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Relation Between Oxidative Stability and Composition in Argentinian Olive Oils

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Abstract The relation between oxidative stability and composition in 58 virgin olive oils from different cultivars and Argentinian regions was studied over four harvest years. The oxidative stability of the oils was assessed using the OSI index (110 °C, 20 L/h air flow). A multiple linear regression model is proposed using OSI values as the dependent variable (multiple $R = 0.933$, $p = 1 \times 10^{-15}$), with positive contributions of the independent variables: fatty acid composition [oleic acid/(linoleic acid + linolenic acid), 55.3%, $p = 1 \times 10^{-15}$], total polyphenols (24.1%, $p = 1.8 \times 10^{-9}$), carotenes (4.8%, $p = 6.1 \times 10^{-5}$), β-tocopherol (1.9%, 6.0×10^{-3}) and other compounds (13.9%). Highly significant correlation was observed between oxidative stability indexes estimated by the compositional model and those experimentally determined by Rancimat method ($b = 0.981$, $R = 0.924$). Chlorophylls and Δ-5-avenasterol contributions to the model were non-significant when variables related with fatty acids and polyphenols were included. The results suggest that the fatty acid composition and the polyphenol content are the main factors that affect the oxidative stability of olive oils. The proposed model allows the estimation of the oxidative stability in olive oils independently of the cultivar. The model was obtained also taking into account samples that lie out of the international legal limits in some compositional values due to natural variations.

Keywords Olive oil · Oxidative stability · Fatty acids · Polyphenols · Tocopherols · Carotenes · Chlorophylls · Sterols

Introduction

Oxidative rancidity development has been recognized as the predominant cause of oil deterioration during storage [1]. This is a reaction between oxygen and unsaturated fatty acids, regardless of whether they are in their free state or esterified as a triacylglyceride molecule. It is also referred to as autoxidation because the activation energies of the first two reaction steps are very low. Exposure to air, heat, light, trace metals, presence of free-fatty acids and moisture enhance the chemical reactivity.

The two compositional factors that determine oil susceptibility to oxidation are the fatty acid composition and anti- and pro-oxidant minor components. The types of fatty acids present in the oil, and in particular their numbers of double bonds, determine the type and extent of chemical reactions that occur during the storage period. More unsaturated fatty acids, such as C18:2 (linoleic acid) and C18:3 (linolenic acid), are more sensitive to autoxidation than monounsaturated fatty acids (C18:1, oleic acid) [2]. Abundance of oleic acid in olive oils, ranging from 55.0 to 83.0%, is the feature that sets these oils apart from other vegetable oils, inasmuch as to their oxidative stabilities. The composition and structure of the triacylglyceride molecule also contribute to determine the level of the autoxidation reactions [3].

Phenolic compounds, which are considered to be the main antioxidant components in virgin olive oil, are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation, scavenging lipid

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radicals and generating stable radicals [4]. The antioxidant activity of polyphenols is also due to chelating properties of pro-oxidant metals [4]. Extra virgin olive oil hydrophilic extracts contain a large number of phenolic compounds, including simple and complex phenols being the latter the most abundant, such as lignans and secoiridoids [1]. Among simple phenols, olive oils contain *p*-hydroxyphenyl ethanol (*p*-HPEA, tyrosol), 3,4-dihydroxyphenyl ethanol (3,4-DHPEA, hydroxytyrosol) and various phenolic acids [5]. Hydroxytyrosol and tyrosol derivatives and lignans predominate among the complex phenols [5, 6].

The antioxidant activity of the phenols depends on the concentration and its chemical structure [7]. In general, the most positive effects in olive oils are observed in 3,4-dihydroxy and 3,4,5-trihydroxy structures, linked to an aromatic ring that confer to the moiety a higher proton dislocation, thus facilitating the scavenging activity [7]. Another structural feature that may increase the antioxidant capacity is the primary hydroxyl group on the alkyl chains of antioxidants, such as *p*-HPEA and 3,4-DHPEA [7]. High antioxidant activity of 3,4-DHPEA has been demonstrated in olive oil, and the most active secoiridoid derivatives were 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to hydroxytyrosol), *p*-HPEA-EDA (dialdehydic form of elenolic acid linked to tyrosol), and 3,4-DHPEA-EA (oleuropein aglycone) [8]. In recent years, some pharmacological effects other than antioxidant capacity have been reported for olive oil phenols [9].

Olive oils also contain lipophilic phenolic compounds (tocopherols). α-Tocopherol is the major isomer representing more than 90% of total tocopherol content [1]. Tocopherols can act as antioxidants by two primary mechanisms, a chain-breaking donor mechanism, in which they donate their phenolic hydrogen atom to lipid free-radicals and a chain-breaking acceptor mechanism, which includes singlet oxygen scavenging or quenching; this inhibits the oxidations induced by electronically excited singlet oxygen [1]. It appears that polar phenolic compounds are more important for the inhibition of autoxidation in olive oils in the initial stages, while α-tocopherol becomes effective when primary products of autoxidation (hydroperoxides) reach a critical concentration [10]. α-Tocopherol acts as an antioxidant or pro-oxidant depending on the concentration of hydrophilic phenols [11]. It has the highest biological activity among the tocopherol isomers [12]. However, the following sequence based on their in vitro antioxidant activity can be established: $\delta > \gamma > \beta > \alpha$ [12]. Argentinian olive oils contain considerable amounts of α-tocopherol (>160 mg/kg) and in some samples measurable quantities of *β*- and *γ*-tocopherols [13].

Olive oil color is a consequence of an appropriate combination between green chlorophylls and orange and brown carotenes, also distinguishing olive oil from other vegetable oils. Carotenes have been found to be potent protectors against photo-oxidation acting as singlet oxygen quenchers, while chlorophylls and their derivatives have pro-oxidant activity due to their ability to transfer energy from light into chemical molecules [14].

Among sterols, Δ-5-avenasterol was effective as an antioxidant, while stigmasterol and cholesterol were ineffective for reducing thermal oxidation of a triacylglyceride mixture similar in composition to olive oil [15]. It was concluded that lipid free radicals react rapidly with sterols at unhindered allylic carbon atoms, leading to relatively stable allylic tertiary free radicals and interrupting the autoxidation chain [15].

Since the Rancimat method is standardized and results are obtained within a short period of time, this technique is one of the most widely used to measure the oil oxidation resistance under accelerated conditions (high air flow and high temperature). The induction time determined by a Rancimat apparatus, known as oxidative stability index (OSI), correlates well with peroxide development during oil storage at room temperature [16]. The method provides useful data on the susceptibility to oxidation under the same operating conditions in oils of high stability, such as olive oil matrices containing antioxidants [3]. An equation has been proposed in order to extrapolate OSI to storage conditions under the assumption that autoxidation is the only relevant decomposition process during storage and temperature dependence is constant [17]. However, the relationship is weak since the mechanism of lipid oxidation changes significantly at elevated temperature [3].

In this work, the relation between OSI and composition from Argentinian extra-virgin olive oil samples obtained from different cultivars and geographic regions was evaluated. In addition, the compositional ranges of fatty acids, tocopherols and sterols, as well as, contents of total polyphenols, chlorophylls, and carotenes are reported.

Experimental Procedures

Olive Oils

Fifty-eight samples of extra virgin olive oils from Arbequina, Barnea, Picual, Arauco, Coratina, Empeltre, Farga, Frantoio and Manzanilla cultivars, from the 2004, 2005, 2006 and 2008 harvests were analyzed. They were provided by small and medium enterprises that in general used two-phase continuous decanters for oil extraction. Only three samples from Córdoba and five samples from La Rioja (mountainous region) were extracted employing a three-phase decanter continuous system. Table 1 shows data about cultivar, origin (latitude and altitude), olive-tree age, olive ripeness index and processing data (beating

Table 1 Cultural and processing data of various olive cultivars grown in different growing environments

Origin[a]	Cultivar	Harvest	Oil numbers	OTA	RI	BTE	BTI
Catamarca							
Central Valley (28.6°S, 525 m)	Arbequina	2004	2	5–6	3.2–3.9	29–35	NP-90
		2005	1	8	3.2	27	90
		2006	1	9	3.9	26	90
	Barnea	2004	2	5–7	3.2–3.4	28–32	NP-90
		2005	1	8	3.8	26	90
		2006	1	9	4.5	31	90
	Picual	2004	2	5–6	3.3–3.5	28–31	NP-90
		2005	1	7	3.1	24	90
		2006	1	8	5.0	24	90
Mountain (28.1°S, 900–1,500 m)	Arbequina	2005	1	3	NP	33	120
	Coratina	2005	1	3	NP	33	120
La Rioja							
Capital Valley (29.4°S, 523 m)	Arbequina	2004	3	5–9	3.1–4.2	30–38	25–60
		2005	2	8–10	NP-2.5	28–32	NP-60
		2006	2	9–11	2.8–3.5	32–33	60
	Barnea	2004	1	6	4.6	33–35	60
		2006	1	9	2.7	24	45
	Picual	2004	1	5	4.2	33–38	60
	Frantoio	2004	1	6	3.9	33–35	60
		2005	1	10	2.4	32	60
		2006	1	11	2.4	33	60
	Arauco	2004	1	9	3.4	34	75
		2006	1	NP	2.7	30	60
	M. Californiana	2004	1	8	4.3	31	45
		2005	1	9	NP	32	NP
		2006	1	10	3.3	30	60
	M. Criolla	2004	1	9	3.2	33	60
		2005	1	10	2.5	32.5	60
		2006	1	11	3.6	32	60
	Coratina	2006	1	8	1.3	27	45
Mountain (29.5°S, 840 m)	Arbequina	2005	2	7	1.3–2.0	30–35	27–60
		2006	1	8	2.0	30	30
		2008	1	8	3.0	<37	40
	Barnea	2005	1	7	3.0	36	60
		2006	1	NP	NP	33	30
	Picual	2005	2	8–12	3.2–3.7	30–35	27–60
	Empeltre	2005	1	10	3.6	30	27
	Frantoio	2005	1	8	3.2	36	60
Córdoba (30.5°S, 500 m)	Arbequina	2004	3	50	G–E–R	29	45
San Juan (32°S, 600 m)	Arbequina	2008	1	9	3.1	<37	40
Mendoza (35°S, 700 m)	Arbequina	2004	1	4–5	4.5	31	30–60
	Empeltre	2004	1	60	4.8	31	30–60
Atlantic Coast							
Buenos Aires (38°S, sea level)	Arbequina	2008	2	50	4.0–5.0	30–33	60–75
	Farga	2008	3	50–60	3.0–5.0	30–33	75–90
Río Negro (41°S, sea level)	Arbequina	2008	1	6	3.0	27	<35

OTA olive tree age (years), *RI* ripeness index (proposed by IOC, based on the color of the skin and flesh of the fruits, ranging 0–7), *BTE* beating temperature (°C), *BTI* beating time (min), *NP* not provided, *M.* Manzanilla, *G* green, *E* envero = semi-ripe, *R* ripe

[a] Latitude and altitude are indicated within parenthesis

temperature and beating time) for analyzed samples. Samples came from regions with a wide range of latitude (28°S–41°S) and altitude (sea level to 1,200 m above sea level) characterized by diverse climate conditions. High temperatures (up to 45 °C in summer), low thermal amplitudes within days and seasons and dry climate distinguish Catamarca and La Rioja valleys, while mountainous regions located at the same latitude present lower medium temperatures and higher rainfall and thermal amplitudes. San Juan and Mendoza provinces with an average altitude of 600 m also have very dry summers and very low rainfall, but remarkable thermal amplitude and lower average temperatures than Catamarca and La Rioja Provinces. Córdoba samples were from 50 old olive trees cultivated in its North Western Region with a highland subtropical climate and annual rainfall of 580 mm. In 2008, some oils from the coast Atlantic (old olive trees from Coronel Dorrego, Buenos Aires province, and new olive trees from Las Grutas, Río Negro province) were included. The Argentinian coast of the Atlantic Ocean has a colder and wetter climate than intra-continental regions. The oil samples were stored at 5 °C under a nitrogen atmosphere and light protected until they were analyzed.

Analytical Methods

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 air flow. Phenolic compounds were removed by three extractions of oil-in-hexane solution with 60% aqueous methanol (3 × 20 mL). The extract obtained was evaporated to dryness at 40 °C under a vacuum and the residue was diluted with methanol. The content of phenolic compounds, as mg/kg of caffeic acid, was determined spectrophotometrically at 725 nm, using Folin-Ciocalteu reagent [18].

Chlorophyll and carotene contents were evaluated from the maximum of absorption for the oil solution in cyclohexane at 670 and 470 nm, respectively, using absorptivities from the literature [19].

Tocopherols were evaluated by HPLC according to AOCS method Ce 8-89 [20]. 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/Doc. No. 24 standard method [21]. Fatty-acid methyl esters (FAME) were analyzed by GLC according to the Ce 1e-91 AOCS method [20]. The FAME were separated on a SP2380 capillary 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, USA) maintained at 170 °C for 15 min, then increased at 4 °C/min to 210 °C for 10 min; using hydrogen as the carrier gas.

Sterol contents were determined by GLC following COI/T.20/Doc. No. 10/Rev. 1 IOC method [22]. 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 was removed with ethyl ether. Then, it was separated by chromatography on a basic silica-gel plate using 65:35 (v/v) a hexane/ethyl ether mixture as developing solvent. After the development, the plate was sprayed with 2,7-dichlorofluorescein solution and observed under ultraviolet light to identify the sterol band. The sterols recovered from the silica gel were transformed into trimethyl-silyl ethers. 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 during 2 min, followed by 1 °C/min change to 265 °C, and then a 20 min hold at 265 °C; injector temperature = 280 °C; FID temperature = 300 °C; injection volume = 1 μL, split injector with 1:90 splitting ratio, and the carrier gas was hydrogen at 37 cm/s.

Statistical Analysis

The analyses were carried out in triplicate, except for the fatty acid determinations that were performed in quadruplicate. Systat for Windows program (5.0 Version, Systat Inc.) was used to perform multiple linear regression analyses. Oxidative stability indexes (OSI, in hours) were used as the dependent variable (n = 58). Parameters related with fatty acids, polyphenols (PP in mg/kg), and compositions of pigments, tocopherols and sterols were used as independent variables to estimate OSI values. More relevant fatty acids and some relations between them, such as monounsaturated/polyunsaturated, oleic/(linoleic + linolenic) [OLLnR] were assayed to fit into the OSI model. The significance of some parameters related with the oil pigmentation such as carotene (CAR) and chlorophyll (CHL) contents (in mg/kg) was studied in order to include them inside the OSI model. The contents of total tocopherols, and alpha, beta (BT, in mg/kg), and gamma isomers were also evaluated to adjust them to the model. Total sterol content, the major sterols, apparent sitosterol percentage, and some relations between sterols, such as Δ-5-avenasterol/stigmasterol (ASR) were also assayed with respect their adjustments to the proposed model. A forward stepwise method was used to multiple linear regressions

selecting the variables that increased multiple R when they were fitted into the model [23]. The values p-to-enter and p-to-remove new variables into statistical model were $p \le 0.01$ and $p > 0.01$. The contribution of each variable into the model was determined from the increase per cent in adjusted squared R observed when each variable was accepted into the OSI model.

Results and Discussion

OSI values and the contents of the most relevant fatty acids and total polyphenols are shown in Table 2. The pigment content, tocopherol composition and the content of total sterols, Δ-5-avenasterol and stigmasterol are presented in Table 3. The compositional characteristics of Argentinian olive oil from new productive zones, during 2004 and 2005 harvests, and their comparison with international limits were discussed previously [13]. The Arbequina oils from intra-continental hot regions located in Catamarca and La Rioja provinces had a low oleic acid content and high contents of palmitic, palmitoleic and/or linoleic acids, high C40–C46 wax content, a high campesterol percentage and, in some samples, low apparent sitosterol percentage, with respect to IOC and Codex limits [13]. However, the oils produced on Atlantic Coast fulfilled the international standards [24].

From the analysis of all parameters related with fatty acid composition, the most highly significant linear regression was observed between OSI and OLLnR ($R = 0.749$, $p = 1.3 \times 10^{-11}$). OLLnR measures the relation between oleic acid, less susceptible to oxidative damage, and (linoleic + linolenic) acids, which are more sensitive to oxidation. Other authors previously concluded

Table 2 Oxidative stability, fatty acids and polyphenols of olive oils obtained from various growing regions

Origin	Cultivar	OSI (h)	Fatty acids (% of oil)			OLLnR	PP (mg/kg)
			C18:1	C18:2	C18:3		
Catamarca							
Central Valley	Arbequina	6.6–11.7	40.6–53.2	19.3–26.6	0.86–1.03	1.47–2.64	34–44
	Barnea	7.7–9.9	56.0–61.1	19.4–22.4	0.90–0.99	2.40–3.01	43–75
	Picual	9.5–17.0	57.8–69.1	7.7–16.5	0.81–1.10	3.30–8.00	22–67
Mountain	Arbequina	8.4–12.1	54.3–55.3	19.0–19.8	0.67–0.83	2.70–2.80	46–102
	Coratina	19.2	68.7	14.3	0.75	4.60	263
La Rioja							
Capital Valley	Arbequina	7.4–14.8	49.5–64.2	14.1–22.9	0.55–1.00	2.07–4.40	35–87
	Barnea	5.6–9.7	60.5–60.9	19.1–19.7	0.87	2.96–3.04	53–82
	Picual	14.7	68.2	9.7	1.10	6.30	36
	Arauco	13.9–14.4	53.7–58.4	17.5–20.0	0.97–1.04	2.56–3.16	165–169
	Coratina	18.8	69.4	11.7	0.82	5.56	332
	Frantoio	9.3–11.0	62.4–63.4	14.8–15.8	0.85–1.06	3.75–4.00	65–121
	M. Californiana	9.5–12.4	59.9–62.6	13.5–15.2	1.01–1.12	3.68–4.30	42–48
	M. Criolla	18.6–23.3	70.0–75.8	4.9–8.2	0.76–1.07	7.54–13.40	73–137
Mountain	Arbequina	9.3–18.1	53.8–59.4	16.5–20.0	0.61–0.79	2.60–3.50	47–158
	Barnea	11.5–11.6	64.6–65.4	16.1–16.3	0.71–0.84	3.80–3.90	65–131
	Picual	18.5–22.8	72.3–73.5	6.2–6.3	0.75–0.76	10.30–10.50	44–93
	Empeltre	12.7	70.6	10.1	0.77	6.50	25
	Frantoio	9.4	63.4	14.2	1.13	4.10	32
Córdoba	Arbequina	7.3–8.5	52.6–53.6	19.5–21.2	0.87–0.92	2.39–2.63	29–36
San Juan	Arbequina	8.6	57.9	17.6	0.75	3.15	41
Mendoza	Arbequina	13.1	63.3	15.5	0.55	3.93	90
	Empeltre	12.2	75.2	8.4	0.72	8.30	53
Buenos Aires	Arbequina	14.1–17.3	71.7–72.0	10.6–10.7	0.69	6.29–6.39	63–90
	Farga	20.0–25.7	71.4–72.6	8.7–10.5	0.80–0.84	6.32–7.62	167–188
Río Negro	Arbequina	27.9	68.7	11.4	0.79	5.62	248
VC (%)		≤7.9	≤0.6	≤4.8	≤14.2	≤5.1	≤13.7

OSI oxidative stability index, *OLLnR* oleic/(linoleic + linolenic) ratio, *PP* polyphenols, *M.* Manzanilla, *VC* variation coefficients

Table 3 Pigments, tocopherols and most significant sterols of olive oils obtained from various growing regions

Origin	Cultivar	CHL (mg/kg)	CAR (mg/kg)	Tocopherols (mg/kg)			Sterols (mg/kg)			ASR
				α	β (BT)	Total	A	S	Total	
Catamarca										
Central Valley	Arbequina	1.0–8.2	0.9–4.2	310–398	8–23	324–413	69–86	23–42	2,007–2,699	1.52–3.50
	Barnea	1.3–6.5	1.5–2.9	321–379	0–5	321–379	71–116	21–26	2,358–2,546	3.24–5.22
	Picual	0.8–7.0	0.9–2.8	282–307	0–11	291–332	78–180	20–34	1,889–2,584	2.39–7.22
Mountain	Arbequina	3.1–9.6	2.8–4.3	243–281	0–9	252–281	140–144	17–24	1,869–2,321	5.90–8.58
	Coratina	4.3	3.6	396	11	437	63	6	1,053	10.59
La Rioja										
Capital Valley	Arbequina	1.6–4.5	1.6–4.0	226–428	8–27	234–463	69–173	15–35	1,780–3,004	2.4–11.27
	Barnea	0.6–1.4	1.0–1.2	247–270	0–3	254–275	98–114	13–21	2,245–2,265	4.80–9.02
	Picual	2.8	3.4	301	8	348	150	20	2,444	7.43
	Arauco	4.1–5.1	4.3–4.4	313–335	3–8	343–377	115–123	15–18	1,545–1,716	6.97–7.92
	Coratina	6.5	4.2	379	0	389	49	11	1,381	4.51
	Frantoio	6.7–14.1	4.2–7.3	281–409	0–4	289–432	83–107	16–17	1,881–2,187	5.22–6.12
	M. Californiana	4.2–8.9	2.5–3.7	180–260	7–21	212–274	81–92	31–43	2,261–2,417	1.89–2.98
	M. Criolla	3.0–4.6	2.5–3.7	221–315	4–8	234–328	137–189	14–18	1,852–2,367	8.05–11.40
Mountain	Arbequina	4.6–10.2	2.8–6.6	252–275	0–9	252–284	113–148	14–25	1,762–2,460	4.46–10.47
	Barnea	4.8–5.6	2.7–2.9	209–259	0–5	218–264	95–113	10–13	1,886–2,323	7.55–11.30
	Picual	9.4–14.7	6.1	264–324	6–9	291–340	111–140	15–16	1,883–2,284	7.72–8.63
	Empeltre	4.8	2.5	283	10	299	145	21	2,007	6.87
	Frantoio	9.0	4.4	311	4	322	126	18	2,226	7.03
Córdoba	Arbequina	2.7–5.6	2.4–2.9	276–317	13–17	289–334	96–118	18–25	1,909–2,403	3.94–5.73
San Juan	Arbequina	4.1	4.0	287	0	287	212	22	2,192	9.70
Mendoza	Arbequina	1.4	2.0	186	3	189	209	11	1,424	19.49
	Empeltre	1.0	2.0	161	2	167	149	15	1,336	9.87
Buenos Aires	Arbequina	6.5–8.9	5.4–6.6	233–245	2	235–247	193–209	23	1,634–1,642	8.32–9.16
	Farga	3.6–10.1	3.7–7.4	248–275	0	248–275	75–99	10–15	1,473–1,537	6.78–7.79
Río Negro	Arbequina	25.6	14.5	283	4	287	158	10	1,689	16.59
VC (%)		≤17.0	≤12.0	≤9.5	≤25.0	≤9.7	≤9.1	≤14.1	≤3.9	≤25.7

CHL chlorophylls, *CAR* carotenes, *A* Δ-5-avenasterol, *S* stigmasterol, *ASR* Δ-5-avenasterol/stigmasterol ratio, *M.* Manzanilla, *VC* variation coefficients

that oxidation principally affects the most unsaturated fatty-acids in olive oils [25–27]. Salvador et al. [26] proposed the use of the following modified ratio (C18:2 + nC18:3)/C18:1 (MLLnOR), where the factor n indicates the difference in the velocity of the autoxidation reaction of the two polyunsaturated fatty acids, whereby $n = 2$ was an adequate value. When the mentioned ratio was tested in the present study, no significant modification were found in the linear regression between OSI and MLLnOR ($R = 0.755$), and in other components contributions to the model.

The PP, CAR, and BT variables presented a highly significant contribution ($p \leq 0.01$) into the OSI model. Table 4 shows the results for multiple linear regression analysis and the estimated coefficients for the significant variables into the OSI model. The independent term (constant) was not significant and the multiple R was 0.933. Polyphenols have been the most studied antioxidant compounds in olive oils and numerous studies have demonstrated the effect of total hydrophilic phenols content on the oxidative stability of olive oil [5, 25, 28–30]. When only pigment composition was considered, the best results were obtained with contents of chlorophylls and carotenes as independent variables [$\mathrm{OSI} = 1.2\times10^{-8} - 0.669 \times \mathrm{CHL} + 2.630 \times \mathrm{CAR}$, multiple $R = 0.646$, p (CHL) = 0.040, and p (CAR) = 7.2×10^{-5}]. The inclusion of other variables into model to estimate OSI values by means multiple linear regression, such as fatty-acid composition (OLLnR) and polyphenol content (PP), lead to non significant p values for chlorophylls (CHL).

In olive oils purified by silicic acid column chromatography to remove non-triacylglyceride components, the

Table 4 Multiple linear regression analyses

Dependent variable = OSI (h)
$n = 58$
Multiple $R = 0.933$

Standard error of estimate = 1.913
ANOVA, $p = 1 \times 10^{-15}$ (**)

Independent variable	Coefficient	Standard error	Adjusted $R^{2\ a}$	Significance (two-tails p)
Constant	1.517	0.759		0.051 (NS)
OLLnR	1.254	0.105	0.553	1.0×10^{-15} (**)
PP (mg/kg)	0.034	0.005	0.794	1.8×10^{-9} (**)
CAR (mg/kg)	0.602	0.138	0.842	6.1×10^{-5} (**)
BT (mg/kg)	0.124	0.043	0.861	6.0×10^{-3} (**)

OLLNR oleic/(linoleic + linolenic) ratio, *PP* polyphenols, *CAR* carotenes, *BT* β-tocopherol, *NS* not significant regression at 1% level

[a] It refers to progressive addition of each component into the model

** Highly significant regression ($p \leq 0.01$)

chlorophylls had a photosensitizing effect, while β-carotenes minimized lipid oxidation under light storage [31]. Carotenes and chlorophylls contributed 6 and 4%, respectively, to the oxidative stability of olive oils [25]. The losses of pigments in olive oils under accelerated oxidation at 100 °C were 67% for carotenes and 58% for chlorophylls [17].

After the inclusion of OLLnR, PP and CAR, the best highly significant variable related to tocopherols into the OSI model was β-tocopherol content (multiple $R = 0.933$, Table 4). Other variables fitted to the model have shown lower multiple regression coefficients and their inclusion was not significant, i.e. total tocopherols (multiple $R = 0.924$, $p = 0.231$), α-tocopherol (multiple $R = 0.923$, $p = 0.347$), and γ-tocopherol (multiple $R = 0.922$, $p = 0.701$). Variables including partial sums or relations between tocopherols, such as (β + γ) tocopherols having higher antioxidant activity in vitro (multiple $R = 0.926$, $p = 0.09$), and equivalent tocopherol (multiple $R = 0.924$, $p = 0.281$), did not significantly contribute to the OSI model. Equivalent tocopherol (ET) relates to the biological activities of vitamin E and can be estimated as ET = 1.10 × α-tocopherol + 0.75 × β-tocopherol + 0.25 × γ-tocopherol + 0.25 × δ-tocopherol. All assayed tocopherol variables had positive coefficients in the OSI model demonstrating their behavior as antioxidants. Significant linear regression has been reported between induction times by the Rancimat method and the contents of total tocopherols [25, 27] and α-tocopherol [3, 26, 32], but no correlation with the γ-tocopherol content in virgin olive oils [25]. Negative [3, 27] and positive [25, 26, 32] regression coefficients have been obtained for tocopherols. In extra virgin olive oils under accelerated oxidation at 60 °C and dark, *o*-diphenols diminished by the highest rate, followed by α-tocopherol [33]. When olive oils were stored at room temperature for 21 months, there was a slight linear fall in α-tocopherol content, although there may be a short lag phase at the beginning of the assay [34]. In the olive oils evaluated in our study, the regression analysis between OSI and tocopherols as the only independent variable showed low regression coefficients ($R = 0.127$–0.178), but revealed a sequence for the isomers that coincides with their in vitro antioxidant activities ($\gamma > \beta > \alpha$). When fatty acid composition (OLLnR) and tocopherols were fitted into the OSI model, the best coefficients ($R = 0.758$) were obtained for variables related with biological activity (total tocopherols, α-tocopherol and equivalent tocopherol). When polyphenols (PP) were included as a new model variable, the best R value corresponded to β-tocopherol and it was highly significant ($R = 0.908$, $p = 0.01$). A synergistic effect of α-tocopherol on the polyphenol antioxidant activity has been demonstrated [35] and it could be in part responsible for the loss of regression for α-tocopherol, when PP entered into the OSI model. Moreover, the most significant regression for β-tocopherol content that was observed in our compositional model may be due to major in-vitro antioxidant activity and higher differentiation between the samples registered for this isomer that ranges from 0 to 27 mg/kg.

Negative linear regressions were obtained between OSI values and the contents of campesterol (CAMP, $R = -0.660$, $p = 1.7 \times 10^{-8}$), stigmasterol ($R = -0.536$, $p = 1.4 \times 10^{-5}$), β-sitosterol ($R = -0.519$, $p = 2.9 \times 10^{-5}$), and total sterols ($R = -0.525$, $p = 2.3 \times 10^{-5}$). The only sterol which had a positive regression coefficient was Δ-5-avenasterol, but it was not significant ($R = 0.178$, $p = 0.181$); however, Δ-5-avenasterol/stigmasterol ratio (ASR) had a highly significant regression and higher R than Δ-5-avenasterol content ($R = 0.532$, $p = 1.7 \times 10^{-5}$). Other sterols (cholesterol, brassicasterol) and sterol ratios had lower R-values or non significant regressions with OSI values.

When all compositional parameters except sterols were fitted into the model for OSI (multiple $R = 0.933$), only the

inclusion of variables related with Δ-5-avenasterol contributed increasing R value up to 0.936, although these contributions were not significant [p(ASR) = 0.104, p(β-sitosterol/Δ-5-avenasterol) = 0.150]. For this reason, none of the variable related with sterols was included in the compositional model proposed to estimate OSI values. Previous studies carried out by other authors evaluated the oxidative-stability indexes by using a model system enriched with the total-sterol fractions of extra-virgin olive oils, and no correlation was found between the OSI time and the added amount of sterols [36].

The values of adjusted squared multiple R (Table 4) were used to estimate the following contributions to the oxidative stability model: 55.3% for fatty-acids (OLLnR), 24.1% for total polyphenols (PP), 4.8% for carotenes (CAR), 1.9% for β-tocopherol (BT), and 13.9% for other compounds. Previous researchers gave contradictory results. While some authors established a contribution to oxidative stability of 30% for phenols, 21% for *o*-diphenols (21%) and 24% for fatty acids [25], others found 73.57 and 8.34% for fatty acid and total phenols contributions, respectively [27]. Some Argentinian olive oils, principally those from Arbequina produced in hot valleys of La Rioja and Catamarca, have oleic acid contents lower than 55.0% and linoleic acid contents higher than 21.0%. Oils from other cultivars, such as Frantoio and Manzanilla, from La Rioja and Catamarca have linolenic acid contents slightly higher than the IOC limit (1.0%). Moreover, Argentinian olive oils from the Arbequina cultivar are not characterized by high polyphenol contents [13]. The higher incidence of fatty acid composition and lower influence of polyphenol content in the OSI model was likely due to the low oleic acid, high polyunsaturated acids and low phenolic contents.

Highly significant linear correlation was observed between OSI values estimated by the proposed model and experimentally determined by Rancimat analysis (Fig. 1, estimated OSI (h) = 0.981 × experimental OSI (h), $R = 0.924$).

It can be concluded that the oxidative stability in olive oils is highly influenced by their fatty acid composition and the level of their natural components, principally polyphenols with antioxidant properties. Minor relevance was observed for carotenes and β-tocopherol as antioxidants. The proposed model also constitutes an estimate of the overall oxidative stability taking into account the synergistic and opposite effects of all components in the unsaponifiable fraction, and not considering the isolated individual compounds. Moreover, the model has been developed for different olive cultivars and productive regions including samples that did not fulfill the international norm due to natural variations.

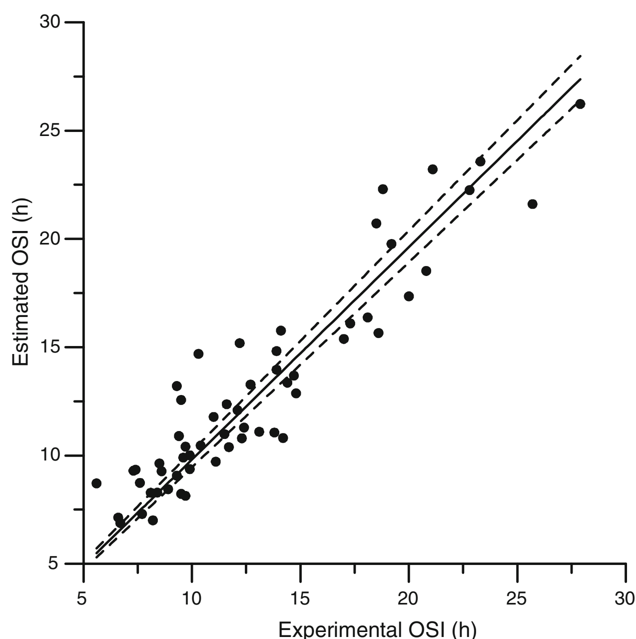

Fig. 1 Linear correlation between OSI values estimated by compositional model and experimentally determined. Estimated straight line ± confidence interval (95%) is indicated

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