Food Research International xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Chemical quality and oxidative stability of extra virgin olive oils from San Juan province (Argentina)

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ARTICLE INFO

Keywords:
Olive oil
Oxidative stability
Fatty acids
Polyphenols
Tocopherols
Pigments

ABSTRACT

This study provides information about the chemical quality (quality indices, fatty acid profile, total polyphenols (PPs), tocopherols and pigments) and oxidative stability index (OSI) of virgin olive oils of Arbequina, Changlot Real and Coratina cultivars (San Juan province, Argentina). The influence of the cultivar and the effect of earlier harvest dates on the yields (OY), quality and OSI of the oils were also evaluated. All the oils were classified as extra virgin. The OY (L/100 kg) averaged: Arbequina = 13.2, Changlot Real = 21.3, Coratina = 18.3. The oleic acid (O) percentage, oleic to linoleic plus linolenic ratio [O / (L + Ln)], PPs and OSI were highly dependent on cultivar (Arbequina < Changlot Real < Coratina). The earlier harvest season associated with lower maturity indices increased the OSI of all the oils (Arbequina: from 6.3–13.8 h up to 10.6–19.0 h, Changlot: from 6.0–12.1 h up to 13.7–36.9 h and Coratina: from 20.5–26.0 h up to 24.6–42.4 h) due to a more favorable O / (L + Ln) ratio and antioxidant composition. Regional producers are recommended to bring forward the harvest season to obtain oils with better chemical and nutritional quality, higher oxidative stability and a fatty acid profile according to the IOC trade standard.

1. Introduction

Argentina produced in average 24,700 t of olive oils between 2010/2011 and 2015/2016 according to the IOC. > 75% of the oils produced in this period were exported, with Argentina ranking fourth after the European Union, Tunisia and Turkey in terms of world exports (IOC, 2016a). Therefore, in the last years, the purchasing countries of olive oils from Argentina (Brazil, USA, Spain, Italy, etc.) and the international organizations such as IOC and Alimentarius Codex have focused their interest on the quality and oxidative stability of these oils. The province of San Juan has increased significantly its export volumes, which represented about 15% of the total olive oil exported by Argentina in the decade 2000/2009 (Antuña, 2010). Owing to its particular soil and climate characteristics and the excellent quality of its irrigation water originated from the thawing snow of the Andes Mountains, the olive oils from the San Juan province are characterized by their excellent quality. However, scarce compositional data for these oils are available (Ceci & Carelli, 2010a; Cobos et al., 2014), and there is great interest in increasing the number of studies that typify, characterize and optimize the quality of these oils.

Oxidation processes are the main cause of olive oil deterioration, and thus of its loss of quality. The main factors affecting the oil shelf life are the glyceride composition and some minor components. Compared to other vegetable oils, olive oil is characterized by its high content of phenolic compounds with recognized antioxidant properties. Studies using purified glyceride matrices from virgin olive oil showed that ortho-diphenolic compounds (hydroxytyrosol or 3,4-DHPEA, hydroxytyrosyl acetate, aldehydic form of oleuropein aglycon, and luteolin) and mixtures thereof exhibited greater antioxidant activities than α-T (Mateos, Trujillo, Pérez-Camino, Moreda, & Cert, 2005). In contrast, tyrosol, squalene and free fatty acids showed low or negligible effects on oil stability. In mixtures of 3,4-DHPEA and α-T, the stability was dependent on the concentration ratio between both antioxidants, and a synergic effect was observed (Baldioli, Servili, Perretti, & Montedoro, 1996).

A multiple linear regression model was developed to study how the

Abbreviations: CARs, carotenoids; CHLs, chlorophylls; EVOO, extra virgin olive oil; FAMEs, fatty acid methyl esters; FA, free acidity; 3,4-DHPEA, hydroxytyrosol; G, gadoleic acid; IOC, International Olive Council; L, linoleic acid; Ln, linolenic acid; MUFAs, monounsaturated fatty acids; OY, oil yield; O, oleic acid; OLLnR, O / (L + Ln) ratio; OSI, oxidative stability index; P, palmitic acid; Po, palmitoleic acid; PV, peroxide value; PPs, polyphenols; PUFAs, polyunsaturated fatty acids; RLV, Reference Labeling Value; S, stearic acid; α-T, α-tocopherol; α-TE, equivalent α-tocopherol; β-T, β-tocopherol; γ-T, gamma-tocopherol; δ-T, delta-tocopherol; TTs, total tocopherols; UFAs, unsaturated fatty acids

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http://dx.doi.org/10.1016/j.foodres.2017.07.074
Received 8 June 2017; Received in revised form 28 July 2017; Accepted 31 July 2017
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different compositional parameters contribute to the oxidative stability determined by the Rancimat method (Ceci & Carelli, 2010b). By means of this model, it was observed that the oxidative stability of the olive oil depended on its fatty acid composition and the level of natural components, mainly PPs. CARs and β-T also contributed to the oxidative stability as antioxidants, although their contribution was less significant.

In a previous study, samples of EVOO from the San Juan province (Argentina) were evaluated, analyzing the profile and content of total and individual biophenols and their relation to flavor (Ceci, Ramírez, Mussio, Mattar, & Carelli, 2017). The contents of total biophenols, secoiridoids and simple phenols strongly depended on the cultivar, with the highest levels being observed for the Coratina oils, variable levels for the Changlot Real oils, and the lowest levels for the Arbequina oils. By bringing forward the harvest date, oils with enhanced biophenol profiles in terms of quality and concentration were obtained, also being harmonious and complex from a sensory point of view.

The objective of this work was to provide information about the chemical quality of EVOO from the San Juan province (Argentina), evaluating the influence of the cultivar and earlier harvest dates on oil yields, classical quality indices, fatty acid profile, total PPs, tocopherols and pigments. The effect of these parameters on the olive oil oxidative stability determined by the Rancimat method was also analyzed.

2. Materials and methods

2.1. Olive oils

Thirty samples of olive fruits of the Arbequina, Changlot Real and Coratina cultivars were harvested in four departments (25 de Mayo, Sarmiento, Zonda and Ullum) of the San Juan province (Argentina). The coordinates of these departments range 31–32°S and 68–69°W, and their altitudes above sea level are 555–785 m. In 2012, the fruits were harvested at the beginning of the harvest season (late April-early May) and at the end of the season (late May-early June). The maturity indices of the fruits were evaluated by the color of the skin and the flesh using a scale ranging from 0 to 7. The maturity indices obtained in 2012 were: Arbequina (6 samples) = 2.52–5.22, Changlot Real (4) = 4.60–5.06, and Coratina (5) = 1.57–2.97.

In 2013, in order to reduce the maturity indices and avoid processing over-ripe olives, the harvest was brought forward 15–17 days, reaping the fruits in early April and early May. The maturity indices obtained in 2013 were: Arbequina (5 samples) = 1.62–3.27, Changlot Real (6) = 2.53–3.33, and Coratina (4) = 0.33–1.36.

About 100 kg of fruits were processed using OLIOMIO two-phase equipment to extract the oil (temperature = 20.0–27.5 °C, time = 40 min). The oil volume was measured at the centrifuge exit, and the OY was expressed as volume of oil in L/100 kg of processed fresh olives.

2.2. Analytical methods

Classical quality indices such as FA [oleic acid (O) content in g/100 g], PV (peroxide oxygen in mEq/kg) and specific UV extinction coefficients (K268 and K232) were determined according to IOC regulations (IOC, 2016b). The OSI, represented as the induction time in hours, was measured with a Metrohm 679 Rancimat apparatus at 110 °C and 20 L/h airflow.

Fatty acids were determined as FAMEs obtained by trans-esterification with a solution of potassium hydroxide in methanol at room temperature (IOC, 2015). Then, FAMEs were analyzed by Capillary GC with a 4809D series gas chromatograph (Agilent Technologies, Hewlett-Packard, Santa Clara, CA, USA), identified by comparing their retention times with Supelco standards (Supelco 37 Component FAME mix, Supelco, Inc.) and quantified as percentage of fatty acids. FAMEs were separated on a SP2380 capillary column [stabilized poly (90% bicyanopropyl/10% cyanopropylphenylsiloxane)] (30 m length × 0.25 mm i.d., 0.25 μm film thickness; Supelco Inc., Bellefonte, PA, USA) using hydrogen as carrier gas. The column temperature was maintained at 170 °C for 15 min, then increased at 4 °C/min to 210 °C and finally maintained at 210 °C for 10 min. The injector was used in split mode with a ratio of 1:50. The temperatures of the injector and Flame Ionization Detector were 220 °C. Data acquisition and peak integration were performed using EZChromElite 332 data system (Agilent Technologies).

Polyphenolic compounds (PPs) were isolated by three extractions of an oil-in-hexane solution with methanol/water (60%, v/v). The content of PPs was determined spectrophotometrically at 725 nm using Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) and caffeic acid (≥ 99%) provided by Sigma (Sigma Chemical Co., St. Louis, MO, USA) as standard. The content of PPs was expressed as caffeic acid equivalents (mg/kg of oil) (Gutfinger, 1981).

Tocopherol content was determined by HPLC according to AOCS method Ce 8-89 (AOCS, 2009). A standard of α-T (purity > 98%) was obtained from Sigma and used as external standard. The equipment used was a HPLC Varian (Vista 5500) chromatograph equipped with a fluorescence detector and a LiChrosorb Si-60 column (l = 25 cm; i.d. = 4 mm; particle size = 5 μm) (Merck). The mobile phase was filtered and degassed isopropanol:hexane (0.5:99.5, v/v) at a flow rate of 1 mL/min. Peak areas were obtained with Waters Empower 2 software (Waters, Milford, MA, USA). TTs, α-T, β-T, γ-T and δ-T were expressed as mg/kg.

CHLs and CARs were evaluated from the absorption maximum for an oil solution in cyclohexane at 670 and 470 nm, respectively. The absorptivity coefficients of pheophytin “a” and lutein were used for CHLs and CARs, respectively, and the results were expressed as mg/kg (Mínguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sánchez-Gómez, & Garrido-Fernández, 1991).

2.3. Statistical analysis

Simple ANOVA (software: INFOSTAT 2014, Universidad Nacional de Córdoba, Argentina) was used for all the assays, and Duncan's test ($p \leq 0.05$) was performed to estimate significant differences between cultivars and harvest years. The analyses were carried out in triplicate, except for the fatty acid determinations that were performed in independent duplicates and each one was twice injected onto the gas chromatograph.

3. Results and discussion

3.1. Oil yield and quality indices

The OY was significantly higher for the Changlot Real and Coratina cultivars, being 21.3 and 18.3 L/100 kg olives in average, respectively (Table 1). The fruits from the Arbequina cultivar presented lower OY (average = 13.2 L/100 kg). According to data from a germplasm bank of Spanish varieties, Changlot Real shows higher oil content (77.1% in dry matter) than Arbequina cultivar (66.2%) (Uceda, Aguilera, Giménez, & Beltrán, 2010). On the other hand, Coratina is a variety characterized by high oil content, reaching up to 22.6% of fresh weight in hot regions (Dag, Harlev, Lavee, Zipori, & Kerem, 2014). No significant differences were observed in the OY of the three cultivars between harvest years. Thus, bringing forward the harvest 15–17 days did not affect the oil extractability.

All samples were classified as EVOO taking into account the maximum limits stated by the IOC Trade Standard for this category: FA = 0.8 g/100 g and PV = 20 mEq/kg (Table 1) (IOC, 2016b). No significant differences were detected between cultivars and harvest years for FA, which was < 0.42 g/100 g. As for PV, the Arbequina oils exhibited significantly lower values in the 2013 season when harvest was brought forward (2013 = 3.36, and 2012 = 7.83 mEq/kg in

Table 1
Oil yield and quality parameters of the studied olive oils.

	Arbequina			Changlot Real			Coratina		
	2012	2013		2012	2013		2012	2013	
Maturity Indices	2.52–5.22b	1.62–3.27a*	B	4.60–5.06b	2.53–3.33a**	B	1.57–2.97b	0.33–1.36a*	A**
OY	9.8–17.1a	7.7–19.2a	A	10.0–25.9a	13.6–28.5a	B	15.8–22.5a	13.6–18.9a	B**
FA	0.13–0.42a	0.13–0.36a	A	0.18–0.27a	0.14–0.25a	A	0.18–0.30a	0.25–0.34a	A
PV	5.7–10.4b	1.8–5.6a**	A	5.0–8.0a	1.8–8.0a	A	3.8–7.6a	2.3–5.4a	A
K268	0.11–0.13a	0.09–0.15a	A	0.12–0.15a	0.11–0.15a	A	0.15–0.18a	0.15–0.22a	B**
K232	2.18–2.99a	1.93–2.77a	B	1.63–2.45a	1.47–2.37a	A	1.48–1.91a	1.49–2.03a	A**

Ranges of average values (n = 3). Different lowercase letters in the same row for each cultivar indicate significant differences between harvest years (Duncan test, $p \leq 0.05$). Different capital letters in the same row indicate significant differences between cultivars (Duncan test, $p \leq 0.05$).
OY = oil yield (L/100 kg); FA = free acidity (g oleic acid/100 g); PV = peroxide value (mEq peroxide oxygen/kg); K268 = extinction coefficient at 268 nm; K232 = extinction coefficient at 232 nm.
* Significance level $p \leq 0.05$.
** Significance level $p \leq 0.01$.

average). The oils of the other cultivars did not present significant changes in PV between harvest years. A sensory panel recognized by the IOC also classified the samples as EVOO (fruity median > 0, median of the defects = 0) (Ceci et al., 2017).

No significant differences between harvest years were observed in the UV extinction coefficients of the oils from the three cultivars (Table 1). The Coratina oils had significantly higher K268 values than those from Arbequina and Changlot cultivars. However, all the oils had K268 values below the maximum limit established for EVOO (≤ 0.22) (IOC, 2016b). On the other hand, the Arbequina oils had significantly higher K232 values than those of the Changlot and Coratina varieties (Table 1). In the 2012 harvest, two samples of the Arbequina variety had K232 values above the maximum limit for EVOO (2.50) (IOC, 2016b), exceeding 2.90. For the Arbequina oils, produced in hot intra-continental regions of Argentina, values of K232 > 2.50 have been previously reported (Ceci & Carelli, 2007). The IOC Standard indicates that K232 determination is solely for application by commercial partners on an optional basis (IOC, 2016b). In the 2013 harvest, only one Arbequina sample presented a value (K232 = 2.77) above the limit established for EVOO. For the Arbequina oils, bringing forward the harvest season decreased the number of samples exceeding the maximum value permitted for K232, and its average value fell from 2.52 to 2.33.

3.2. Fatty acid profiles

Significant differences were observed between cultivars in the fatty acid profiles (Table 2, capital letters). The Arbequina oils were mainly characterized by the lowest contents of C18:0 (S) and C18:1 (O), and the highest contents of C16:0 (P), C16:1 (Po), and C18:2 (L). On the other hand, the Coratina oils had the highest contents of O and Ln (C18:3), and the lowest content of Po. Finally, Changlot oils presented medium contents of O and Po, and P and L that were similar to those of Coratina oils but lower than those of Arbequina oils.

The O / (L + Ln) ratio [OLLnR] was dependent on the cultivar in a highly significant level (Arbequina < Changlot < Coratina, Table 2). In a study carried out in Tunisia using cluster analysis on eighteen varieties of Mediterranean origin including the three varietals analyzed in this study, three groups of oils based on their fatty acid profiles were proposed (Zarrouk et al., 2009). In this study, the Coratina and Changlot oils were included in group 1 showing high O/L ratios (6.29–21.53%), while Arbequina oils were included in group 3 with low O/L ratios (O/L ratios for groups 2 and 3 = 1.72–5.50%), but richer in P (16.32–19.24%) and Po (2.07–2.89%) and poorer in UFAs (77.72–80.20%) when compared to cultivars from group 2 (P = 11.22–14.14%, Po = 1.00–1.32% and UFAs = 82.54–86.01%). In the Arbequina oils, the lowest OLLnR were observed, which can be explained by their lower O content and higher L content, whereas Coratina oils showed the highest OLLnR levels due to their higher O content and lower L content (Table 2).

As it can be observed in Table 2 (lowercase letters), the fatty acid profiles of the oils significantly changed when the harvest was brought forward in 2013. Three samples of Arbequina oils harvested in 2012 had O values lower than 55.00%. Low O contents have been observed in Arbequina olive oils from hot intra-continental regions of Argentina (Ceci & Carelli, 2007, 2010b). However, the Arbequina oils obtained in 2013 had higher O content, with values within the 55.00–83.00% range established by the IOC Trade Standard (IOC, 2016b). The Arbequina samples of 2013 also presented lower contents of other UFAs: C16:1, C17:1, C18:2 and C18:3 (Table 2). These changes led to significantly higher OLLnR values for the Arbequina oils extracted in 2013 (average OLLnR: 2012 = 2.96, 2013 = 3.48).

All these changes observed in the fatty acid profile of the oils are related to the statistically significant reduction of the maturity indices of the olives in 2013 as a consequence of the earlier harvest (Table 1). Recently a work was published on the dynamics of fatty acid, tocopherol and phenolic compound biogenesis during fruit ontogeny from Arbequina and Manzanilla olives of the San Juan province (Bodoira et al., 2016). In the oils extracted from Arbequina olives, after reaching maximum O content, the levels of this fatty acid decreased markedly during fruit ripening, demonstrating the greater sensitivity of fatty acid metabolism in this cultivar. Parallel to the decrease in O, increases in L and Po contents were also observed (Bodoira et al., 2016). This opposite relation between O and L may be due to the activity of oleate desaturase enzyme transforming O into L (Hernández, Guschina, Martínez-Rivas, Mancha, & Harwood, 2008). Ln is also formed from L by the linoleate desaturase enzyme (Hernández et al., 2008).

The same behavior (increase in O and decrease in L and Ln) was observed in Changlot oils for the earlier harvest (Table 2). As a consequence of these changes, a higher OLLnR was recorded in 2013 (average = 7.16) with respect to 2012 (average = 5.36). The content of G (C20:1) was higher than 0.40% in all Changlot oils in 2012. The average content of this fatty acid decreased from 0.46% to 0.42% when the harvest season was brought forward in 2013 (Table 2). Recently, the IOC raised the maximum limit for C20:1 from 0.40% to 0.50% (IOC, 2016b). Therefore, all the Changlot samples fit this new limit value.

Bringing forward the harvest in 2013 also produced a significant change in the OLLnR of the Coratina oils, which increased from 6.10 up to 8.10 in average (Table 2). In the oils from this cultivar, O increased, L decreased, but Ln showed no significant differences between harvests. In the 2012 harvest, one Coratina sample presented a G content equal to 0.51%, but in 2013 the G values for all the oils from this cultivar did not exceed the new maximum limit (≤ 0.50%).

Samia Dabbou et al. (2015) observed that the fatty acid profiles of Coratina oils from northwestern Tunisia, cultivated under similar conditions to the area of origin (Italy), varied with the irrigation level. They

Table 2
Fatty acid profiles (FAMEs, % m/m) of the studied olive oils.

FAME	Arbequina			Changlot Real			Coratina		
	2012	2013		2012	2013		2012	2013	
C14:0	0.02a	0.02a	C	0.01–0.02b	0.01a**	B	0.01a	0.01a	A**
C16:0	18.03–19.21a	16.50–18.89a	B	11.46–12.89a	11.07–13.22a	A	11.15–13.48a	11.11–13.64a	A**
C16:1	1.88–3.15b	1.50–2.74a*	C	0.75–1.07a	0.74–1.06a	B	0.46–0.69a	0.44–0.57a	A**
C17:0	0.09–0.16a	0.11–0.12a	B	0.13–0.15a	0.11–0.15a	C	0.05–0.06a	0.05–0.07a	A**
C17:1	0.24–0.32b	0.23–0.27a*	B	0.24–0.30a	0.25–0.29a	C	0.07–0.10a	0.08–0.12a	A**
C18:0	1.50–1.84a	1.56–1.76a	A	2.19–2.54a	2.00–2.44a	C	2.08–2.23a	2.16–2.31b**	B**
C18:1	53.89–61.00a	56.38–62.60b**	A	67.92–72.02a	72.20–74.50b**	B	70.96–72.82a	73.45–75.66b**	C**
C18:2	15.29–20.86b	14.19–18.93a**	B	11.45–14.48b	8.74–10.40a**	A	9.69–13.12b	7.17–10.31a**	A**
C20:0	0.30–0.35a	0.30–0.34a	A	0.37–0.44b	0.33–0.41a**	B	0.39–0.43b	0.39–0.40a**	C**
C18:3	0.72–0.81b	0.61–0.76a**	B	0.60–0.81b	0.53–0.77a**	A	0.77–0.95a	0.76–0.87a	C**
C20:1	0.20–0.25a	0.20–0.24a	A	0.44–0.48b	0.39–0.45a**	C	0.40–0.51a	0.36–0.46a	B**
C22:0	0.08–0.10a	0.07–0.10a	A	0.10–0.11b	0.08–0.10a**	B	0.10–0.11a	0.10–0.12a	C**
C24:0	0.03–0.05a	0.03–0.05a	A	0.05–0.07b	0.03–0.06a**	C	0.05–0.06b	0.03–0.04a**	B**
OLLnR	2.51–3.80a	2.87–4.20b**	A	4.44–5.93a	6.63–8.00b**	B	5.04–6.95a	6.62–9.39b**	C**

Ranges of average values of two replicates injected twice into the gas chromatograph. Different lowercase letters in the same row for each cultivar indicate significant differences between harvest years. Different capital letters in the same row indicate significant differences between cultivars. OLLnR = Oleic / (Linoleic + Linolenic) Ratio.

* Significance level $p \leq 0.05$.

** Significance level $p \leq 0.01$.

considered two degrees of olive maturation: early harvest (first harvest = FH) and late harvest (second harvest = SH). The oils obtained in the FH from trees exposed to moderate irrigation had higher O content (75.8%) than those from the SH (72.6%). The highest L contents (8.9%) were observed for the oils of the FH from trees exposed to moderate and full irrigation regimes, whereas lower contents were registered in the SH for the same irrigation levels. However, the L content in the oils appeared to be slightly affected by the ripening degree of the olives. Finally, Ln contents were only slightly affected or unaffected by the irrigation regime and the harvest period.

On the other hand, the fatty acid profiles of olive oils from nine Italian cultivars (including Coratina) grown in the Calabria region during fruit maturity were studied for three seasons (Poiana & Mincione, 2004). The Coratina oils showed an increasing trend in O content, but at a very low rate in the late stages of fruit maturity. The L content had an oscillating trend, although it declined at the end of the process. The Ln content showed a slight drop during fruit maturity, with a notable increase in the last month. However, the O/L ratio was a constant parameter throughout olive ripening, or decreased at the later harvest according to the year considered. The decrease in the MUFAs/PUFAs ratio of the oils during fruit maturity was attributed to the higher temperatures that prevail in hot summers (Dag et al., 2014).

3.3. Polyphenols

As shown in Table 3, significant differences were observed in the content of PPs between cultivars (Arbequina < Changlot < Coratina). Five categories have been proposed by a germplasm bank of Spanish varietals to classify olive oils by their content of PPs determined with the Folin-Ciocalteu reagent using caffeic acid as standard and expressing the result in mg/kg: very high (> 600), high (450–600), medium (300–450), low (150–300), and very low (< 150) (Uceda et al., 2010). According to their content of PPs, the Arbequina oils were mainly classified into category “low” and the Changlot oils into category “medium” (Uceda et al., 2010). On the other hand, among Italian varietals, Coratina and Moraiolo oils have very high concentrations of PPs (Vossen, 2007). Compared to the traditional three-phase centrifuges, using two-phase decanters allows obtaining oils with higher contents of PPs, being the reported levels as hydroxytyrosol equivalents > 600 mg/kg for oils from the Coratina cultivar in their region of origin (Servili et al., 2004).

In the present work, the content of PPs significantly increased for the oils from the Arbequina and Changlot cultivars when the harvest was brought forward in 2013 (Table 3). This increase was in average of 46% for Arbequina and 106% for Changlot. The increase in the content of PPs was more significant for the Changlot oils because these oils registered a higher decrease in the maturity indices of the olives (Table 1). The Coratina oils did not show significant changes in the content of PPs with the earlier harvest in 2013 (Table 3) despite the significant fall observed in the maturity indices of the olives (Table 1). The content of total phenolic compounds in Arbequina olives from the same region as those studied in the present work showed an increase until 95 days after full flowering, but then, from fruit set to maturity, decreasing concentrations were observed (Bodoira et al., 2016). The negative effect of fruit ripening on the phenolic concentration of virgin olive oil is particularly clear. The hydrophilic phenols showed the lowest concentration in the oils obtained from overripe olives (Servili et al., 2004). The early harvest in 2013, involving olives with lower maturity indices, could explain the higher contents of PPs in the oils from the Arbequina and Changlot cultivars. Other authors, using the same analytical method as in this study, observed higher total phenol contents for the oils obtained from the Coratina cultivar in earlier harvests, then those contents sharply decreased and increased again, until eventually remaining virtually constant for the last 45 days (Dag et al., 2014). However, in the present work no statistically significant changes were observed when the harvest dates were brought forward 15 days in 2013. In contrast, in a previous study we detected higher levels of biophenols in the oils from the Coratina cultivar in 2013 using the HPLC-UV method (Ceci et al., 2017). This confirms that the results from the spectrophotometric method depend on the differences in the molar absorptivity per reactive group between phenols.

Other factors such as the irrigation levels and temperature also contributed to the differences in the content of PPs. It is known that increasing available water induces a clear reduction of the total content of phenolic compounds in the oils, especially for fruits harvested early (Samia Dabbou et al., 2015). Moreover, high temperatures during the summer might induce a reduction of phenolic compounds in the oil (Samia Dabbou et al., 2015). Hence, it was suggested that with higher temperatures (due to global warming), earlier harvests involving a lower maturity index are recommendable to enhance the total phenol levels in the oils.

3.4. Tocopherols

Significant differences between cultivars were observed in the content of TTs (Table 3), with the mean values being 133 mg/kg for

Table 3
Compounds from the unsaponifiable fraction and oxidative stability of the studied olive oils.

	Arbequina			Changlot Real			Coratina		
	2012	2013		2012	2013		2012	2013	
PPs	70–158a	129–194b**	A	39–157a	125–364b**	B	362–657a	394–487a	C**
TTs	211–298a	219–327a	B	93–151a	107–214a	A	322–473b	303–391a**	C**
α-T	203–280a	206–310a	B	89–144a	99–195a	A	299–434b	280–358a**	C**
β-T	6–16a	7–13a	C	3–5a	5–10b**	A	6–10a	6–8a	B**
γ-T	1–3b	0–2a*	A	1–2a	1–6a	A	13–26b	11–18a**	B**
δ-T	0a	0a	A	0a	0a	A	1–4a	1–3a	B**
α-TE	207–289a	212–317a	B	90–147a	102–202a	A	307–446b	286–366a**	C**
CHLs	0.3–6.5a	5.5–8.7b**	A	0.6–3.8a	6.4–17.2b**	A,B	2.3–10.3a	6.6–35.6b**	B**
CARs	1.4–5.1a	2.7–4.3b*	A	1.6–3.3a	2.4–6.9b**	A	5.0–9.1a	6.1–14.1a	B**
CHLs/CARs	0.21–1.26a	1.88–2.47b**	A,B	0.36–1.16a	1.96–2.92b**	B	0.46–1.17a	1.09–2.53b**	A*
OSI	6.3–13.8a	10.6–19.0b**	A	6.0–12.1a	13.7–36.9b**	B	20.5–26.0a	24.6–42.4b**	C**

Ranges of average values (n = 3). Different lowercase letters in the same row for each cultivar indicate significant differences between harvest years. Different capital letters in the same row indicate significant differences between cultivars.

PPs = polyphenols (mg caffeic acid/kg); TTs = total tocopherols (mg/kg); *α-, β-, γ-, δ-T* = alpha-, beta-, gamma- and delta-tocopherol (mg/kg); α-TE (mg/kg) = 1xα-T + 0.5xβ-T + 0.25xγ-T + 0.1xδ-T (Scientific Committee of Food-European Commission SCF/CS/NUT/UPPLEV/31, 2003); CHLs = chlorophylls (mg/kg); CARs = carotenoids (mg/kg); OSI = Oxidative Stability Index (h).

* Significance level $p \leq 0.05$.

** Significance level $p \leq 0.01$

Changlot oils, 248 mg/kg for Arbequina oils and 366 mg/kg for Coratina oils. Data on the TTs of 21 varietals of a Spanish germplasm bank showed contents of 230 ± 29 mg/kg for the oils from Arbequina (Uceda et al., 2010). The tocopherol level of these oils exhibited a strong influence of the varietal (79%) with respect to the harvest date associated with the fruit maturity index (20%) and the harvest year (1%). Recently it was reported that oils extracted from Arbequina olives from the San Juan province (Argentina) have higher tocopherol content than their Spanish counterparts (Bodoira et al., 2016). These higher contents could be due not only to geographic and climatic conditions, but also to the oil extraction procedure used for the Argentinean olives, consisting in hexane extraction of freeze-dried fruit samples, which are different from those used at commercial scale. Among the most representative Spanish varietals, the highest tocopherol content (488 mg/kg) was reported for Changlot oils (Serrano-Morago & Lezcano-Martín, 2005), with a value much higher than those obtained in the present study. As for the Coratina cultivar, a very wide range of contents of TTs can be found between the oils, with values ranging from 200 mg/kg in the Mediterranean region (Cinquanta, Esti, & Di Matteo, 2001) up to almost 800 mg/kg in hotter areas (Benincasa et al., 2011). The oils containing higher amounts of polar phenols (responsible for the bitter/pungent hues in taste) generally also contain high levels of tocopherols (Kalogeropoulos & Tsimidou, 2014). This fact was confirmed in the Coratina oils studied in this work (Table 3).

The early harvest in 2013 produced no significant changes in the content of TTs of the oils from Arbequina and Changlot cultivars (Table 3). However, in the Coratina oils the level of TTs was lower in 2013. The general pattern of variation of tocopherol levels (α-, β- and γ-isomers) in the Arbequina olives from the San Juan province (Argentina) was characterized by high amounts in the young drupes, pronounced decrease during fruit development, and little or no change during fruit ripening (Bodoira et al., 2016). The lower content of TTs observed in the Coratina oils of 2013 is in disagreement with lower maturity indices of the olives in 2013 (Table 1). This could be explained by the influence of other factors such as water availability and harvest year, given that the agronomic practices and technological factors were the same for both harvests. A negative correlation between water availability and the concentrations of TTs or α-T is known (Samia Dabbou et al., 2015).

Alpha-T is the predominant isomer in EVOO (> 85–90% of TTs), and has the highest in vivo biological activity as vitamin E. The α-T content showed the same differences between cultivars and harvests as those observed for the content of TTs (Table 3). The other isomers (β-, γ- and δ-T) were absent or present in small amounts (Table 3). Delta-T was only detected in the Coratina oils, not showing significant differences between harvests. These isomers, on the other hand, exert a high in vitro antioxidant capacity, with δ-T exhibiting the greatest activity, followed by γ-T and β-T.

The Coratina oils were characterized by the highest contents of γ-T (11–26 mg/kg), while the Arbequina and Changlot oils showed the lowest contents (0–6 mg/kg) (Table 3). As a general rule, it can be argued that the presence of γ-T is influenced by a genetic component more than by other factors, so that some cultivars contain rather high levels of γ-T whereas others contain negligible amounts. Beltrán et al. (2010) studied oils from three olive varietals (Frantoio, Hojiblanca and Picual) for three consecutive crop years and different harvests biweekly, and they observed a slight decrease in γ-T at the beginning, achieving a minimum and later increasing. The increase in γ-T for the last harvesting dates was attributed to the generation of phytol from the breakdown of CHLs and its reuse in the tocopherol synthesis during chloroplast senescence, although this is rather unlikely. This increase was strongly cultivar-dependent, being irrelevant in Frantoio oils and more significant in Hojiblanca and Picual oils (Beltrán et al., 2010). The results obtained for the oils from the San Juan province confirm that γ-T content is dependent on the cultivar, and also on the maturity indices of the processed olives. Thus, a decrease was observed in γ-T content of the Arbequina and Coratina oils when the harvest season was brought forward in 2013 and the processed olives exhibited lower maturity indices (Tables 1 and 3). However, the γ-T content did not vary significantly for the Changlot oils between harvest years, despite the lower maturity indices of the olives in 2013.

The RLV for the vitamin E in the European Union is 12 mg of α-TE per day, with values ranging between10–15 mg/day depending on age, sex and geographic region. Based on RLV = 12 mg/day, a daily intake of EVOO of 40 g/day (the generally recommended intake) and concentrations of α-TE (Table 3) estimated by the Scientific Committee on Food of the European Commission, the following statements can be made: 1) despite having a lower vitamin E content, the Coratina oils obtained in 2013 provided virtually 100% of the RLV (2012 = 102–149%, and 2013 = 95–122%); 2) the Arbequina oils ensured > 70% of the RLV in both harvests (69–106%); and 3) the Changlot oils provided intermediate coverage in both harvests (30–67% of RLV).

3.5. Pigments

Total contents of CHLs and CARs (the pigments responsible for the color of EVOO) are shown in Table 3. First, a strong influence of the varietal was observed, with Coratina oils having the highest levels of both pigments, and Arbequina oils the lowest. Secondly, as expected, by bringing forward the harvest date in 2013, the contents of CHLs and CARs significantly increased in the Arbequina and Changlot oils. In the Coratina oils, the content of CHLs was also significantly higher in 2013, but the content of CARs was not statistically different, although the mean value increased from 6.7 in 2012 to 8.2 mg/kg in 2013. Finally, the ratio between the total contents of CHLs and CARs (CHLs/CARs) significantly increased in the oils of the three varietals in 2013.

Good quality olive oil is best defined by its golden green color, although the green hue may vary depending on the varietal and the maturity index of the fruits processed. The chlorophyll pigments include CHLs “a” and “b” and pheophytins “a” and “b”. CHLs “a” and “b” (major pigment constituents of the fresh fruit) are lost on ripening, and virtually disappear in the oil, being transformed into their magnesium-free derivatives, the pheophytins (Boskou & Clodoveo, 2016). This structural transformation is favored by the liberation of acids during olive oil extraction. The components of the carotenoid fraction in fresh olive fruits are mainly β-carotene and lutein, and other minor CARs. During the extraction process, the acidity of the medium causes isomerization, producing other CARs that have been found in olive oils. The pigment loss observed in the olive oil during fruit maturity is higher for the chlorophyll fraction (Mínguez-Mosquera, Gandul-Rojas, Garrido-Fernández, & Gallardo-Guerrero, 1990). Thus, a lower CHLs/CARs ratio is observed in the oils as fruit maturity progresses. The oils from the San Juan province were obtained from greener olives in 2013, and therefore they presented higher ratios CHLs/CARs.

Greener oils from the three cultivars were obtained in 2013 when the harvest was brought forward. The quality perception of olive oils is strongly influenced by the color when it is evaluated by consumers. Generally, in the Mediterranean area, consumer preferences about the color of a good quality olive oil are oriented toward darker green compared to lighter colors (Boskou & Clodoveo, 2016). Conversely, in non-traditional areas of olive oil production, where seed oils are predominantly consumed, consumers prefer yellowish olive oils. In fact, some people who do not usually consume olive oil can reject too green oils, qualifying their taste as unpleasant, strange or defective. The sensory analysis of the samples from the San Juan province using a tasting panel recognized by the IOC described the oils harvested in 2013 as harmonious and complex, also revealing an enhanced profile in their positive attributes (fruity, bitterness and pungency) (Ceci et al., 2017)). Although the sensory analysis recognized by the IOC does not include color evaluation, the tasting panel did not make any negative comment about the quality of the olive oil from the 2013 harvest.

3.6. Oxidative stability

A strong influence of the cultivar was observed in the OSI: Coratina > Changlot > Arbequina (Table 3). The higher oxidative stability of the oils from the Coratina cultivar is based on their better fatty acid profile (highest OLLnR, Table 2) and higher content of PPs (Table 3). In the Arbequina oils, the OLLnR (Table 2) and the contents of PPs and CARs (Table 3) presented the lowest values, reducing the oxidative stability of these oils.

As a consequence of the decrease in the maturity indices of the processed olives with the earlier harvest, the oxidative stability increased in the oils from the three cultivars in 2013 (Tables 1 and 3). The largest effect was observed for the Changlot oils, whose OSI improved in average from 9.8 h in 2012 up to 23.7 h in 2013. The decrease in maturity indices was more significant for the olives of this cultivar (Table 1). The higher oxidative stability of these oils in 2013 is a result not only of a better fatty acid profile (higher OLLnR in 2013, $p < 0.0001$), but also of the higher contents of all the antioxidant compounds (PPs: $p = 0.0001$, β-T: $p < 0.0001$, CARs: $p = 0.0001$).

The improvement in oxidative stability observed in the 2013 harvest was lower for the oils of the Coratina and Arbequina cultivars, increasing in average from 23.5 h up to 34.6 h and from 9.6 h up to 13.7 h, respectively. In the Arbequina oils, the most significant effect was observed on the content of PPs ($p < 0.0001$), with the increase in OLLnR also being important but less significant ($p = 0.0010$) than that exhibited in the Changlot oils (Tables 2 and 3). In these oils no significant effect was observed on β-T content ($p = 0.3804$), and the increase in the content of CARs in the 2013 harvest was less significant ($p = 0.0264$). Finally, as presented in Tables 2 and 3, in the Coratina oils a significant effect was only observed for OLLnR ($p < 0.0001$), and no changes were registered in the antioxidant compounds between harvests (PPs: $p = 0.0766$, β-T: $p = 0.2830$, and CARs: $p = 0.1684$).

The results obtained in the present work confirm those previously observed about the strong influence of the OLLnR (55.3%) on the olive oil oxidative stability (Ceci & Carelli, 2010b). In the Arbequina oils, both the lowest OLLnR and OSI levels were observed, which can be explained by their lowest O content and highest L content, whereas Coratina oils showed both the highest OLLnR and OSI due to their highest O content and lowest L content (Tables 2 and 3). The content of PPs (determined by the same method used in this study) was ranked second after fatty acid profile, with a contribution of 24.1% (Ceci & Carelli, 2010b). In the case of the oils from the Arbequina and Changlot cultivars, bringing forward the harvest about 15–17 days can be a tool to increase the contents of PPs, and thus improve their oxidative stability (Table 3).

Other authors have reported high oxidative stability when 3,4-DHPEA and its derivatives (3,4-DHPEA-EDA and 3,4-DHPEA-EA) were added to triglyceride matrices of purified olive oil (Baldioli et al., 1996). In a previous paper, we reported the biophenol profile of the oils analyzed in this study, observing for the three cultivars significantly higher 3,4-DHPEA and 3,4-DHPEA-EDA contents in 2013 (Ceci et al., 2017), coincident with the higher oxidative stability observed in this study. This confirms that, in addition to the increase in the total content of PPs, the changes induced in the biophenol profile when the harvest season was brought forward in 2013 also enhanced the oxidative stability of the oils.

As for the tocopherols, β-T was the isomer that contributed as antioxidant in the model designed to estimate the OSI in EVOO, although its input was of only 1.9% (Ceci & Carelli, 2010b). As shown in Table 3, β-T contents varied the most in Arbequina (6–16 mg/kg) and Changlot oils (3–10 mg/kg), and were less scattered in the Coratina oils (6–10 mg/kg). No changes were observed in β-T content for Arbequina and Coratina oils when the harvest was moved forward in 2013, however a significant increase was observed for the Changlot oils (Table 3) which contributes to enhance the oxidative stability of the oils.

The pigments are also involved in auto-and photo-oxidation mechanisms. CHLs are very active in lipid photo-oxidation, but exhibit weak antioxidant activity in darkness. CHLs take part in co-oxidation reactions involving lipid peroxides and the lipoxygenase enzyme (Mínguez-Mosquera et al., 1990). CARs, especially β-carotene, provide efficient protection to the virgin olive oil against photo-oxidation because they absorb light between 400 and 500 nm, and are capable of quenching the singlet oxygen. In a previous study it was found that the total content of CARs contributed by 4.9% to the oxidative stability of EVOO, while the total content of CHLs did not show a statistically significant contribution (Ceci & Carelli, 2010b). In the present work, it was possible to obtain oils from the Arbequina and Changlot cultivars with higher total content of CARs by bringing forward the harvest 15–17 days (Table 3), thus improving their oxidative stability. Taking into account that CARs act as precursors of vitamin A (pro-vitamin A), and that they are among the main components of the carotenoid fraction in EVOO, the increase in the total content of CARs in 2013 could also enhance the nutritional quality of these oils.

4. Conclusions

This work provides information about the chemical quality of EVOO from the San Juan province (Argentina). It was observed that the oxidative stability of the EVOO was strongly dependent on cultivar. The Coratina oils were the most stable, while the Arbequina oils had lower oxidative stability. Bringing forward the harvest date about 15–17 days did not decrease the yield and improved the oxidative stability of the oils. All the analyzed oils had an enhanced fatty acid profile with a higher ratio of O (included between the MUFAs) to L plus Ln (PUFAs). The levels of antioxidant compounds (PPs, β-T, and CARs) also increased depending on the cultivar. Finally, no losses were observed in the nutritional quality of the oils (vitamin E and provitamin A) by bringing forward the harvest. A valuable recommendation for the regional producers would be, therefore, to move forward the harvest season to further improve the chemical and nutritional quality and the oxidative stability of virgin olive oils. In view of its exportation to the most demanding markets, the oil thus obtained would completely conform to the international trade standard in regard to its fatty acid profile.

Acknowledgements

This work was supported by “Agencia Nacional de Promoción Científica y Tecnológica” [ANPCyT, PICTO 105, 2009], “Consejo Nacional de Investigaciones Científicas y Técnicas” [CONICET, PIP 243, 2013], “Universidad Católica de Cuyo” (UCCUYO, PICTO 105, 2009), and “Universidad Nacional del Sur” (UNS, PGI 24M/152, 2017), Argentina. The authors wish to thank F. Delucchi for his kind assistance during the chromatographic analysis.

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