Research Paper

Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina

Marcela L. Martínez and Damián M. Maestri

Instituto Multidisciplinario de Biología Vegetal, Instituto de Ciencia y Tecnología de los Alimentos, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

Walnut (*Juglans regia* L.) oil (WO) from the varieties Chandler, Franquette, Hartley, Lara, Mayette, Serr, Sorrento and Tulare were studied in order to evaluate genotypical variations in fatty acid (FA) and volatile compositions, tocopherol content and oxidative parameters. Oil content was found to range between 71.4 and 73.9%. Oils obtained by pressing presented low acid (0.05–0.22% oleic acid), peroxide (0.05–0.47 meq O_2/kg oil), K_{232}, and K_{270} values, and moderate (247–365 μg/g oil) total tocopherol contents. Variations in unsaturated fatty acid contents were between 16.1 and 25.4% (oleic acid), 52.5 and 58.9% (linoleic acid), and 11.4 and 16.5% (linolenic acid). Oxidative stability (OS), as measured by the Rancimat method, was poor (2.64–3.44 h) and it correlated positively with oleic and negatively with linolenic acid contents. In contrast to their low OS, the antioxidant capacity evaluated through the 2,2-diphenyl-1-picrylhydrazyl radical assay showed that the WO analyzed here have good radical-scavenging activity. Tocopherols appear to be the most important contributors to this biochemical property. The findings connected with volatile composition showed a similar qualitative pattern where aldehydes were present at higher concentration. Most of them seem to come from unsaturated FA mainly through a chemical pathway.

Keywords: Fatty acids / Oxidative parameters / Tocopherol content / Volatile compounds / Walnut genotypes

Received: May 7, 2008; accepted: August 15, 2008

DOI 10.1002/ejlt.200800121

1 Introduction

Walnut (*Juglans regia* L.) is an ancient species originating from Central Asia. At present, it is cultivated commercially throughout Southern Europe, Northern Africa, Eastern Asia, the USA and Western South America. The world production of whole walnut (with shell) was around 1,664,000 metric tons in 2006 [1]. China and the USA were the major producers, with about 25 and 20%, respectively, of the total world production, but yields in other countries such as Argentina have increased rapidly in the recent years.

The walnut seed (kernel) represents between 40 and 60% of the nut weight. The kernel contains high levels (60% in average) of oil; the range varies from 52 to 72% depending on the cultivar, geographic location and irrigation rate [2–4]. The major components of walnut oil (WO) are triacylglycerols (up to 98% of the oil), in which monounsaturated (oleic mainly) and polyunsaturated fatty acids (PUFA; linoleic and α-linolenic) are present in high amounts [2–5]. The high oil and essential fatty acid (FA) contents of the walnut kernel make it a good source for commercial production of edible oil. In a recent work [6], we studied the oil extraction process by screw-pressing followed by supercritical CO_2 extraction.

The FA composition of WO is strongly affected by the genotype. Interestingly, it has been demonstrated that the FA profile of walnut genotypes may influence flavor stability and, therefore, it may affect walnut palatability [7, 8]. The major cause of decreased flavor stability is oil oxidation, and this phenomenon depends mainly on the type and amount of FA present, but other factors such as the presence of antioxidant or pro-oxidant substances must be considered as well. Although WO have been widely studied for their FA and tocopherol composition [2, 4, 8–11], research findings associated with the screening and identification of volatile flavor components are limited. This work analyzes the relationships among

Correspondence: Damián M. Maestri, Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET – UNC), Instituto de Ciencia y Tecnología de los Alimentos (ICTA), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, X5016GCA Córdoba, Argentina.
E-mail: dmaestri@efn.uncor.edu
Fax: +54 0351 5334439

FA and volatile profiles, tocopherol content and oxidative parameters of eight walnut genotypes cultivated in Argentina. The data obtained are useful to identify suitable genotypes for walnut oil production.

2 Materials and methods

2.1 Collection site and plant material

Fruits from eight varieties (Chandler, Franquette, Hartley, Lara, Mayette, Serr, Sorrento and Tulare) of walnut (*Juglans regia* L.) were collected, at the same growing season, from commercial plantations located at Guanchín, La Rioja province, Argentina. Guanchín is located in the Monte phytogeographical area, at about 2000 m above sea level. The climate in this area represents a typical arid climate with great daily and annual temperature variations. The average monthly temperatures range from a minimum of –9 °C to a maximum of 40 °C. The average value of annual rainfall is about 300 mm, with rains mostly falling in summer and dry and short winters. Walnut plants were grown under natural rainfall, plus supplemental irrigation of 500 mm/year/plant.

From each variety, three samples (1.5 kg each) of healthy and mature fruits were picked by hand from the trees. Fruits were devoid of pericarp and then were dried at 30 ± 2 °C during 24 h.

2.2 Oil extraction and analyses

Dried nuts were shelled manually and whole kernels were used to obtain three oil samples from each variety, 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.

Acid, peroxide, K_{232}, K_{270} and color values of the oil samples were determined according to standard AOCS methods [13]. Total tocopherol content was measured according to Wong *et al.* [14]. The oxidative stability was determined by Rancimat analysis. The air flow rate was set at 20 L/h and the temperature of the heating block was maintained at 110 °C [6].

To evaluate the radical-scavenging activity of WO, two sets of experiments were carried out. In the first one, 100 mg (in 1 mL toluene) of each oil was vortexed (20 s, ambient temperature) with 3.9 mL toluene solution (10^{-4} M) of the free stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (DPPH•). Against a blank of pure toluene, the absorption at 515 nm was measured in 1-cm quartz cells after 1, 30 and 60 min of mixing using a UV-visible spectrophotometer (Perkin-Elmer Lambda 25, Shelton, CT, USA). The radical-scavenging activity toward DPPH• was estimated by means of the following equation:

$$DPPH\bullet_r = 1 - \left[\frac{\text{absorbance of control} - \text{absorbance of testsample}}{\text{absorbance of control}} \times 100\right]$$

where $DPPH\bullet_r$ expresses the amount of the radical that remains in the medium after antioxidants present in the oils are depleted. In the second experiment, three concentrations (75, 100 and 150 mg oil in 1 mL toluene) were prepared for each WO variety. The oil/toluene solutions were added separately to 3.9 mL DPPH• solution (10^{-4} M) and the absorbance of each mixture was determined at 515 nm after 30 min of mixing. The radical-scavenging activity was expressed as EC_{50}, which was defined as the concentration at which 50% of the initial absorbance was reduced. A lower EC_{50} value indicates a higher antiradical activity.

For the determination of FA composition, each oil sample (0.5 g) was subjected to alkaline saponification by reflux (45 min) using 30 mL 1 N KOH in methanol. Unsaponifiable matter was extracted with *n*-hexane (3 × 30 mL). The fatty acids were converted to methyl esters (FAME) by reflux (45 min) using 50 mL 1 N H_2SO_4 in methanol and analyzed by GC (Perkin-Elemer Clarus 500, Shelton, CT, USA) 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, USA); carrier gas: N_2 at 1 mL/min; column temperature programmed from 180 °C (5 min) to 240 °C at 4 °C/min; injector and detector temperatures at 250 °C; FID. The identification of FAME was carried out by comparison of their retention times with those of reference compounds (Sigma-Aldrich, St. Louis, MO, USA).

Iodine values (IV) were calculated from FA percentages by using the formula:

IV = (% oleic acid × 0.899) + (% linoleic acid × 1.814) + (% linolenic acid × 2.737)

Volatile compounds were analyzed by solid-phase microextraction (SPME) coupled to GC-MS as reported earlier [15]. Briefly, fresh oil samples (7.5 mL) were put in 15-mL headspace vials, fitted with silicon septa, and heated at 50 °C. Volatiles were sampled for 30 min from the headspace of the vial, with a 100-μm fiber coated with divinylbenzene/carboxene on polydimethylsiloxane (DVB/CAR-PDMS), conditioned prior to use as recommended by the producer. Among diverse fiber coatings tested, DVB/CAR-PDMS was found to be the most suitable for the analysis of oil volatiles because it provides the highest number of compounds detected [16]. After sampling, the fiber was immediately inserted into the injection port (250 °C) of a HP 5890 II gas chromatograph coupled to a HP 5972 A mass-selective detector (Hewlett Packard, Palo Alto, CA, USA), and it was thermally desorbed for 1 min. The GC separations were performed using a HP 5 fused-silica capillary column (30 m long × 0.25 mm i.d.), coated with a 0.25-μm layer of 5% phenyl methyl siloxane, and helium (flow rate 1 mL/min) as carrier gas. The GC oven temperature was initially maintained at 50 °C (2 min) and then increased to 250 °C (5 °C/min). Volatile compounds were identified by comparison of their mass spectra data with those of the Wiley 275 mass

spectra search library. Identification of the components was also based on their GC retention indices (RI) on the HP 5 column, determined relative to the retention times of a series of *n*-alkanes (C6–C30) and compared with literature data [16]. To make analytical data comparable, the peak areas of each identified compound in WO samples were percent normalized.

For oil content determination, samples of dry, finely chopped walnuts (10 g) were extracted with *n*-hexane in accordance with AOCS [13] method Am-2-93.

2.3 Statistical analyses

The analytical determinations reported in this study are the average of triplicate measurements from three independent oil samples of each walnut variety. Statistical differences among varieties were estimated from the ANOVA test. Whenever ANOVA indicated a significant difference, a pairwise comparison of means by least significant difference (LSD) was carried out. Correlation analysis was performed employing Pearson's test.

3 Results and discussion

The oil content was found to vary between 71.4 and 73.9% (Table 1). There were minor differences among genotypes; all of them contained more oil than identified in walnut varieties cultivated in several countries [11]. No significant correlations were observed between oil content and kernel size or kernel weight (data not shown), suggesting that oil accumulation is not influenced by these physical characteristics of the seeds.

The freshly extracted oils from all selected genotypes had very low acid values (AV) (0.05–0.22% oleic acid) and peroxide values (PV) (0.05–0.47 meq O_2/kg oil) (Table 1). The highest values of both AV and PV were registered in the Lara variety. However, these values were significantly smaller than those observed in solvent-extracted WO [2, 8, 17, 18]. Extraction of WO by pressing at room temperature would not result in thermal and/or hydrolytic cleavage of FA from the glycerol backbone of the triacylglycerols. Hence, the free fatty acid (FFA) content in cold-pressed WO would be expected to reflect the real level present in the seed. However, lipases, such as those present in bacteria and moulds, might catalyze the FA cleavage. Possibly, some fruits of the Lara variety could have been infected and this fact may have led to increased hydrolysis of WO triacylglycerols. Coincidently, oil from Lara growing in Portugal exhibited a very high AV [2]. As expected, a higher AV in this variety increased the PV of the oil, suggesting a pro-oxidant effect of FFA as reported by Frega *et al.* [19] in diverse vegetable oils. This hypothesis is supported by a highly significant positive correlation between AV and PV ($r = 0.93$, $p \leq 0.01$).

Table 1. Oil content, fatty acid composition and chemical quality parameters of eight walnut varieties.

	Walnut varieties							
Parameter	Chandler	Franquette	Hartley	Lara	Mayette	Serr	Sorrento	Tulare
OC	$72.5^{bc} \pm 0.10$	$72.0^{abc} \pm 0.40$	$71.4^{ab} \pm 0.001$	$71.2^{a} \pm 0.30$	$73.2^{cd} \pm 0.70$	$72.8^{cd} \pm 0.001$	$73.9^{d} \pm 0.90$	$73.6^{d} \pm 0.70$
AV	$0.05^{a} \pm 0.01$	$0.05^{a} \pm 0.01$	$0.07^{ab} \pm 0.01$	$0.22^{d} \pm 0.01$	$0.05^{a} \pm 0.01$	$0.08^{b} \pm 0.001$	$0.11^{c} \pm 0.03$	$0.05^{a} \pm 0.01$
PV	$0.08^{a} \pm 0.001$	$0.05^{a} \pm 0.01$	$0.08^{a} \pm 0.001$	$0.47^{d} \pm 0.001$	$0.16^{b} \pm 0.001$	$0.13^{b} \pm 0.01$	$0.26^{c} \pm 0.06$	$0.06^{a} \pm 0.001$
K_{232}	$1.10^{ab} \pm 0.001$	$1.06^{a} \pm 0.02$	$1.15^{bc} \pm 0.03$	$1.05^{a} \pm 0.001$	$1.05^{a} \pm 0.01$	$1.09^{ab} \pm 0.02$	$1.21^{c} \pm 0.07$	$1.04^{a} \pm 0.001$
K_{270}	$0.05^{a} \pm 0.001$	$0.05^{ab} \pm 0.001$	$0.07^{bc} \pm 0.02$	$0.06^{ab} \pm 0.001$	$0.06^{ab} \pm 0.001$	$0.05^{a} \pm 0.001$	$0.07^{c} \pm 0.001$	$0.05^{ab} \pm 0.001$
Color (R)	$1.15^{a} \pm 0.07$	$1.20^{a} \pm 0.001$	$1.60^{b} \pm 0.001$	$1.20^{a} \pm 0.001$	$1.20^{a} \pm 0.28$	$1.50^{b} \pm 0.001$	$1.00^{a} \pm 0.14$	$0.95^{a} \pm 0.07$
Color (Y)	$11.0^{b} \pm 0.001$	$11.0^{b} \pm 1.40$	$14.0^{b} \pm 0.001$	$12.0^{b} \pm 0.001$	$12.0^{b} \pm 0.20$	$13.0^{b} \pm 0.001$	$10.5^{b} \pm 0.70$	$6.50^{a} \pm 0.71$
TTC	$280^{b} \pm 0.20$	$318^{d} \pm 0.10$	$328^{de} \pm 1.20$	$335^{e} \pm 1.40$	$299^{c} \pm 5.40$	$247^{a} \pm 1.1$	$365^{f} \pm 13.2$	$279^{b} \pm 1.2$
EC_{50}	$692.7^{e} \pm 10.7$	$655.2^{d} \pm 8.45$	$621.0^{bc} \pm 19.9$	$609.2^{b} \pm 9.72$	$639.6^{cd} \pm 1.23$	$796.3^{g} \pm 7.79$	$569.5^{a} \pm 8.67$	$736.6^{f} \pm 4.76$
OSta	$2.64^{a} \pm 0.16$	$3.39^{cd} \pm 0.16$	$3.13^{bcd} \pm 0.24$	$2.87^{ab} \pm 0.25$	$3.40^{cd} \pm 0.19$	$3.04^{bc} \pm 0.06$	$3.02^{b} \pm 0.02$	$3.44^{d} \pm 0.01$
FA								
Palmitic	$6.81^{abc} \pm 0.10$	$7.05^{bc} \pm 0.19$	$8.14^{d} \pm 0.06$	$8.15^{d} \pm 0.28$	$6.63^{ab} \pm 0.02$	$6.60^{ab} \pm 0.08$	$7.16^{c} \pm 0.43$	$6.38^{a} \pm 0.08$
Stearic	$1.65^{b} \pm 0.05$	$1.89^{bc} \pm 0.03$	$0.93^{a} \pm 0.05$	$1.63^{b} \pm 0.35$	$1.77^{b} \pm 0.02$	$1.80^{b} \pm 0.01$	$1.71^{b} \pm 0.08$	$2.16^{c} \pm 0.11$
Oleic	$16.1^{a} \pm 0.01$	$23.2^{f} \pm 0.20$	$17.9^{c} \pm 0.20$	$19.7^{d} \pm 0.01$	$22.3^{e} \pm 0.01$	$25.4^{h} \pm 0.20$	$17.2^{b} \pm 0.01$	$24.1^{g} \pm 0.10$
Linoleic	$58.9^{c} \pm 0.01$	$55.3^{b} \pm 0.50$	$58.4^{c} \pm 0.90$	$57.9^{c} \pm 0.10$	$55.4^{b} \pm 0.30$	$52.5^{a} \pm 0.50$	$58.9^{c} \pm 0.10$	$55.9^{b} \pm 0.10$
Linolenic	$16.5^{c} \pm 0.20$	$12.5^{ab} \pm 0.50$	$14.6^{cd} \pm 1.10$	$12.5^{ab} \pm 0.01$	$13.9^{cd} \pm 0.40$	$13.6^{bc} \pm 0.70$	$15.0^{d} \pm 0.40$	$11.4^{a} \pm 0.20$
IV	$166.0^{e} \pm 0.46$	$155.0^{ab} \pm 0.20$	$162.0^{d} \pm 1.07$	$157.1^{bc} \pm 0.12$	$158.6^{c} \pm 0.47$	$155.4^{ab} \pm 0.90$	$163.5^{d} \pm 1.26$	$154.3^{a} \pm 0.31$

OC, oil content (%); AV, acid value (% oleic acid); PV, peroxide value (meq O_2/kg oil); K_{232}, conjugated dienes; K_{270}, conjugated trienes; color (R, red), (Y, yellow); TTC, total tocopherol content (μg/g oil); EC_{50} (mg oil/mg DPPH•); OSta, oxidative stability (h); FA, fatty acids (% of total fatty acids); IV, iodine value.

Mean values of each variety are the average of three independent measurements. Values in each row with different superscript letters present significant differences ($p \leq 0.05$) among walnut varieties.

Minor differences in the UV absorption coefficients (K_{232} and K_{270} values) were observed among the genotypes (Table 1). The values obtained from all samples analyzed were in agreement with those from WO extracted by solvent or pressing [17].

The color of the oils varied from clear amber to yellow. Lovibond values for red color were in the range of 0.95–1.60, whereas yellow color varied from 6.5 to 14.0 (Table 1). A significant positive correlation between both parameters was found ($r = 0.85$, $p \leq 0.01$).

Statistically significant differences among varieties were found for all FA analyzed (Table 1). Saturated (palmitic and stearic) FA accounted for 8.4–9.8% of the total FA. Variations in unsaturated FA composition were as follows: Oleic acid was between 16.1 (Chandler variety) and 25.4% (Serr variety); linoleic acid varied from 52.5 (Serr variety) to 58.9% (Chandler and Sorrento varieties); linolenic acid changed between 11.4 (Tulare variety) and 16.5% (Chandler variety). Similarly to other oily seeds [20, 21], highly significant negative correlations were found between the oleic acid percentage and each of two PUFA (linoleic and linolenic) ($r = -0.93$ and $r = -0.73$, respectively, $p \leq 0.01$). The values for the main FA were, in general, similar to those reported elsewhere [2, 4, 8, 11], but three genotypes (Franquette, Serr and Tulare) had high proportions (>23%) of oleic acid, which are significantly higher than those registered in such reports. For example, Amaral *et al.* [2], when analyzing the FA composition of six walnut varieties (including Franquette, Mayette and Lara) grown in Portugal, found that oleic acid ranged from 14.26 to 18.09%. Savage *et al.* [8] studied 13 walnut varietal oils from New Zealand, Europe and the USA and obtained, on average, lower values of oleic acid than those determined in the present work. A worldwide study of WO from unspecific varieties [11] showed proportions of saturated FA within the range reported here, but the average proportion of oleic acid was much lower and that of linoleic acid was higher than reported here. Finally, the percentages of the main FA in Chandler and Franquette oils were in general agreement with those from the Catamarca province, Argentina, analyzed earlier [4]. All works mentioned previously confirm that the genotype is the main variability source for FA composition of WO, but it may be influenced by geographical origin or other factors such as agricultural practices. The mean calculated IV of the oils studied herein were comparable to those reported earlier [11], but with a narrower range.

The DPPH• assay measures the ability of the antioxidants present in the oil to scavenge free radicals. Tocopherols and polyphenols in nut oils have previously been identified as possibly being the main compounds responsible for their free-radical-scavenging capacity [22, 23].

A highly significant variation among genotypes was observed for total tocopherol content (Table 1). Overall, the values obtained in this work (247–365 μg/g oil) were similar to those observed in walnuts from Europe, Asia and the USA [11].

Recently, we have reported a high phenolic content with antioxidant activity in whole kernels from different walnut varieties [24]. In the present work, an attempt was made to determine the presence of phenolic compounds in the oils, but the results were negative. Therefore, the activity of phenolics other than tocopherols appears to be negligible in providing some protection against oxidation in WO. By means of spectrophotometric recordings obtained in a kinetic assay (Fig. 1), a strong decrease in DPPH• concentration was observed when it was added to WO. After 30 min of incubation, 40.3–55.5% of DPPH• radicals were quenched and the reaction reached a plateau. The highest percentage of DPPH• inhibition was found for Sorrento varietal oil, and the lowest one for Serr oil. The EC_{50} values from the DPPH assay using different oil concentrations (Table 1) correlated significantly and inversely with the total tocopherol contents ($r = -0.95$, $p \leq 0.01$). The order of effectiveness of varietal oils in inhibiting DPPH• was as follow: Sorrento > Lara > Hartley > Mayette > Franquette > Chandler > Tulare > Serr. All EC_{50} values were found to be lower than those reported earlier in some WO from different origins [22, 23].

In agreement with data reported elsewhere [2, 8], oxidative stability determined by Rancimat (Table 1) ranged from 2.64 (Chandler variety) to 3.44 (Tulare variety). The correlation analysis showed that the Rancimat values of the oils were negatively associated with linolenic acid percentages ($r = -0.63$, $p \leq 0.01$), but positively correlated with oleic acid content ($r = 0.61$, $p \leq 0.01$). Surprisingly, neither PV nor tocopherol content were correlated with oxidative stability.

Table 2 shows the headspace volatile composition of WO sampled after heating (30 min) at 50 °C. The majority of the identified volatile components were those reported previously as constituents of varietal WO using the SPME-GC-MS method [15]. Most of them were saturated and unsaturated aldehydes which represented 55–81% of the total volatile components. In all samples, 2,4-decadienal was quantitatively the largest carbonyl compound, followed by hexanal. These aldehydes arise from two main linoleate hydroperoxide isomers: 2,4-Decadienal is formed exclusively from the linoleate 9-hydroperoxide, whereas hexanal is a typical linoleate 13-hydroperoxide derivative. At 50 °C, these hydroperoxide isomers are produced in similar amounts [25]. Other carbonyl compounds found in significant quantities were C8–C11 aldehydes. Evidence indicates that these oil volatile components are formed mainly through a chemical pathway from oleate 8-hydroperoxide (2-undecenal), oleate 9-hydroperoxide (2-decenal), oleate 10-hydroperoxide (nonanal), and oleate 11-hydroperoxide (octanal). These hydroperoxide isomers are produced in similar amounts during the oxidation of oleic acid [25]. In spite of the relative abundance of linolenic acid in the oils analyzed, 2-hexenal and 2,4-heptadienal (two of the most abundant linolenate hydroperoxide isomers) were found in small amounts. Although a few hydrocarbons, particularly pentane which is formed primarily from linoleic acid, were present in high amounts in some varieties, their contribution to the WO flavor could be negligible owing to their

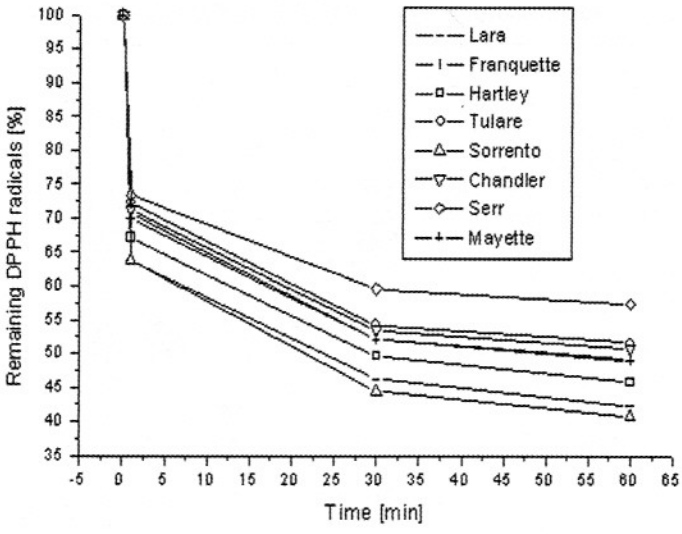

Figure 1. Radical-scavenging activity of walnut varietal oils during DPPH kinetic assay.

flavor thresholds being significantly higher than those of aldehydes [26]. The formation of some volatiles (pentanal, 2-heptenal, 2-octenal) could be better explained through an enzymatic pathway rather than a chemical (non-enzymatic) one. In walnuts, as in a wide variety of beans, peas, peanuts and almonds, substantial quantities of lipoxygenases (Lox) may be present [27, 28]. Lox catalyze the oxidation of PUFA containing *cis*,*cis*-1,4-pentadiene units, such as linoleic and linolenic acids. The hydroperoxides produced by Lox undergo cleavage to give short- and medium-chain hydrocarbons, aldehydes and alcohols, similar to those formed by non-enzymatic autoxidation, which contribute to the headspace volatile flavors [25]. So, in many of these decomposition reactions, it may be difficult to distinguish between enzymatic and non-enzymatic processes.

4 Conclusions

The results presented in this study indicate significant differences in total oil and tocopherol contents, fatty acid composition, oxidative stability and radical-scavenging activity among eight walnut varieties cultivated in Argentina. The oil yields were higher than the mean value (69%) reported for walnuts from different origins. The variability found in tocopherol and individual FA contents was in general agreement with that reported for the Eurasian and North American walnut distribution range. Considering that the walnut varieties were grown under the same experimental conditions, it is assumed that the differences among them were mainly due to genotypical variations.

In relation to oxidative stability parameters, low values of peroxide and UV absorption coefficients were registered in fresh extracted oils, but when they were subjected to an accelerated stability test, a poorer oxidative stability was observed as compared to other common vegetable oils.

In contrast with their low oxidative stability, the antioxidant capacity evaluated through the DPPH assay showed that the WO analyzed here have good radical-scavenging activity. Tocopherols appear to be the most important contributors to this biochemical property.

Findings connected with volatile composition showed a similar qualitative pattern where aldehydes were present at higher concentration. Most of them seem to come from unsaturated FA, mainly through a chemical pathway.

This work adds and updates the available compositional tables for some WO components. Showing that most of the chemical parameters analyzed have been influenced by the genotype, the results obtained may contribute to establish quality criteria in order to select walnut varieties for oil production.

A number of investigations have demonstrated that WO is an excellent source of edible oil with one of the highest known amounts of unsaturated FA. Although WO is not yet described by the current Committee on Fats and Oils of the Codex Alimentarious, its consumption is gaining popularity owing to the beneficial effects on human health, particularly on blood lipid levels, with a decrease in total and LDL cholesterol as well as triacylglycerols, and an increase in HDL cholesterol [29, 30], all of which represent a protective factor against cardiovascular risk.

Acknowledgments

This work was financed with grants from CONICET, Secretaría de Ciencia y Tecnología – Universidad Nacional de Córdoba (SeCyT-UNC), and the Ministerio de Ciencia y Tecnología de la provincia de Córdoba. M.L.M. is a fellow and D.M.M. is a career researcher at CONICET (Argentina).

Table 2. Volatile composition (peak area %) of walnut oil varieties determined by headspace SPME at 50 °C followed by GC-MS analysis.

		Walnut varieties							
Compound†	RI§	Chandler	Franquette	Hartley	Lara	Mayette	Serr	Sorrento	Tulare
n-Pentane	500	$26.5^{ab} \pm 8.98$	$18.4^{a} \pm 1.34$	$21.2^{a} \pm 6.01$	$34.7^{b} \pm 3.61$	$16.2^{a} \pm 1.34$	$16.5^{a} \pm 1.34$	$20.6^{a} \pm 4.95$	$33.1^{b} \pm 4.67$
n-Hexane	600	$4.56^{a} \pm 0.93$	$6.70^{a} \pm 0.44$	$2.68^{a} \pm 0.49$	$5.85^{a} \pm 1.02$	$2.96^{a} \pm 0.67$	$5.48^{a} \pm 1.87$	$2.91^{a} \pm 4.11$	$11.7^{b} \pm 2.26$
Pentanal	670	$10.2^{c} \pm 1.92$	$8.24^{bc} \pm 2.63$	$7.60^{bc} \pm 0.72$	$7.71^{bc} \pm 0.55$	$6.25^{ab} \pm 1.10$	$4.20^{a} \pm 0.52$	$6.77^{ab} \pm 1.07$	$4.32^{a} \pm 0.59$
1-Pentanol	753	Tr	$1.94^{a} \pm 0.88$	$1.49^{a} \pm 0.71$	Tr	Tr	$1.60^{a} \pm 0.19$	Tr	Tr
Hexanal	774	$12.3^{a} \pm 5.59$	$14.1^{a} \pm 1.91$	$13.8^{a} \pm 2.97$	$8.06^{a} \pm 1.30$	$20.8^{b} \pm 2.90$	$14.4^{ab} \pm 1.84$	$14.2^{a} \pm 1.36$	$9.82^{a} \pm 1.96$
2-Hexenal	828	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Heptanal	882	Tr	$2.10^{b} \pm 1.03$	$2.11^{b} \pm 0.88$	Tr	$1.52^{ab} \pm 0.54$	$2.11^{b} \pm 0.65$	$1.38^{ab} \pm 1.94$	Tr
2-Heptenal	934	$4.46^{b} \pm 2.05$	$3.35^{b} \pm 1.87$	$3.98^{b} \pm 0.24$	$2.71^{ab} \pm 1.58$	$3.21^{b} \pm 1.00$	$4.32^{b} \pm 0.83$	$4.75^{b} \pm 0.78$	Tr
2,4-Heptadienal	973	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
2-Pentylfuran	979	$3.02^{b} \pm 1.46$	$2.04^{ab} \pm 1.63$	$3.10^{b} \pm 1.04$	$1.96^{ab} \pm 0.83$	$2.58^{ab} \pm 0.35$	$2.93^{ab} \pm 0.59$	$1.80^{ab} \pm 2.55$	Tr
Octanal	985	$2.03^{abcd} \pm 1.34$	$3.77^{d} \pm 0.95$	$2.38^{bcd} \pm 1.39$	$1.89^{abcd} \pm 0.99$	Tr	$3.39^{cd} \pm 1.40$	$0.39^{ab} \pm 0.55$	
$1.23^{abc} \pm 0.38$									
2-Octenal	1036	Tr	$1.84^{a} \pm 0.51$	$1.86^{a} \pm 1.12$	Tr	$2.18^{ab} \pm 0.23$	$2.91^{b} \pm 0.27$	Tr	Tr
Nonanal	1087	$4.96^{a} \pm 2.61$	$6.63^{ab} \pm 1.04$	$5.05^{a} \pm 1.71$	$5.45^{a} \pm 1.12$	$5.19^{a} \pm 1.56$	$6.91^{ab} \pm 1.75$	$4.79^{a} \pm 2.30$	$11.0^{b} \pm 3.32$
2-Nonenal	1137	Tr	0.55 ± 0.33	Tr	Tr	Tr	Tr	Tr	Tr
Decanal	1189	Tr	$1.64^{bc} \pm 0.46$	$1.0^{ab} \pm 0.13$	$2.88^{c} \pm 1.12$	Tr	$2.32^{c} \pm 0.55$	$0.66^{ab} \pm 0.93$	Tr
2-Decenal	1241	$3.27^{ab} \pm 1.17$	$5.51^{bcd} \pm 0.54$	$3.77^{ab} \pm 1.67$	$2.99^{a} \pm 0.40$	$4.16^{abc} \pm 0.41$	$6.77^{d} \pm 1.22$	$3.73^{ab} \pm 0.23$	$6.44^{cd} \pm 1.35$
2,4-Decadienal	1292	$24.6^{a} \pm 5.94$	$23.5^{a} \pm 4.53$	$24.0^{a} \pm 8.20$	$23.5^{a} \pm 11.7$	$29.2^{a} \pm 2.97$	$19.4^{a} \pm 3.68$	$34.1^{a} \pm 17.2$	$15.7^{a} \pm 0.85$
2-Undecenal	1350	$3.72^{ab} \pm 1.34$	$6.49^{b} \pm 0.38$	$3.70^{ab} \pm 1.30$	$2.27^{a} \pm 0.21$	$5.81^{b} \pm 3.01$	$6.71^{b} \pm 2.13$	$3.94^{ab} \pm 0.68$	$6.51^{b} \pm 0.83$
Dodecanal	1411	Tr	Tr	0.76 ± 0.08	Tr	Tr	Tr	Tr	Tr

Mean values of each variety are the average of three independent measurements. Tr, trace (<0.3%). Values in each row with different superscript letters present significant differences ($p \leq 0.05$) among walnut varieties.

† Order of elution and percentages of components are given on the apolar HP 5 column.

§ Retention indices as determined on the HP 5 column using the homologous series of *n*-alkanes.

Conflict of interest statement

The authors have declared no conflict of interest.

References

[1] FAOSTAT, Food and Agriculture Organization: *FAOSTAT data*. Rome (Italy) 2006.

[2] J. S. Amaral, S. Casal, J. A. Pereira, R. M. Seabra, B. B. P. Oliveira: Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. *J Agric Food Chem.* 2003, **51**, 7698–7702.

[3] R. B. N. Prasad: Walnuts and pecans. In: *Encyclopaedia of Food Science and Nutrition*. Academic Press, London (UK) 2003, pp. 6071–6079.

[4] M. L. Martínez, M. Mattea, D. M. Maestri: Varietal and crop year effects on lipid composition of walnut (*Juglans regia*) genotypes. *J Am Oil Chem Soc.* 2006, **83**, 791–796.

[5] L. C. Greve, G. McGranahan, J. Hasey, R. Snyder, K. Kelly, D. Goldhamer, J. M. Labavitch: Variation in polyunsaturated fatty acids composition of Persian walnut. *J Am Soc Hort Sci.* 1992, **117**, 518–522.

[6] M. L. Martínez, M. Mattea, D. M. Maestri: Pressing and supercritical carbon dioxide extraction of walnut oil. *J Food Eng.* 2008, **88**, 399–404.

[7] F. Sinesio, E. Moneta: Sensory evaluation of walnut fruit. *Food Qual Pref.* 1997, **8**, 35–43.

[8] G. P. Savage, P. C. Dutta, D. L. McNeil: Fatty acid and tocopherol contents and oxidative stability of walnut oils. *J Am Oil Chem Soc.* 1999, **76**, 1059–1063.

[9] F. Lavedrine, A. Ravel, A. Poupard, J. Alary: Effect of geographic origin, variety and storage on tocopherol concentrations in walnuts by HPLC. *Food Chem.* 1997, **58**, 135–140.

[10] J. S. Amaral, M. Rui Alvez, R. M. Seabra, B. P. P. Oliveira: Vitamin E composition of walnuts (*Juglans regia* L.): A 3-year comparative study of different cultivars. *J Agric Food Chem.* 2005, **53**, 5467–5472.

[11] C. Crews, P. Hough, J. Godward, P. Brereton, M. Lees, S. Guiet, W. Winkelmann: Study of the main constituents of some authentic walnut oils. *J Agric Food Chem.* 2005, **53**, 4853–4860.

[12] L. Tobares, C. A. Guzmán, D. M. Maestri: Effect of the extraction method and bleaching on jojoba (*Simmondsia chinensis*) wax quality. *Eur J Lipid Sci Technol.* 2003, **105**, 749–753.

[13] AOCS: *Official Methods and Recommended Practices of the American Oil Chemists' Society*. 5th Edn. AOCS Press, Champaign, IL (USA) 1998.

[14] M. L. Wong, R. E. Timms, E. M. Goh: Colorimetric determination of total tocopherols in palm oil, olein and stearin. *J Am Oil Chem Soc.* 1988, **65**, 258–261.

[15] M. M. Torres, M. L. Martínez, D. M. Maestri: A multivariate study of the relationship between fatty acids and volatile flavor

components in olive and walnut oils. *J Am Oil Chem Soc.* 2005, **82**, 105–110.

[16] S. Vichi, A. I. Castellote, L. Pizzale, L. S. Conte, S. Buxaderas, E. López-Tamames: Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. *J Chromatogr A.* 2003, **983**, 19–33.

[17] T. D. Crowe, T. W. Crowe, L. A. Johnson, P. J. White: Impact of extraction method on yield of lipid oxidation products from oxidized and unoxidized walnuts. *J Am Oil Chem Soc.* 2003, **79**, 453–456.

[18] L. S. Maguire, S. M. O'Sullivan, K. Galvin, T. P. O'Connor, N. M. O'Brien: Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *Int J Food Sci Technol.* 2004, **55**, 171–178.

[19] N. Frega, M. Mozzon, G. Lercker: Effects of free acid acids on oxidative stability of vegetable oils. *J Am Oil Chem Soc.* 1999, **76**, 325–329.

[20] J. J. Salas, J. Sánchez, U. S. Ramli, A. M. Manaf, M. Williams, J. L. Harwood: Biochemistry of lipid metabolism in olive and other oil fruits. *Prog Lipid Res.* 2000, **39**, 151–180.

[21] M. A. Askin, M. F. Balta, F. E. Tekintas, A. Kazankaya, F. Balta: Fatty acid composition affected by kernel weight in almond [*Prunus dulcis* (Mill.) D.A. Webb.] genetic resources. *J Food Comp Anal.* 2007, **20**, 7–12.

[22] L. Li, R. Tsao, R. Yang, J. K. G. Kramer, M. Hernández: Fatty acid profiles, tocopherol contents, and antioxidant activities of hearnut (*Juglans ailanthifolia* var. *cordiformis*) and Persian walnut (*Juglans regia* L.). *J Agric Food Chem.* 2007, **55**, 1164–1169.

[23] S. Arranz, R. Cert, J. Pérez-Jiménez, A. Cert, F. Saura-Calixto: Comparison between radical scavenging capacity and oxidative stability of nut oils. *Food Chem.*, in press.

[24] D. O. Labuckas, D. M. Maestri, M. Perelló, M. L. Martínez, A. L. Lamarque: Phenolics from walnut (*Juglans regia* L.) kernels: Antioxidant activity and interactions with proteins. *Food Chem.* 2008, **107**, 607–612.

[25] E. N. Frankel: *Lipid Oxidation.* P.J. Barnes & Associates, Bridgwater (UK) 2005.

[26] S. P. Kochhar: Oxidative pathways to the formation of off-flavours. In: *Food Taints and Off-Flavours.* Ed. M. J. Saxby, Blackie Academic & Professional, London (UK) 1993, pp. 150–201.

[27] J. R. Whitaker: Lipoxygenases. In: *Oxidative Enzymes in Foods.* Eds. D. S. Robinson, N. A. M. Eskin, Elsevier, London (UK) 1991, pp. 175–215.

[28] A. Buranasompob, J. Tang, J. R. Powers, J. Reyes, S. Clark, B. G. Swanson: Lipoxygenase activity in walnuts and almonds. *LWT – Food Sci Technol.* 2007, **40**, 893–899.

[29] A. Chisholm, J. Mann, M. Skeaff, C. Frampton, W. Sutherland, A. Duncan, S. Tiszavari: A diet rich in walnuts favourably influences plasma fatty acid profile in moderately hyperlipidaemic subjects. *Eur J Clin Nutr.* 1998, **52**, 12–16.

[30] F. Lavedrine, D. Zmirou, A. Ravel, F. Balducci, J. Alary: Blood cholesterol and walnut consumption: A cross-sectional survey in France. *Prev Med.* 1999, **28**, 333–339.

