multivariate analysis was carried out to select chemical parameters as a means of variety differentiation. All chemical parameters that presented significant differences were included in a PCA (Fig. 1), but the parameters that made the major contribution to the discrimination power were oleic and linolenic acids, tetradecane, eicosane, tetracosane, cycloartenol, and 24-methylenecycloartanol. The first principal component (PC1) explained 73% of the data variability and allowed separation of Franquette from Chandler variety. The former was associated mainly with oleic acid, whereas the Chandler variety was related to linolenic acid and, to a lesser extent, to 24-methylenecycloartanol and tetradecane. The Criolla variety was weakly related to cycloartenol and to C_{20} and C_{24} *n*-alkanes. PC2 (27% of the data variability) stressed the separation of Criolla variety from Franquette and Chandler varieties. Nevertheless, the Criolla variety appeared to be more related to the Franquette variety rather than to the Chandler variety.

The present investigation showed that both the variety and the crop year affect the chemical composition of WO. Different crop years significantly influence the tocopherol and volatile compositions, and different walnut cultivars had specific responses to this variation. FA composition and related parameters (PUFA/MUFA and iodine values) as well as some minor unsaponifiable lipid constituents constitute the major varietal influence. PCA adequately reduces the multidimensional structure of the data and may be a useful tool to determine the identity of walnut genotypes. However, future studies, focusing on the effect of other factors that could influence the oil composition, i.e., geographical location, climatic effects, the ripening grade of fruits, and their handling after harvest, should be made to arrive at stronger conclusions.

The high oil and oleic acid contents found in the Franquette variety are noteworthy. This variety is now being evaluated

with the goal of bringing it into commercial production of WO in Argentina.

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