A Multivariate Study of the Relationship Between Fatty Acids and Volatile Flavor Components in Olive and Walnut Oils

Mariela M. Torres, Marcela L. Martínez, and Damián M. Maestri*

Instituto Multidisciplinario de Biología Vegetal (IMBIV), Consejo Nacional de Investigaciones Científicas y
Técnicas–Universidad Nacional de Córdoba (CONICET-UNC), Cátedra de Química Orgánica, Facultad de Ciencias
Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, X5016GCA Córdoba, Argentina

ABSTRACT: The relationships between FA and the volatile profiles of olive and walnut oils from Argentina were studied using GC and solid-phase microextraction coupled with GC–MS. The major volatiles were aldehydes and hydrocarbons, produced mainly through the oxidative pathways. *n*-Pentane, nonanal, and 2,4-decadienal were predominant in walnut oils, whereas nonanal, 2-decenal, and 2-undecenal were the most abundant components in olive oils. A multivariate analysis applied to the chemical data emphasized the differences between the oils and allowed us to see a pattern of covariation among the FA and the volatile compounds. The main differences between walnut and olive oils were the presence of larger amounts of short-chain (C_5–C_6) saturated hydrocarbons and aldehydes in the former and the greater quantities of medium-chain (C_7–C_{11}) compounds in olive oil. This can be explained by their different origins, mainly from the linoleic acid in walnut oil or almost exclusively from the oleic acid in olive oil.

Paper no. J10947 in *JAOCS 82*, 105–110 (February 2005).

KEY WORDS: Fatty acids, multivariate analysis, olive oil, volatile compounds, walnut oil.

In Argentina, two important economic activities are the commercial production of olives and walnuts, especially in semiarid regions that are considered marginal areas for conventional crops. Most of the Argentinean olive production is destined for oil manufacture, and the oil is appreciated internationally. Virgin olive oils have a complex flavor owing to the presence of many volatile compounds, such as aldehydes, hydrocarbons, alcohols, ketones, acids, and esters (1–3). Some of the volatiles are produced through the lipoxygenase pathway and are always present in the headspace of virgin olive oils, although at different concentrations, depending on the olive cultivar (4–6). Walnut oil has been used as an alternative to olive oil for salad dressing. Although it possesses an abundant flavor, which is greatly enjoyed by consumers, the volatile composition of walnut oil has been scantly documented (7,8).

One of the most important characteristics of olive oils is the presence of a high content of oleic acid (9). Walnut oils are rich in linoleic acid, but oleic and linolenic acids also may be present in significant amounts (10,11). Although these facts are considered to be nutritionally favorable, the high content of unsaturated FA plays a crucial role in the generation of fat-derived flavor components.

In this work, the FA and volatile fractions of olive and walnut oils were examined and the data obtained were further analyzed by a multivariate statistical method to verify its ability to distinguish the two groups of oils. Another objective was to analyze the interrelation between flavor development and FA composition. The results from this analysis are discussed in relation to available chemical knowledge.

EXPERIMENTAL PROCEDURES

Plant material. Oils from five olive and three walnut varieties, chosen from among the most representative of the Argentine production, were studied.

Olive (*Olea europea* L.) fruits of the varieties Arbequina, Manzanilla, Nevadillo, Farga, and Frantoio were collected from Traslasierra Valley, Córdoba province, Argentina. Three samples (10 kg each) of each variety were picked by hand at the same degree of ripening. After homogenization and cleaning, each sample was crushed, the olive paste was kneaded for 50 min at 22 ± 2°C and then squeezed. The liquid obtained (aqueous and oily) was separated in a stainless-steel decanter.

Walnut (*Juglands regia* L.) fruits of the varieties Criolla, Chandler, and Franquette were collected from commercial plantations at the Belén location, Catamarca province, Argentina. Three samples (10 kg each) of each variety were picked by hand from the trees and were then dried at 30 ± 2°C in a day's time. Fruits were shelled, and the whole kernels were used to obtain oil samples, using a manually operated pilot-plant hydraulic press as described previously (12).

All oil samples were filtered through anhydrous Na_2SO_4 and stored at −10°C in dark glass bottles until analysis.

FA analysis. FA were analyzed by GC. Briefly, oils were subjected to alkaline saponification (1 N KOH) and the unsaponifiable matter was extracted with *n*-hexane. FAME were obtained using 1 N H_2SO_4 in methanol. The identification of FAME was carried out by GC–MS (13) and by comparison of the retention times with those of reference compounds.

Analysis of volatile compounds. Fresh oil samples (5 mL)

---

*To whom correspondence should be addressed at Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET-UNC), Cátedra de Química Orgánica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina. E-mail: dmaestri@efn.uncor.edu from each olive and walnut variety were added to 15-mL headspace vials 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 an HP 5890 II gas chromatograph coupled to an HP 5972 A mass selective detector. The GC separations were performed using an HP 5 fused-silica capillary column (30 m long × 0.25 mm i.d.; Hewlett-Packard, Palo Alto, CA) coated with a 0.25-µm layer of 5% phenyl methyl siloxane, with helium (flow rate 1 mL/min) as the carrier gas. The injector temperature was held at 250°C, and the GC oven temperature was initially maintained at 50°C (2 min) and then increased at 5°C/min 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 spectra matching using the Wiley mass spectra search library.

Statistical analysis. All measurements were replicated three times. A correlation analysis was performed using Pearson's test. A multivariate statistical analysis of the data set from the FA and volatile fractions was performed using principal component analysis (PCA).

RESULTS AND DISCUSSION

FA profiles are presented in Table 1. The variability observed in FA composition was within the normal range found in olive and walnut oils from different geographic origins (9–11). With the exception of palmitoleic acid, which was present only in olive oils, the remaining FA—namely, palmitic, stearic, oleic, linoleic, and linolenic acids—were found in both olive and walnut oils.

Oleic acid was the main FA in olive oils, ranging from 64.98 to 72.45%, with an overall mean value of 69.5%. Palmitic acid was the second most prevalent FA (14.36–16.72%), followed by linoleic acid (7.90–14.30%). The concentration of linolenic acid was the lowest. On the other hand, the highest percentages of linoleic acid were found in walnut oils (more than 52%), representing about 55.4% of the total FA on average. A large variation was observed in the oleic acid content (16.51–26.28%). Differences among varieties were also evident in the linolenic acid content, which averaged 15.2%, with the highest value (18.58%) being observed in the Chandler variety. Hence, olive oils contained a high proportion of monounsaturated FA (mainly oleic acid), whereas walnut oils were abundant in PUFA (linoleic + linolenic).

Twenty-two components were identified that contribute to the flavor of olive oil (Table 2). These included hydrocarbons, alcohols, aldehydes, and furan derivatives. Most of the identified compounds were previously reported in the literature as constituents of olive oil flavor (2). The major components in all oil samples were saturated and unsaturated aldehydes, which represented from 57.68 (var. Nevadillo) to 70.58% (var. Manzanilla) of the total volatile compounds. Nonanal, 2-decenal, and 2-undecenal were predominant in all samples. Saturated hydrocarbons (C_4–C_8) were second in abundance; *n*-heptane and *n*-octane had the greatest values. Aliphatic alcohols were found in small quantities, whereas furan derivatives were present only in the Manzanilla and Nevadillo varieties.

Similar to olive oils, in walnut oils the most abundant group of volatiles was the aldehydes and hydrocarbons (Table 3). *n*-Pentane (8.84–19.46%) was the principal component in all varieties, followed by 2,4-decadienal and nonanal. Unlike olive oils, the walnut oils contained significantly higher amounts of pentanal and hexanal. Others aldehydes and alcohols were produced and formed an important fraction of volatiles in walnut oil of the variety Criolla. Furthermore, 2-hexanone, 2-pentylfuran, and 2-octylfuran were present only in this variety. Oils from the Chandler and Franquette varieties were characterized by a low number of volatile compounds.

Published works on the volatile composition of virgin olive oil have focused on C_5 and C_6 compounds. However, the focus on these compounds has been criticized by Reiners and Grosch (14), who found that high M.W. compounds (up to C_{10}) are also abundant components of the volatile flavor fraction of virgin olive oils. The volatile composition of olive oils analyzed in this study was quite different from that of European olive oils. The main differences that characterize the Argentinean olive oils

TABLE 1
FA Composition (% of total FA) from Olive and Walnut Varieties

	FA					
Oil variety	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
Olive						
Arbequina	16.42[a]	2.01	1.56	64.98	14.30	0.74
Manzanilla	15.33	2.08	1.46	72.39	7.90	0.83
Nevadillo	14.43	1.60	1.64	72.45	8.97	0.90
Farga	16.72	1.87	1.49	66.90	12.10	0.91
Frantoio	14.36	1.30	1.71	70.62	11.22	0.80
Walnut						
Criolla	7.67	ND[b]	1.71	17.31	57.62	15.61
Chandler	6.94	ND	1.50	16.51	56.45	18.58
Franquette	7.54	ND	2.12	26.28	52.12	11.93

[a] Mean values ($n = 3$).
[b] Not detected.

TABLE 2
Volatile Compounds (% normalized areas) of Olive Oil Varieties

	Olive varieties				
Compounds	Arbequina	Manzanilla	Nevadillo	Farga	Frantoio
Hydrocarbons					
n-Butane	Tr[a]	1.24[b]	Tr	Tr	Tr
n-Pentane	4.33	3.21	5.19	5.28	4.98
n-Hexane	1.53	1.89	2.62	2.11	3.13
n-Heptane	6.02	4.70	9.05	5.97	7.13
n-Octane	7.73	1.89	11.59	6.98	7.01
Alcohols					
Ethanol	3.51	Tr	3.36	4.28	Tr
Cyclobutanol	0.72	Tr	Tr	Tr	Tr
1-Octanol	Tr	Tr	Tr	1.09	Tr
Aldehydes					
Pentanal	Tr	0.86	Tr	Tr	Tr
Hexanal	Tr	0.46	Tr	Tr	Tr
2-Hexenal	0.90	0.76	Tr	Tr	1.67
Heptanal	2.72	2.19	1.84	2.75	2.53
Octanal	6.62	2.83	5.98	3.20	2.28
2-Octenal	ND[c]	ND	ND	0.87	ND
Nonanal	10.45	6.73	13.33	13.04	15.11
2-Nonenal	1.15	1.69	1.00	2.08	1.52
Decanal	Tr	0.87	0.65	Tr	Tr
2-Decenal	5.99	11.87	11.57	9.17	12.72
2, 4-Decadienal	Tr	Tr	Tr	1.15	Tr
2-Undecenal	5.84	9.23	10.33	8.00	11.32
Furan derivatives					
2-Pentylfuran	ND	ND	0.99	ND	ND
2-Octylfuran	ND	0.51	ND	ND	ND
Total	57.51	53.12	77.50	65.97	69.40

[a] Trace (<0.3%).
[b] Mean values ($n = 3$).
[c] Not detected.

studied are the low amounts of short-chain (C_4–C_6) aldehydes and alcohols and the presence of significantly higher amounts of many aldehydes with chain lengths of C_8 to C_{11}. Evidence indicates that, in olive fruits, the C_5–C_6 compounds are produced mainly through biochemical (enzymatic) pathways (4,15), whereas the formation of higher M.W. components occurs through chemical pathways (16). Thus, the differences observed in volatiles from Argentinean and European olive oils may indicate a difference in the relative importance of these pathways.

Table 4 shows the theoretical hydroperoxide precursors of the main flavor components. As expected, the major headspace components in olive oils arose from oleate 8-hydroperoxide (2-undecenal), oleate 9-hydroperoxide (2-decenal), oleate 10-hydroperoxide (*n*-octane, nonanal), and oleate 11-hydroperoxide (*n*-heptane, octanal). These hydroperoxide isomers are formed in similar amounts during the oxidation of oleic acid (16), the most abundant FA in olive oil.

Linoleic was the major FA in walnut oil (Table 1), and most of the fat-derived flavors arose from it. According to Frankel (16), the main hydroperoxides are formed from the 9- and 13-positions. The volatile composition of walnut oils analyzed here reflected this fact. 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. In spite of the relative abundance of linolenic acid in walnut oils, 2-hexenal and 2,4-heptadienal (two of the most important linolenate hydroperoxide derivatives) were found in trace amounts.

A correlation analysis between FA and volatile compounds was performed by considering the olive and walnut oils as a whole data set; the results are presented in Table 5. *n*-Heptane, nonanal, 2-decenal, and 2-undecenal were positively correlated with oleic acid, but they showed a negative correlation with linoleic acid. On the other hand, *n*-pentane, pentanal, hexanal, and 2,4-decadienal had significant positive correlations with linoleic acid, but they were negatively correlated with oleic acid content.

PCA was applied to the chemical data set from the olive and walnut oils. The resulting score plot provided an overview of the oil varieties, showing a total of 92% of the variance (Fig. 1). This plot reflected the differences among the oil types and allowed us to see the pattern of covariation among the chemical variables: The variables found in a similar direction and far from the origin were positively correlated. Oils from the walnut varieties appeared close together in a group, whereas those from the olive varieties were found clustered in another group, indicating that variations within groups were smaller than variations between groups.

TABLE 3
Volatile Compounds (% normalized areas) of Walnut Oil Varieties

	Walnut varieties		
Compounds	Criolla	Chandler	Franquette
Hydrocarbons			
n-Pentane	8.84[a]	16.93	19.46
n-Octane	Tr[b]	4.11	5.78
n-Nonane	0.38	ND[c]	ND
Alcohols			
Ethanol	2.12	4.31	Tr
Cyclobutanol	2.22	Tr	Tr
1-Pentanol	0.85	ND	ND
1-Hexanol	0.57	ND	ND
1-Heptanol	1.32	ND	ND
1-Octanol	1.13	ND	ND
Aldehydes			
Pentanal	6.09	5.38	8.33
Hexanal	6.74	5.61	4.89
2-Hexenal	Tr	Tr	Tr
Heptanal	4.53	3.67	Tr
2,4-Heptadienal	0.89	ND	ND
Octanal	5.60	4.56	Tr
Nonanal	6.37	6.05	7.19
2-Nonenal	1.38	ND	ND
Decanal	0.66	ND	ND
2-Decenal	5.99	3.57	6.27
2,4-Decadienal	7.49	8.28	9.85
Ketones			
2-Hexanone	0.67	ND	ND
Furan derivatives			
2-Pentylfuran	0.77	ND	ND
2-Octylfuran	0.45	ND	ND
Total	65.06	62.47	61.78

[a] Mean values (n = 3).
[b] Trace (<0.3%).
[c] Not detected.

TABLE 4
Theoretical Hydroperoxide Precursors of the Main Volatile Compounds from Olive and Walnut Oils

Compounds	Precursor	Compounds	Precursor
Aldehydes		Hydrocarbons	
Pentanal	13-LOOH	*n*-Pentane	13-LOOH[a]
Hexanal	13-LOOH	*n*-Heptane	11-OOOH[b]
Heptanal	11-LOOH	*n*-Octane	10-OOOH
Octanal	11-OOOH		
Nonanal	10-OOOH		
2-Decenal	9-OOOH		
2,4-Decadienal	9-LOOH		
2-Undecenal	8-OOOH		

[a] LOOH, linoleic acid hydroperoxide.
[b] OOOH, oleic acid hydroperoxide.

The analysis of the score plot emphasized a separation on the first principal component (PC1) since it explained 85% of the total variance. The amounts of 2,4-decadienal and C_5–C_6 aldehydes and hydrocarbons were related mainly to linoleic acid, and all these compounds were associated with the walnut varieties. Oils from the olive varieties were characterized by larger amounts of higher M.W. aldehydes, all of which were associated with oleic acid. So far in our discussion, we have tried to establish whether any chemical correlation exists between FA and volatile profiles. However, we remain fully aware of factors other than the degree of oil unsaturation, such as the TG structures or the presence of anti- and/or prooxidant compounds, that also can influence flavor development.

The results of the present investigation showed that the most abundant headspace volatile compounds in olive and walnut oils were short- and medium-chain aldehydes and hydrocar-

TABLE 5
Correlation Coefficients Between FA and Volatile Compounds from Olive and Walnut Oils

	n-Pentane	*n*-Heptane	Pentanal	Hexanal	Nonanal	2-Decenal	2,4-Decadienal	2-Undecenal
Oleic acid	–0.83[a]	0.94[b]	–0.93[b]	–0.99[b]	0.73[a]	0.82[a]	–0.96[b]	0.96[b]
Linoleic acid	0.84[a]	–0.94[b]	0.94[b]	0.99[b]	–0.72[a]	–0.80[a]	0.97[b]	–0.96[b]

[a] $P \leq 0.01$.
[b] $P < 0.001$.

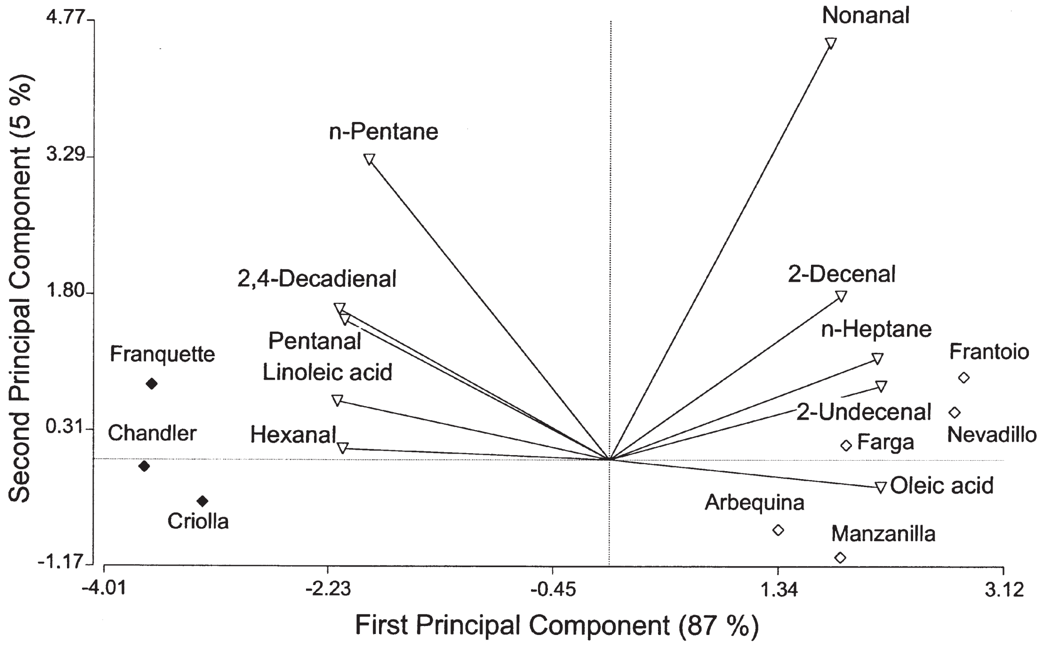

FIG. 1. Score plot of principal components 1 and 2 for chemical data (▽) from olive (◇) and walnut (◆) varieties.

bons, indicating that flavor components are produced mainly through oxidative pathways. The application of a multivariate analysis to the chemical data showed that the pattern of variation for the FA and flavor compounds could be visualized by the loading plot obtained by PCA. The remarkable features that differentiated the flavor composition of walnut oils from those of olive oils were the presence of larger amounts of short-chain (C_5–C_6) saturated hydrocarbons and aldehydes in the former, and the increased quantities of medium-chain (C_7–C_{11}) compounds in the latter. The explanation could be their different origins, mainly from linoleic acid in walnuts or almost exclusively from oleic acid in olives.

ACKNOWLEDGMENTS

This work was financed by grants from CONICET and Secretaría de Ciencia y Tecnología (SeCyT)–UNC. M.M.T. is a fellow and D.M.M. a career researcher at CONICET (Argentina).

REFERENCES

1. Morales, M.T., J.J. Ríos, and R. Aparicio, Changes in the Volatile Composition of Virgin Olive Oil During Oxidation: Flavors and Off-flavors, *J. Agric. Food Chem. 45*:2666–2673 (1996).
2. Kiritsakis, A.K., Flavor Components of Olive Oil—A Review, *J. Am. Oil Chem. Soc. 75*:673–681 (1998).
3. Angerosa, F., R. Mostallino, C. Basti, and R. Vito, Virgin Olive Oil Odour Notes: Their Relationships with Volatile Compounds from the Lipoxygenase Pathway and Secoiridoid Compounds, *Food Chem. 68*:283–287 (2000).
4. Angerosa, F., C. Basti, and R. Vito, Virgin Olive Oil Volatile Compounds from Lipoxygenase Pathway and Characterization of Some Italian Cultivars, *J. Agric. Food Chem. 47*:836–839 (1999).
5. Cavalli, J., X. Fernandez, L. Lizzani-Cuvelier, and A. Loiseau, Characterizacion of Volatile Compounds of French and Spanish Virgin Olive Oils by HS-SPME: Identification of Quality-Freshness Markers, *Food Chem. 88*:151–157 (2004).
6. Tura, D., P.D. Prenzler, D.R. Bedgood, Jr., M. Antolovich, and K. Robards, Varietal and Processing Effects on the Volatile Profile of Australian Olive Oils. *Ibid. 84*:341–349 (2004).
7. Clark, R.G., and H.E. Nursten, Volatile Flavour Components of Walnuts (*Juglans regia* L.), *J. Sci. Food Agric. 27*:902–908 (1976).
8. Caja, M.M., M.L. Ruiz del Castillo, R. Martínez Alvarez, M. Herraiz, and G.P. Blanch, Analysis of Volatile Compounds in Edible Oils Using Simultaneous Distillation-Solvent Extraction and Direct Coupling of Liquid Chromatography with Gas Chromatography, *Eur. Food Res. Technol. 211*:45–51 (2000).

9. Uceda, M., and M. Hermoso, La Calidad del Aceite de Oliva, in *El Cultivo del Olivo*, edited by D. Barranco, R. Fernández-Escobar, and L. Rallo, Ediciones Mundi-Prensa, Madrid, 1999, pp. 571–596.
10. Savage, G.P., P.C. Dutta, and D.L. McNeil, Fatty Acid and Tocopherol Contents and Oxidative Stability of Walnut Oils, *J. Am. Oil Chem. Soc. 76*:1059–1063 (1999).
11. Demir, C., and M. Çetin, Determination of Tocopherols, Fatty Acids and Oxidative Stability of Pecan, Walnut and Sunflower Oils, *D. Lebens.-Runds. 95*:278–282 (1999).
12. Tobares, L., C. Guzmán, and D.M. Maestri, Effect of the Extraction and Bleaching Processes on Jojoba (*Simmondsia chinensis*) Wax Quality, *Eur. J. Lipid Sci. Technol. 105*:749–753 (2003).
13. Maestri, D.M., and C.A. Guzmán, A Comparative Study of Seed Lipid Components of Nicotianeae (Solanaceae), *Biochem. Syst. Ecol. 23*:201–207 (1995).
14. Reiners, J., and W. Grosch, Odorants of Virgin Olive Oils with Different Flavor Profiles, *J. Agric. Food Chem. 46*:2754–2763 (1998).
15. Olías, J.M., A.G. Pérez, J.J. Ríos, and L. Sanz, Aroma of Virgin Olive Oil: Biogenesis of the “Green” Odor Notes, *Ibid. 41*:2368–2373 (1993).
16. Frankel, E.N., Chemistry of Autoxidation: Mechanism, Products and Flavor Significance, in *Flavor Chemistry of Fats and Oils*, edited by D.B. Min and T.H. Smouse, American Oil Chemists’ Society, Champaign, IL, 1985, pp. 1–37.

[Received September 28, 2004; accepted February 21, 2005]