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ORIGINAL PAPER

Characterization of Monovarietal Argentinian Olive Oils from New Productive Zones

Liliana N. Ceci · Amalia A. Carelli

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Abstract Quality characteristics (acidity, peroxide value, K_{232}, K_{270}, ΔK, oxidative stability index) and chemical data (antioxidant compound, fatty-acid, sterol, erythrodiol-uvaol, and wax compositions) were studied in monovarietal virgin-olive oil samples (2004–2005 harvests) from different regions of Argentina. The data obtained according to standard methods were compared with international quality and purity criteria. The total-polyphenol content ranged from 25 to 263 mg/kg, showing the highest values for Coratina and Arauco oils. The α-tocopherol content varied between 160 and 428 mg/kg; these values are generally stated to belong to good quality oils. Most of the samples from the new productive zones failed at least one purity criterion. Arbequina samples presented the highest deviations from the International Olive Oil Council criteria in fatty acids, waxes, and sterol percentages, indicating a poor adaptation of this cultivar to the agronomic medium and its sensibility to adverse climatic conditions. Principal component analysis revealed that the harvest-year influence was attributable to environmental factors.

Keywords Olive oil · Antioxidants · Quality · Fatty acids · Waxes · Phytosterols · Tocopherols · Polyphenols · Erythrodiol-uvaol

L. N. Ceci (✉) · A. A. Carelli
Planta Piloto de Ingeniería Química (PLAPIQUI),
Universidad Nacional del Sur-Consejo Nacional de Investigaciones Científicas y Técnicas (UNS-CONICET),
Camino La Carrindanga km. 7, CC 717,
8000 Bahía Blanca, Argentina
e-mail: lceci@plapiqui.edu.ar

Introduction

Argentine has greatly extended the production zones of olive oil in recent years principally through the application of governmental projects supported by a program for agro-industrial promotion. New areas, such as Catamarca and La Rioja, have been added to the traditionally productive Provinces (Mendoza, Córdoba, and San Juan). These two areas have different climatic conditions. Nowadays the olive-growing zone is wide in terms of latitude and altitude, and there are no precedents on the performance of the Mediterranean varietals in our regions. The lack of studies to typify and characterize the Argentinian olive oils constitutes one of the weaknesses of the national productive sector.

The production increase has been accomplished by a change in marketing: the Argentinian olive oils are marketed not only inside the MERCOSUR but also among member countries and non-member states that adopt the directives of the International Olive Oil Council (IOOC). The IOOC attributes different designations to olive oils and olive-pomace oils and establishes quality and purity criteria [1]. The main quality assays include organoleptic characteristics, free acidity, peroxide values and absorbance in the ultraviolet region. The fatty-acid and sterol compositions, the total sterol content, the erythrodiol-uvaol and wax contents are among those measures proposed to evaluate the genuineness of olive oils.

The two compositional factors of oils that determine their susceptibility to oxidation are the fatty acid profile and the concentration of minor compounds with anti- or pro-oxidant characteristics. The kind of fatty acids and in particular the number of double bonds, determines the type and extent of chemical reactions that occur during the oil storage period. The major abundance of oleic acid (C18:1)

in olive oils is the feature that sets them apart from other vegetable oils. In addition, virgin olive oil is a rich source of natural antioxidants. These include chlorophylls, carotenes, tocopherols and phenolic compounds that may act, by different mechanisms, to confer an effective defense system against free radical attacks. Some authors have estimated the contribution to oil stability to be around 29% for phenols, 24% for fatty acids, 22% for orthodiphenolic compounds, 11% for tocopherols, 6% for carotenoids, and 4% for chlorophylls [2]. Chlorophylls are antioxidants in the dark and pro-oxidants in presence of light. The contents of chlorophyll and carotene pigments are also interesting due to their contribution to the virgin-olive oil color. In addition, phenolic compounds are related to the characteristic bitter taste of the oils that are obtained either from green olives or olives whose color is turning.

The sterol fraction is an important determinant of the genuineness of an olive oil and can be used to assess the degree of purity of the oil and the absence of admixture with other vegetable oils. The sterol composition in the olive oils can be influenced by genetic factors [3], the agroclimatic conditions [4], the maturation stage of the fruits [5], and the oil-extraction system [6]. The campesterol/stigmasterol ratio has been reported as the quality index for olive oils with the highest values for good-quality oils. The virgin olive oils of the Koroneiki cultivar have ratios of 5.4; the refined oils, 3.8; and the solvent-extracted oils, 2.5 [6]. The oils with poor quality and acidity values higher than 5.0% have a campesterol/stigmasterol ratio lower than 1 [7].

Wax and erythrodiol-uvaol contents in virgin olive oil have been proposed to detect adulteration with lower-quality olive oil or olive-pomace oil because they have higher values of these minor components [8].

The aim of the present work is to study and characterize Argentinian olive oils from the new production areas through classical quality indexes and through the compositions of fatty acids and minor components, which are related to both oxidative stability and oil purity. This study will contribute to typify Argentinian olive oils, allowing the comparison of their composition with international quality and genuineness criteria.

Experimental Procedures

Olive Oils

Thirty-seven samples of virgin olive oils from Arbequina, Barnea, Picual, Frantoio, Empeltre, Manzanilla, Arauco and Coratina cultivars corresponding to 2004–2005 harvests from different Argentinian regions were evaluated. Nine commercial plants provided oil samples indicating the olive-fruit ripeness indexes calculated according to the IOOC maturation scale based on olive-fruit pigmentation color. Table 1 shows data about cultivar, origin, olive-tree age, and ripeness index for each sample. Three oil samples from Arbequina (A1 = A10, A2 = A11, A3 = A12), two from Manzanilla (MC1 = MC2, MCR1 = MCR2), one from Picual and Frantoio (P1 = P4, FR1 = FR2), and two from Barnea (B1 = B4, B2 = B5) were collected from the same olive trees in both 2004 and 2005 harvests. The 2004 samples came from valley regions with different latitudes (Mendoza 35°, Córdoba 30.5°, La Rioja 29.4° and Catamarca 28.6°), which allowed us to compare traditional productive zones with new ones. Some 2005 samples (A13, A14, A15, B5, C, E2, P5, P6) came from mountainous regions with an altitude of 900–1,200 m above sea level (latitudes of 28.1° and 29.5°), whose thermal amplitudes are wider than those in the valley regions. Two-phase continuous decanters were used in the oil extractions, except for the samples A6, A7, A8, A14, B5, and P6 where three-phase decanter continuous systems were employed. Temperatures and times for paste beating varied from 26 to 38 °C and from 25 min to 120 min, respectively. The oil samples were stored at 5 °C under a nitrogen atmosphere and light protected until they were analyzed.

Analytical Methods

The analyses for acidity and the peroxide value (PV) were carried out with the IUPAC 2.201 and AOCS Cd 8–53 standard methods, respectively [9, 10]. The oxidative stability index, which is represented as the induction time in hours, was measured with a Metrohm 679 Rancimat apparatus at 110 °C and 20 L/h airflow. The extinction coefficients (K) at wavelengths 232 and 270 nm and the variation of the specific extinction (ΔK) at 270 nm were determined following the IOOC method [11].

Phenolic compounds were isolated by three extractions of oil in hexane solution with a 60% *v*/*v* water/methanol mixture. The content of phenolic compounds as mg/kg of caffeic acid was determined spectrophotometrically at 725 nm using Folin-Ciocalteau reagent [12]. Chlorophyll and carotenoid contents were evaluated from the maximum of absorption for the oil solution in cyclohexane at 670 and 470 nm, respectively [13].

Tocopherols were evaluated by HPLC according to the Ce 8–89 AOCS method [10]. A fluorescence detector and a LiChrosorb Si-60 column (l = 25 cm; i.d. = 4 mm; particle size = 5 μm from Merck, Darmstadt, Germany) were used.

Fatty acids were determined as their methyl esters obtained by trans-esterification with a cold methanolic solution of potassium hydroxide following the COI/T.20/

Table 1 Cultural data

Cultivar	Harvest	Sample	Origin	Olive tree age (Years)	Ripeness Index
Arbequina	2004	A1	Catamarca[a]	6	3.2
		A2	La Rioja[a]	7	3.3
		A3	La Rioja[a]	9	3.1
		A4	La Rioja[a]	5	4.2
		A5	Catamarca[a]	5	3.9
		A6	Córdoba[a]	50	Green
		A7	Córdoba[a]	50	Envero
		A8	Córdoba[a]	50	Ripe
		A9	Mendoza[a]	5	4.5
	2005	A10	Idem A1[a]	7	3.2
		A11	Idem A2[a]	8	NP
		A12	Idem A3[a]	10	2.5
		A13	La Rioja[b]	7	1.3
		A14	La Rioja[b]	7	2.0
		A15	Catamarca[b]	3	NP
Picual	2004	P1	Catamarca[a]	6	3.5
		P2	La Rioja[a]	5	4.2
		P3	Catamarca[a]	5	3.3
	2005	P4	Idem P1[a]	7	3.1
		P5	La Rioja[b]	8	3.7
		P6	La Rioja[b]	12	3.2
Barnea	2004	B1	Catamarca[a]	7	3.4
		B2	La Rioja[a]	6	4.6
		B3	Catamarca[a]	5	3.2
	2005	B4	Idem B1[a]	8	3.8
		B5	La Rioja[b]	7	3.0
Frantoio	2004	FR1	La Rioja[a]	6	3.9
	2005	FR2	La Rioja[b]	8	3.2
		FR3	La Rioja[a]	10	2.4
Empeltre	2004	E1	Mendoza[a]	60	4.8
	2005	E2	La Rioja[b]	10	3.6
Manzanilla Californiana	2004	MC1	La Rioja[a]	8	4.3
	2005	MC2	Idem MC1[a]	9	NP
Manzanilla Criolla	2004	MCR1	La Rioja[a]	9	3.2
	2005	MCR2	Idem MCR1[a]	10	2.5
Arauco	2004	AU	La Rioja[a]	9	3.4
Coratina	2005	C	Catamarca[b]	3	NP

Ripeness index index proposed by IOOC, based on the color of the skin and flesh of the fruits, ranging from 0 to 7 [8]; *NP* not provided

[a] From valleys

[b] From mountain regions

Doc. No. 24 standard method [14]. Fatty-acid methyl esters were analyzed by GLC according to the IUPAC 2.302 method [9]. A SP2380 column [stabilized poly (90% bi cyanopropyl/10% cyanopropylphenyl siloxane)], 30 m length × 0.25 mm i.d., 0.25 μm film thickness (Supelco Inc., Bellefonte, PA) was used.

Sterol and erythrodiol-uvaol contents were determined by GLC with IOOC and European Union analytical methods [15, 16]. The oil sample containing 5-α-cholestan-3-β-ol (from Fluka Switzerland, purity 95%) as internal standard was saponified with potassium hydroxide solution in ethanol. The unsaponifiable fraction containing sterols, erythrodiol, and uvaol, was removed with ethyl ether and then was separated by Silicagel plate chromatography. Separation and quantification of the silanized compounds was carried out by GLC using a 30 m SE 54 column of 0.25 mm i.d. and 0.2 μm film thickness (Supelco Inc., Bellefonte, PA). The operating conditions were as follows: oven temperature, 260 °C (2 min)–1 °C/min–265 °C (20 min); injector temperature, 280 °C; FID temperature,

300 °C; injection volume, 1 μL, and carrier gas, hydrogen at 37 cm/s.

The contents and composition of waxes were evaluated by GLC after fractionation by chromatography on a hydrated silica gel column using COI/T.20/Doc. No. 18 method [17]. A Varian 3700 GLC chromatograph equipped with FID, cold injector for on-column injection, and capillary column (HP 5, 11 m length × 0.32 mm i.d., 0.52 μm film thickness) from Hewlett–Packard (Palo Alto, CA) was used. The oven programming temperature was: initial temperature, 80 °C, increase at 30 °C/min to 200 °C, hold for 1 min, increase at 3 °C/min to 340 °C, hold for 20 min. On-column injector was programmed from 80 to 320 °C at 40 °C/min. The other operation conditions were: FID temperature, 350 °C; injection volume, 3 μL; carrier gas, hydrogen at 3 mL/min.

Statistical Analysis

The analyses were carried out in triplicate, except for the fatty acid and wax determinations that were performed in quadruplicate. The differences in mean values between samples were assessed with Student's *t* test, being statistically different at a significance level of 5%. Multivariate analysis based on principal component analysis (PCA) and cluster analysis (CA) was applied using an academic statistical package (Análisis Multivariado, Departamento Matemática, Universidad Nacional del Sur, Bahía Blanca, Argentina). The selected variables were the following: OSI, K_{232}, polyphenols (PP), carotenoids (CAR), oleic-acid content (O), oleic/(linoleic + linolenic) ratio (OLLnR), total-sterol content (STE), apparent-sitosterol percentage (ASP), C40–C46 wax content (WC), and C44–C46 wax relative percentage or insoluble-wax percentage (IWP). These variables had representation percentage higher than 70% in the space of the first three principal components. Acidity, PV, K_{270}, ΔK, tocopherols, chlorophylls, erythrodiol-uvaol, were not considered because their representation percentages were lower than 70%. A correlation matrix with 37 rows corresponding to samples and with 10 columns of variables standardized by standard deviation was used for the PCA. Euclidean distances between samples and complete linkage (the furthest neighbor) were used for CA.

Results and Discussion

Quality Indexes

The evaluated samples were classified as "extra-virgin olive oil", according to the IOOC norms for quality indexes (acidity ≤ 0.8%, PV ≤ 20 mequiv/kg, $K_{270} \leq 0.22$ and $\Delta K \leq 0.01$) [1]. PV and acidity were in the ranges of 1.80–12.70 mequiv/kg and 0.14–0.69 (% oleic acid), respectively. K_{270} was between 0.076 and 0.161 and ΔK in the range of –0.006 to 0.007. Two samples from Arbequina and one from Barnea and Coratina presented K_{232} higher than 2.50 (A9: 2.54, A15: 2.67, B3: 2.58, C: 2.83). The IOOC establish that commercial partners in the country of retail sale may require compliance with these samples when the oil is made available to the end consumer.

Antioxidant Compounds and Oxidative Stability Index

The total-polyphenol content ranged from 25 to 263 mg/kg showing the highest values for oils from Coratina and Arauco (Table 2). The Arbequina samples had very variable contents (29–158 mg/kg). The Picual oils presented more polyphenol contents in 2005. The concentration of total phenols in olive oils usually ranges from 50 to 400 ppm, but oils with concentrations up to 600–700 ppm can be found [18, 19]. The analyzed Argentinian oils are evidently situated among those oils with small concentrations of phenolic substances. A poor adaptation of some Mediterranean cultivars to local agro-climatic conditions, and a non-optimized control in processing parameters such as temperature and time in beating process, would cause these low values.

The chlorophyll and carotene contents were respectively 1.0–14.7 and 1.2–6.6 mg/kg (Table 2). These values are in accordance with the typical ranges reported by other authors [8, 13], with amounts of 0.2–55 ppm and 1–23 ppm for chlorophylls and carotenes, respectively. The Argentinian oils from the Arbequina cultivar had a large range of total-pigment content: 3.2–12.4 mg/kg in 2004, and 2.2–16.8 mg/kg in 2005. Around 20% of the analyzed samples showed a chlorophylls/carotenes ratio between 1.06 and 1.22. This is the proposed range, with a limited variability, for "best-quality" Spanish olive oils, independent of the cultivar and the production date [20]. About 20% of the samples presented signs of over ripening [20] with chlorophyll/carotene ratios lower than 1.06. The remaining oils had chlorophyll/carotene ratios significantly greater than unity, thus suggesting a lower level of fruit ripeness. Another factor to be taken into account is that the chlorophyll and carotene contents are related not only with the olive-fruit ripeness indexes, but also with the oil processing method. Analysis of the content and type of pigments present in both fruits and corresponding oil showed that the oil extraction causes a structural transformation of pigments, due to the liberation of acids, and also a considerable loss of pigmentation, mainly in the chlorophyll fraction [20]. Substances with acid character and/or

Table 2 Oxidative stability indexes and antioxidant contents

Sample	PP (mg/kg)	CHL (mg/kg)	CAR (mg/kg)	TOC (mg/kg)	OSI (h)
A1	42.4[a]	6.3[a]	3.3[a]	403[a]	9.7[a]
A2	34.8[b]	3.3[b]	2.8[a]	463	14.2[b]
A3	68.4[c]	3.0[b,c]	3.4[a]	364[b]	13.8[b]
A4	46.8[a,d]	1.6[d]	1.6[b]	344[b,c]	8.1[a,c]
A5	34.4[b,e]	8.2[e]	4.2[c]	414[a,d]	11.7[a,b,d]
A6	29.3[b,e,f]	5.6[f]	2.9[a,d]	316[e]	7.3[c]
A7	36.0[a,b,e–g]	4.4[g]	2.8[a,d]	334[c,e,f]	8.5[c]
A8	34.2[b,e–g]	2.7[c]	2.4[e]	289[g]	7.6[c]
A9	90.4[h]	1.4[d,h]	2.0[f]	190	13.1[b,d,e]
A10	37.2[a,b,e,g,i]	1.0[i]	1.2[g]	356[b,c,h]	6.7[c]
A11	46.5[a,d,j]	4.5[g,j]	4.0[c,h]	399[a,d]	9.9[a,c,d]
A12	71.6[c]	4.0[c,g,k]	4.0[h,i]	234[i]	14.8[b,e]
A13	47.4[a,d,j]	5.2[l]	2.8[a,d]	270[g,j]	9.3[c,d]
A14	158.1[k]	10.2[m]	6.6	284[g,j]	18.1[f]
A15	102.4[l]	9.6[e,m,n]	4.3[c,h–j]	252[j]	12.1[b–d]
P1	38.8[a,b,e,g,i,m]	7.0[o]	2.9[a,d]	303[c,e–g,j,k]	11.1[c,d]
P2	36.2[b,e,g,i,m]	2.8[c,p]	3.4[a]	348[b,c,f,k]	14.7[b,d,e]
P3	28.3[e–g]	4.1[c,k]	2.5[e,k]	332[c,e,f,k]	17.0[f]
P4	66.9[c]	2.0[c]	1.7	315[e,f,k]	13.9[b,d,e]
P5	43.9[a,d,g,j]	14.7	6.1[l]	291[g,j,k]	18.5[f,g]
P6	92.7[h,l,n]	9.4[n]	6.1[l]	340[c,f,k]	22.8[h]
B1	45.4[a,d,j]	6.5[a]	2.9[a,d]	321[e,f,k]	8.9[c,d]
B2	53.3[j]	1.4[d,h]	1.2[g]	254[j]	9.7[a,c–e]
B3	75.2[c]	2.4[c]	1.5[b]	345[b,c,f,k]	9.9[a,c,d]
B4	46.3[a,d,i,j,m]	1.3[h]	1.5[b]	370[b,h]	8.2[c,d]
B5	65.1[c]	5.6[f]	2.9[a,d]	218[l]	11.5[c,d]
FR1	65.4[c]	7.6[e]	4.2[c,h,j]	432[a,d]	11.0[c, d]
FR2	32.2[b,e–g]	9.0[n]	4.4[j]	322[e,f,k]	9.4[a,c,d]
FR3	120.9	6.7[a,o]	5.9[l]	289[g,j,k]	10.3[c,d]
E1	53.2[j]	1.0[i]	2.0	167	12.2[d,e]
E2	25.3[f]	4.8[q]	2.5[d–f,k]	299[e,g,k]	12.7[d,e]
MC1	45.4[a,d,j,m]	4.2[g,k]	2.5[e,k]	212[l]	12.3[d,e]
MC2	48.4[d,j]	6.0[a]	3.1[a]	270[g,j,k]	12.4[d,e]
MCR1	78.9[n,o]	3.0[c,r]	2.5[e,k]	328[c,e,f,h,k]	18.6[g]
MCR2	73.1[c,o]	4.6[j,q]	3.0[a,d]	234[i]	23.3[h]
AU	165.0[k]	4.1[c,k]	4.4[j]	377[a,b]	14.4[b,d,e]
C	263.2	4.3[b,f,g,j–l,p–r]	3.6[a,i]	437[d]	19.2[f,g]

The means within a column followed by the same letter are not significantly different at the $P = 0.05$ level. Variation coefficients (%): VC = 1.5–10.7 for PP, VC = 0.1–17.0 for CHL, 0.04–8.1 for CAR and VC = 1.0–9.7 for TOC

OSI oxidative stability index, *PP* polyphenols, *CHL* chlorophylls, *CAR* carotenes, *TOC* total tocopherols (alpha, beta and gamma isomers)

Mg-dechelating properties can be released during fruit milling and paste beating. The reactions of pigments degradation could be catalyzed by these substances and the enzyme lipoxygenase could be also involved in these reactions.

The main tocopherol that is present in olive oil is α-tocopherol. The reported values of tocopherol contents for olive oils range from 5 to 300 mg/kg, and between 100 and 300 mg/kg for good quality oils [8, 21]. The tocopherol contents in the analyzed samples presented the following ranges in mg/kg: α-tocopherol = 160–428, β-tocopherol = 0–27, γ-tocopherol = 0–39. These values correspond to good quality oils. The Coratina sample presented the highest tocopherol content (alpha: 396 mg/kg, beta: 11 mg/kg, and gamma: 30 mg/kg). Tocopherols have demonstrated antioxidant properties under determined conditions during processing and storage of olive oils [8].

Appreciable differences were observed between cultivars and harvests with respect to the oxidative stability of the analyzed oils (Table 2). The samples from Manzanilla Criolla, Coratina and Picual showed the highest oxidative-stability indexes on average, and those corresponding to

Barnea the lowest ones. In addition, the oils from Arbequina revealed a large range of oxidative-stability indexes (6.7–18.1 h). These results suggest that the geographical and agro-climatic conditions may influence the olive oil stability, in addition to the genetic factor.

Fatty Acids

In Table 3 the fatty-acid compositions obtained from Arbequina samples are shown. Four samples (A3 and A9 in 2004, A12 and A14 in 2005) that represent only about 27% of the analyzed samples from this cultivar were within the legal ranges stated by the IOOC for fatty acids. All samples beyond these limits had oleic acid contents lower than 55.0%, and some had higher values than 20% for palmitic acid, 21.0% for linoleic acid, and/or 3.5% for palmitoleic acid. The oils from Arbequina showed the lowest mean values (2.8) for oleic/(linoleic + linolenic) ratio (OLLnR), despite its wide range of oxidative stability.

The fatty-acid compositions corresponding to the oils from the other cultivars are shown in Table 4. The oil from Arauco also presented a low content of oleic acid (53.7%) and one sample from Barnea had linoleic acid in excess (22%) with respect to IOOC limits. Two oils from Picual (2004 harvest), two from Frantoio (2004 and 2005 harvests) and two from Manzanilla (Californiana and Criolla, harvested in 2004) had values slightly above the IOOC limit for linolenic acid. One sample from Picual and other from Manzanilla Criolla had the highest average values for OLLnR (10.5 and 13.4, respectively) in accordance with their higher oxidative stability indexes.

Empeltre and Coratina samples were within fatty-acid legal ranges. Although Coratina sample showed a medium OLLnR value (4.6), its oxidative stability was high (19.2 h). This is due to the fact that oxidative stability is related not only to fatty-acid composition, but also to several other factors, such as pro- and/or anti-oxidant substances. In fact, this sample had the highest tocopherol and polyphenol contents.

The analysis of the fatty acid composition for oils from the same olive trees in both harvests (A1–A10, A2–A11, A3–A12, P1–P4, B1–B4, B2–B5, FR1–FR2, MC1–MC2, MCR1–MCR2 in 2004–2005, respectively) revealed significant differences between harvests for most of the fatty acids (Tables 3, 4). Sample A2 had palmitoleic acid higher than 3.6% in 2004 however satisfied the limit in 2005 (A11) with 2.7%. Samples A2 and B1 had percentages for linoleic acid lower than the pre-established limit (21.0%) in 2004, but this limit was exceeded in 2005 (A11 and B4). In addition, the oils from Manzanilla had values slightly high (1.1%) for linolenic acid in 2004, but satisfied the legal range in 2005 (1.0% for Manzanilla Californiana and 0.7% for Manzanilla Criolla).

The values established by the IOOC as purity criteria were fixed by statistical studies applied to several analyses performed on samples from different cultivars and origins. However, wider ranges of 43.7–93.5% for oleic acid and 1–30% for linoleic acid have been found mainly due to genetic and climatic conditions [8]. Few previous works about the fatty-acid composition have been carried out with

Table 3 Fatty acid composition (methyl esters, % m/m) of the Arbequina oil samples

Sample	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C22:0	C24:0	OLLnR
A1	**21.8**	**4.3**	0.1	0.3	1.7	**47.9**	**22.0**	0.4	1.0	0.3	0.1	0.1	2.1
A2	20.0	**3.6**	0.1	0.3	1.7	**52.1**	20.3	0.4	1.0	0.3	0.1	0.1	2.5
A3	19.1	3.2	0.1	0.3	1.6	57.5	16.5	0.4	0.8	0.3	0.1	0.1	3.3
A4	**20.1**	3.3	0.1	0.2	1.8	**49.5**	**22.9**	0.5	1.0	0.3	0.2	0.1	2.1
A5	**20.1**	3.5	0.1	0.3	1.7	**53.2**	19.3	0.4	0.9	0.3	0.1	0.1	2.6
A6	**20.1**	2.6	0.1	0.3	1.8	**53.6**	19.5	0.5	0.9	0.3	0.2	0.1	2.6
A7	**20.1**	2.9	0.1	0.3	1.8	**52.6**	20.4	0.4	0.9	0.3	0.1	0.1	2.5
A8	19.5	2.8	0.1	0.2	1.8	**52.6**	**21.2**	0.4	0.9	0.3	0.1	0.1	2.4
A9	15.8	1.6	0.1	0.3	1.9	63.3	15.5	0.4	0.6	0.3	0.1	0.1	3.9
A10	**22.5**	**3.9**	0.1	0.3	1.6	**46.8**	**23.3**	0.3	0.9	0.2	0.1	0.0	1.9
A11	**20.1**	2.7	0.1	0.2	1.5	**52.7**	**21.3**	0.3	0.8	0.3	0.0	0.0	2.4
A12	16.6	2.0	0.1	0.2	1.6	64.2	14.1	0.3	0.5	0.3	0.1	0.0	4.4
A13	**20.1**	2.8	0.1	0.3	1.5	**53.8**	20.0	0.3	0.8	0.3	0.0	0.0	2.6
A14	18.4	2.5	0.1	0.2	1.6	59.4	16.5	0.3	0.6	0.3	0.1	0.0	3.5
A15	19.8	2.7	0.1	0.2	1.8	**54.3**	19.8	0.3	0.7	0.2	0.1	0.0	2.7

Values outside IOOC limits are in bold. Precision: VC ≤ 7.2% for FA ≥ 1%, VC ≤ 24.5% for FA between 0.1 and 1%, and VC ≤ 69.2% for FA ≤ 0.1%; $n = 4$

Table 4 Fatty-acid composition (methyl esters, % m/m) of Barnea, Frantoio, Empeltre, Arauco, Coratina, Picual, and Manzanilla Oils

Sample	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C22:0	C24:0	OLLnR
P1	18.2	3.1	0.1	0.2	2.2	57.8	16.4	0.4	**1.1**	0.3	0.1	0.1	3.3
P2	14.5	2.0	0.0	0.1	3.3	68.2	9.7	0.4	**1.1**	0.4	0.2	0.1	6.3
P3	14.7	2.0	0.0	0.1	4.4	69.1	7.7	0.5	1.0	0.3	0.1	0.1	8.0
P4	17.0	2.5	0.1	0.2	2.2	67.4	9.2	0.3	0.8	0.3	0.0	0.0	6.7
P5	14.6	2.1	0.1	0.1	3.2	72.3	6.3	0.3	0.7	0.3	0.0	0.0	10.3
P6	14.5	1.7	0.0	0.1	2.5	73.5	6.2	0.3	0.8	0.3	0.1	0.0	10.5
B1	13.8	1.3	0.1	0.1	2.4	60.6	19.9	0.4	0.9	0.3	0.1	0.1	2.9
B2	14.0	1.4	0.1	0.1	2.1	60.9	19.7	0.4	0.9	0.2	0.1	0.1	3.0
B3	14.1	1.2	0.0	0.1	2.3	61.1	19.4	0.4	0.9	0.3	0.1	0.1	3.0
B4	15.8	2.0	0.1	0.1	2.0	56.0	**22.4**	0.3	0.9	0.2	0.1	0.1	2.4
B5	13.4	1.3	0.1	0.1	2.1	65.4	16.1	0.4	0.8	0.2	0.1	0.0	3.9
FR1	15.8	1.7	0.1	0.1	2.4	63.1	14.8	0.4	**1.1**	0.3	0.1	0.1	4.0
FR2	16.5	1.9	0.0	0.1	2.0	63.4	14.2	0.3	**1.1**	0.3	0.1	0.1	4.1
FR3	16.5	1.5	0.1	0.1	2.1	63.4	14.8	0.3	0.8	0.3	0.1	0.0	4.0
E1	11.7	1.0	0.1	0.3	1.8	75.2	8.4	0.3	0.7	0.3	0.1	0.1	8.3
E2	14.5	1.5	0.1	0.3	1.6	70.6	10.1	0.2	0.8	0.3	0.0	0.0	6.5
MC1	16.5	2.1	0.1	0.2	3.7	59.9	15.2	0.6	**1.1**	0.3	0.2	0.1	3.7
MC2	16.6	2.2	0.1	0.3	2.9	62.6	13.5	0.4	1.0	0.3	0.1	0.0	4.3
MCR1	15.6	2.0	0.1	0.3	1.7	70.0	8.2	0.4	**1.1**	0.4	0.1	0.1	7.5
MCR2	14.2	1.8	0.1	**0.4**	1.5	75.8	4.9	0.2	0.7	0.4	0.0	0.0	13.4
AU	19.0	2.3	0.1	0.1	2.8	**53.7**	20.0	0.5	1.0	0.2	0.2	0.1	2.6
C	12.8	0.6	0.0	0.1	2.0	68.7	14.3	0.3	0.7	0.4	0.1	0.0	4.6

Values outside IOOC limits are in bold. Precision: VC ≤ 7.2% for FA ≥ 1%, VC ≤ 24.5% for FA between 0.1 and 1%, and VC ≤ 69.2% for FA ≤ 0.1%; $n = 4$

Argentinian olive oils [22, 23]. The Argentinian olive oils had 54.0–79.1% oleic acid and 5.3–22.7% linoleic acid in a study on fatty-acid composition in oils from different countries [22]. The Arbequina oils from the 1998–2003 harvests from Catamarca Province (Central Valley) gave the following percentages: 46.9–55.8 for C18:1, 17.2–23.9 for C18:2, 0.8–1.4 for C18:3, 2.9–4.4 for C16:1, and 20.3–23.4 for C16:0 [23]. One can see that these oils presented some fatty-acid values outside legal limits.

Sterols and Erythrodiol-Uvaol

The total sterol content ranged from 1,053 to 3,004 mg/kg (Tables 5, 6), satisfying the IOOC legal limit (≥1,000 mg/kg) for virgin olive oil.

The oils from Picual, Empeltre and Manzanilla were in accordance with the IOOC norms in both evaluated harvests (Table 6). All the samples from Barnea and the 70% from Arbequina had campesterol values higher than 4.0% (Tables 5, 6). The campesterol content evidenced important differences between harvests in Arbequina cultivar. The samples A2 and A3 with 5 and 4.4% of campesterol in 2004 satisfied the limit of 4.0% in 2005 (A11 and A12, respectively). Besides, high percentages of cholesterol or brassicasterol were found in some samples from Arbequina (Table 5). A sample from Frantoio harvested in 2004 and those from Arauco and Coratina also had some of these sterols outside the legal limits (Table 6). In brief, about 50% of the analyzed samples had values higher than 4.0% for campesterol, while 20% of them had more than 0.5% for cholesterol and/or 0.1% for brassicasterol. Other authors have found campesterol contents up to 4.5% in Spanish olive oil from Cornicabra [24]. High campesterol was also observed in Greek oils from Koroneiki during early stages of fruit ripeness [25] and when the olives are grown under drought stress [4].

The Coratina oil presented Δ-7-stigmastenol content slightly greater than the IOOC limit (Table 6). Δ-7-stigmastenol values higher than 0.5% were observed in Empeltre oils during extremely dry seasons in Spain [26]. These values reached 1.3% in Spanish Cornicabra oils [21]. The same behavior was reported from the Italian Nebbio cultivar [27].

The apparent-sitosterol percentage (ASP) measures both the most abundant sterol (β-sitosterol) and some adjacent sterols in the chromatogram. It was lower than the legal limit for two samples from Arbequina and one each from

Table 5 Methylsterols and erythrodiol-uvaol contents from Arbequina oil samples

Sample	Sterol (%)															STE* (mg/kg)	ASP (%)	CSR	EU (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
A1	0.2	0.0	0.0	**4.5**	0.3	1.3	0.3	0.2	1.0	87.9	0.7	2.6	0.6	0.2	0.2	2,625	93.0	3.4	1.6
A2	0.2	0.0	0.5	**5.0**	0.6	1.4	0.2	1.6	1.2	83.5	0.8	4.2	0.5	0.1	0.2	2,457[a]	**91.8**	3.5	1.4
A3	0.1	0.0	0.1	**4.4**	0.2	1.0	0.2	0.2	1.0	86.1	0.7	5.0	0.6	0.1	0.3	2,127[b]	93.6	4.2	0.9
A4	0.1	0.0	0.1	**4.6**	0.2	1.2	0.3	0.2	1.1	86.1	0.6	4.6	0.6	0.1	0.2	3,004	93.2	4.0	1.0
A5	0.2	0.0	0.0	**4.8**	0.4	1.2	0.2	0.5	1.0	85.9	0.7	4.1	0.5	0.2	0.3	2,007[c]	92.7	4.1	1.9
A6	**0.6**	0.0	0.0	**4.5**	0.2	1.1	0.2	0.2	1.0	86.0	0.8	4.3	0.7	0.1	0.3	2,218[d]	93.0	4.1	1.8
A7	**0.6**	0.0	0.1	**4.3**	0.2	1.0	0.3	0.1	1.0	85.8	0.7	4.9	0.6	0.1	0.3	2,403[a,e]	93.1	4.2	1.5
A8	0.1	**0.7**	0.1	**5.5**	0.2	0.9	0.2	0.7	0.7	84.0	0.3	5.3	0.8	0.2	0.3	1,909[f]	**91.8**	5.9	1.6
A9	0.1	0.0	0.3	4.0	0.2	0.7	0.1	0.4	0.9	76.7	0.5	14.7	0.8	0.2	0.4	1,424	94.0	5.3	1.1
A10	0.2	0.1	0.0	**4.5**	0.5	1.3	0.2	0.4	0.9	86.3	1.0	4.0	0.4	0.1	0.1	2,172[b,d]	93.0	3.6	0.9
A11	0.2	0.0	0.1	4.0	0.2	1.1	0.2	0.1	0.7	86.2	0.3	6.1	0.5	0.1	0.2	2,366[e,g]	93.9	3.7	0.9
A12	0.3	0.0	0.1	3.7	0.6	0.9	0.2	0.1	0.8	81.0	1.7	9.7	0.4	0.1	0.2	1,780[h]	93.7	4.5	1.1
A13	0.2	0.0	0.0	**4.1**	0.4	0.9	0.3	0.0	0.8	86.4	0.8	5.1	0.6	0.2	0.2	2,460[a]	93.7	4.5	1.4
A14	**0.9**	0.0	0.1	3.8	0.5	0.8	0.2	0.6	1.0	80.3	2.3	8.4	0.5	0.4	0.2	1,762[h]	93.1	4.6	1.8
A15	0.3	0.0	0.0	**4.2**	0.2	0.9	0.2	0.1	0.8	83.6	0.8	7.7	0.7	0.2	0.3	1,869[f]	93.7	4.7	1.5

Variation coefficients (Tables 5, 6): VC ≤ 1.9% for = β-Sitosterol, VC ≤ 23.4% for sterols in the range 1.0–15.0%, VC ≤ 82.2%for sterols in percentages lower than 1.0%, VC ≤ 1.7% for STE and VC ≤ 18.3% for EU

* Means followed by the same letter within the column in Tables 5 and 6 are not significantly different ($P \leq 0.05$). Values in bold letters are outside IOOC limits

STE total sterol content (mg/kg), *ASP* apparent sitosterol percentage (sum 8-13), *CSR* campesterol/stigmasterol ratio, *EU* erythrodiol-uvaol. Nomenclature: *1* Cholesterol, *2* Brassicasterol, *3* 24-Methylene-Cholesterol, *4* Campesterol, *5* Campestanol, *6* Stigmasterol, *7* Δ-7-Campesterol, *8* Δ-5,23-Stigmastadienol, *9* Clerosterol, *10* β-Sitosterol, *11* Sitostanol, *12* Δ-5-Avenasterol, *13* Δ-5,24-Stigmastadienol, *14* Δ-7-Stigmastenol, *15* Δ-7-Avenasterol

Frantoio and Arauco in the 2004 harvest (Tables 5, 6). Sample A2, with a low ASP in 2004, satisfied the limit in 2005 (sample A11). Other authors reported that more than 15–20% of the Cornicabra oils analyzed in their study had ASP below 93.0% with values lower than 91.9% [24]. Small ASP values were observed simultaneously with high campesterol values in oils from under-ripe olive fruits, in those extracted by three-phase centrifugation system [25], and also in olive oils with high acidity values [26]. The campesterol/stigmasterol ratio ranged between 1.7 and 10.2 for the analyzed oils and the average values were particularly high for Barnea oils (Tables 5, 6).

The Arbequina oils showed very variable Δ-5-avenasterol contents in both harvests (2.6–14.7% in 2004 and 4.0–9.7% in 2005), as shown in Table 5. Empeltre and Manzanilla Criolla oils presented high Δ-5-avenasterol contents (Table 6). The samples with high Δ-5-avenasterol content also had a high oxidative stability (OSI greater than 12.0 h). A negative correlation between Δ-5-avenasterol and β-sitosterol contents ($r = -0.861$) was observed. Δ-5-avenasterol has been associated with antioxidant activity and a negative correlation with β-sitosterol has been also observed for Spanish virgin olive oil [6].

The erythrodiol and uvaol contents ranged between 0.5 and 3.0% satisfying the 4.5% limit proposed by the IOOC for edible virgin olive oils (Tables 5, 6). The Manzanilla Criolla and Empeltre oils exhibited the lowest contents (<18 mg/kg), being less than the 1.0% of total sterols in both harvests. Erythrodiol and uvaol contents of more than 2.0% of total sterols (around 50 mg/kg) were observed in two samples, one from Manzanilla Californiana, and the other one from Arauco. When samples from the same olive trees were compared, lower erythrodiol-uvaol contents were observed in 2005. The influence of the cultivar on the erythrodiol and uvaol contents in virgin olive oils has been pointed out [28, 29] and variable contents between different harvests have also been reported [26, 30].

Waxes

In 2004, eight samples from Arbequina (A1–A8, between 280 and 646 mg/kg) and one from Picual (P1: 327 mg/kg) failed to satisfy the IOOC norms for edible virgin olive oils (C40–C46 waxes ≤250 mg/kg). In 2005 only two samples from Arbequina (A10: 312 mg/kg; A11: 299 mg/kg) had wax contents higher than the limit. When samples from the same olive trees were compared in both harvests, a reduction between 5.0 and 78.3% in C40–46 wax contents was observed in 2005. In addition, two samples (A3:

Table 6 Methylsterols and Erythrodiol-uvaol in oil samples from Picual, Barnea, Frantoio, Empeltre, Manzanilla, Arauco and Coratina

Sample	Sterol (%)															STE* (mg/kg)	ASP (%)	CSR	EU (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
P1	0.2	0.0	0.1	3.8	0.2	1.3	0.3	0.2	1.1	87.8	0.7	3.2	0.7	0.2	0.2	2,509[a]	93.7	2.8	1.5
P2	0.2	0.0	0.1	3.6	0.3	0.8	0.2	0.6	0.7	86.0	0.3	6.1	0.5	0.2	0.4	2,444[a,e]	94.2	4.4	0.9
P3	0.3	0.0	0.1	3.3	0.2	1.1	0.2	0.6	1.0	87.6	0.5	4.1	0.4	0.3	0.3	1,889[f]	94.2	3.2	0.7
P4	0.3	0.0	0.1	2.7	0.3	1.0	0.2	0.4	0.9	83.9	1.7	7.4	0.5	0.2	0.4	2,419[a,e,g]	94.8	2.7	0.9
P5	0.1	0.0	0.1	3.2	0.3	0.7	0.2	0.0	0.7	87.4	0.3	6.1	0.4	0.2	0.3	2,284[i]	94.9	4.6	1.1
P6	0.2	0.0	0.0	3.3	0.2	0.8	0.2	0.2	0.8	86.7	0.8	5.9	0.4	0.2	0.3	1,883[f]	94.8	4.3	0.8
B1	0.2	0.0	0.1	**4.8**	0.3	0.9	0.2	0.0	1.1	88.3	0.5	2.9	0.4	0.1	0.2	2,401[a,e,g]	93.2	5.3	1.5
B2	0.2	0.0	0.1	**4.8**	0.4	0.6	0.3	0.4	1.0	85.7	0.6	5.0	0.5	0.2	0.2	2,245[d]	93.2	8.6	1.0
B3	0.2	0.0	0.1	**4.5**	0.3	0.8	0.2	0.6	1.2	86.0	0.6	4.5	0.6	0.1	0.3	2,457[a,e]	93.5	5.2	1.1
B4	0.2	0.0	0.1	**4.4**	0.4	1.1	0.2	0.4	0.8	85.9	0.9	4.9	0.5	0.1	0.1	2,358[e,g,j]	93.5	3.9	0.9
B5	0.2	0.0	0.1	**4.5**	0.4	0.4	0.1	0.0	0.8	87.6	0.5	4.9	0.3	0.1	0.1	2,323[g,i–k]	94.1	10.2	1.1
FR1	**2.3**	0.0	0.0	3.2	0.2	0.8	0.2	1.0	0.8	84.1	0.5	4.9	1.0	0.4	0.6	2,187[d]	**92.3**	4.0	1.1
FR2	0.2	0.0	0.0	3.3	0.3	0.8	0.1	0.0	0.8	86.6	1.1	5.6	0.6	0.3	0.3	2,226[d]	94.7	4.1	0.9
FR3	0.3	0.0	0.0	3.5	0.1	0.8	0.2	0.0	0.9	87.6	1.1	4.4	0.7	0.1	0.3	1,881[f]	94.7	4.2	0.6
E1	0.1	0.0	0.1	3.0	0.2	1.1	0.2	0.5	0.9	81.0	0.5	11.2	0.5	0.2	0.5	1,336	94.6	2.6	0.5
E2	0.1	0.0	0.1	3.4	0.3	1.1	0.3	0.0	0.9	85.0	0.7	7.2	0.5	0.1	0.3	2,007[c]	94.3	3.3	0.9
MC1	0.2	0.0	0.0	2.9	0.3	1.8	0.3	0.3	1.2	87.5	0.7	3.9	0.4	0.2	0.3	2,261[d,i,k]	94.0	1.7	2.3
MC2	0.2	0.0	0.0	2.7	0.4	1.3	0.1	0.0	0.8	89.2	0.6	3.8	0.4	0.2	0.3	2,409[a,e,j]	94.8	2.1	1.5
MCR1	0.2	0.0	0.2	3.3	0.3	0.8	0.2	0.5	1.0	84.5	0.6	7.4	0.4	0.1	0.5	1,852[f]	94.5	4.4	0.6
MCR2	0.1	0.0	0.1	3.5	0.3	0.7	0.2	0.1	0.9	84.2	0.9	8.0	0.4	0.1	0.5	2,366[e,g,j,k]	94.5	4.9	0.6
AU	**1.5**	**0.5**	0.2	**4.5**	0.2	0.9	0.2	0.3	1.0	81.4	0.5	7.4	0.8	0.2	0.4	1,545	**91.4**	4.7	3.0
C	**0.6**	0.0	0.1	3.1	0.5	0.6	0.5	0.0	1.1	84.3	1.8	6.0	0.5	**0.6**	0.3	1,053	93.7	5.5	2.0

The values in bold are outside IOOC limits. Abbreviations, nomenclature and variation coefficients are indicated in Table 5

* The means followed by the same letter within the column in Tables 5 and 6 are not significantly different at $P = 0.05$ level

294 mg/kg and P1: 327 mg/kg), which were outside the limits in 2004, satisfied the norm in 2005 (A12: 156 mg/kg and P4: 71 mg/kg).

A highly significant linear correlation ($b = 0.843$, $r = 0.994$) was observed between the total wax contents and the partially soluble wax contents (C40–C42), as shown in Fig. 1. Insoluble waxes (C44–C46) evidenced a slight increase with the C40–C46 wax content. The high content of waxes in the oils outside the legal wax limit is evidently due to the augmentation of partially soluble waxes.

It is widely known that waxes are found on the surface of the leaves, seeds and fruits in order to protect them against the water loss and insect attack. In addition, waxes increase the resistance to abrasive damage, which can facilitate the entry of pathogens and toxic chemicals into the plant. In dry and hot seasons the plants produce more waxes to control the rate of transpiration in order to reduce water loss [31]. Since waxes are extracted with the oil, the lower wax content observed in 2005 production could be due to a rainier autumn season in Catamarca and La Rioja valleys.

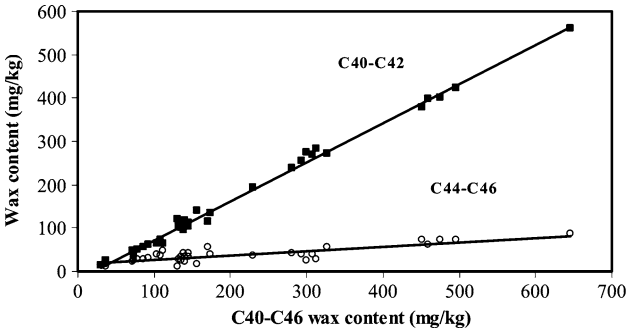

Fig. 1 Correlations between both insoluble and partially soluble waxes, and the total wax content

Principal Component and Cluster Analysis (PCA and CA)

About 80% of the total variance was associated with the three first principal components in the PCA. Figure 2 shows the biplot on the plane of the first two principal components with the centroids for Arbequina, Picual and Barnea in 2004 and 2005. The following variables

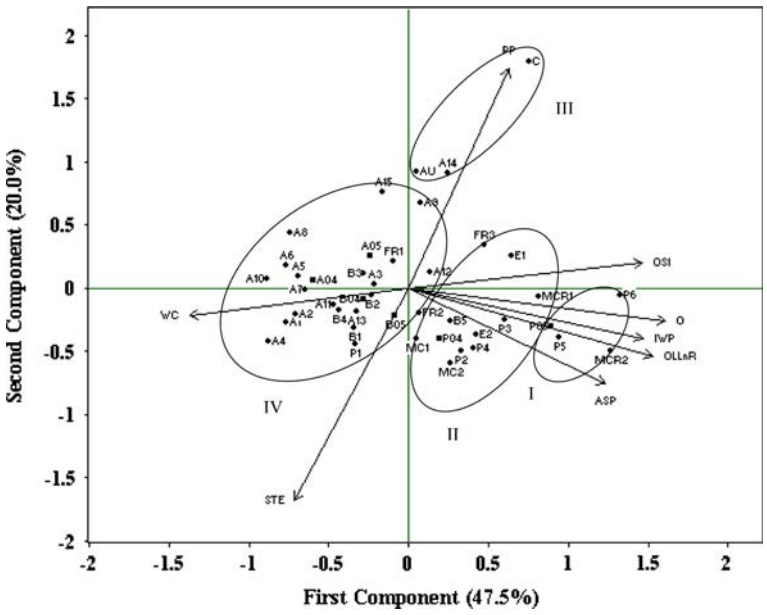

Fig. 2 Biplot for the first and the second principal components and clusters formed by complete linkage for analyzed olive oils. A04, A05, B04, B05, P04 and P05 centroids for Arbequina, Barnea and Picual in 2004 and 2005, respectively. I–IV = Groups separated by CA (cut-off distance = 5.7)

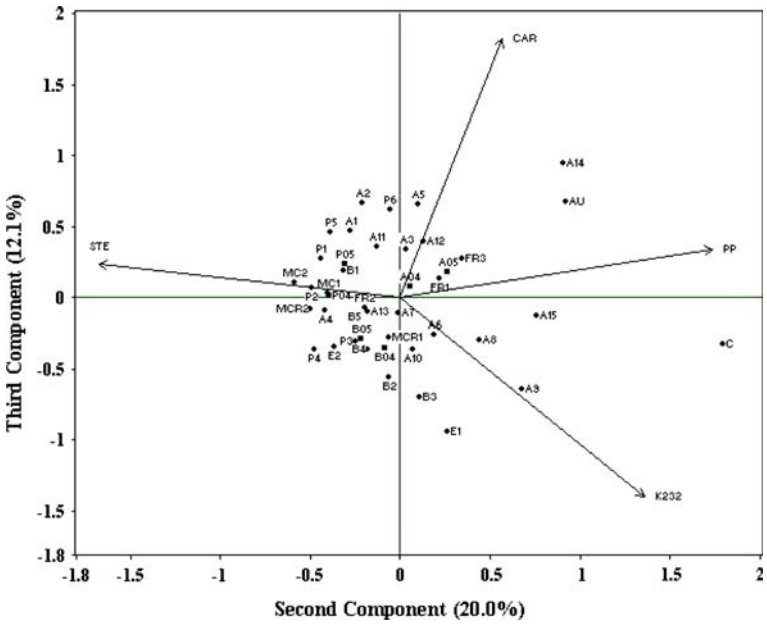

Fig. 3 Biplot for the second and third principal components for analyzed olive oils. A04, A 05, B04, B05, P04 and P05 centroids for Arbequina, Barnea and Picual in 2004 and 2005, respectively

contribute to the first principal component: OSI, O, OLLnR, IWP, and ASP opposite to WC. The PP variable is represented in the second principal component in contrast to the total sterol content (STE) variable. The CAR variable opposed to the K_{232} variable contributes to the third principal component, as shown in the biplot for the second and third principal components (Fig. 3). Using the Euclidean distances between samples, the CA separated four groups (Fig. 2). The oils from Group I have a high oleic-acid content and high OLLnR, together with low C40–C46 wax content and a high percentage of insoluble waxes. The apparent sitosterol percentage and oxidative stability are also high. The oils from Group I have K_{232} values lower than those corresponding to the average oil (Fig. 3). The C40–C46 wax content is low for this group in comparison with the one corresponding to the average oil. The oxidative stabilities were high in this group.

Group III is characterized by high polyphenol contents and low total sterol contents (Fig. 2). The C40–C46 wax contents are low for this group in comparison with those corresponding to the average oil, especially in the sample from Coratina cultivar but this sample has a higher percentage of insoluble waxes. The oils from this group have significant oxidative stabilities and as shown in Fig. 3 have

also high carotenoid contents which contribute together with polyphenols to facilitate the resistance in the presence of oxidative processes.

Group IV includes two Frantoio oils, a sample from Picual, and everything analyzed from Arbequina and Barnea, except for A14 and B5. Low oleic-acid content, low OLLnR, and high C40–C46 wax content with prevalence of the C40–C42 fraction (low IWP) mainly characterize this group. The PP and the OSI for the majority of these samples are low. With respect to 2004, the centroids for Arbequina and Barnea showed a significant shift in 2005 from second–third quadrants towards first-fourth quadrants. For these cultivars and for Picual, higher O, higher OLLnR, and lower WC were observed in 2005.

The results show a poor adaptation to local agronomic media, mainly by Arbequina, and its sensitivity to adverse weather conditions, such as high temperatures, low thermal amplitudes and drought. The national productive sector should recommend the selection of those cultivars best adapted to the agronomical media, and the analysis and implementation of the best cultural and processing conditions. Moreover, additional studies are necessary to establish normal composition values for Argentinian olive oils. The knowledge of oil composition from olives grown in areas different to the Mediterranean region will contribute to the update of the market conditions and the international rules affecting trade in olive oils.

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