J Am Oil Chem Soc (2017) 94:643–654
DOI 10.1007/s11746-017-2985-z

ORIGINAL PAPER

Biophenols and Flavor in Extra Virgin Olive Oils from San Juan Province (Argentina)

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Received: 12 September 2016 / Revised: 14 February 2017 / Accepted: 30 March 2017 / Published online: 7 April 2017

Abstract This work studied phenolic compounds and their relationship with flavor in extra virgin olive oils from Arbequina, Changlot Real, and Coratina cultivars from San Juan province (Argentina) in the 2012 and 2013 harvests. In 2013, the harvesting was brought forward by 15–17 days. The total and individual biophenols were analyzed by HPLC–UV. A taste panel recognized by the International Olive Council (IOC) carried out the sensory analysis. Oxidative stability was measured by accelerated oxidation assays using Rancimat equipment. The oils obtained in 2013 from the three cultivars had higher biophenol contents and particularly higher contents of secoiridoids and derivatives. The positive attributes (fruitiness, bitterness, and pungency) of the oils were more harmonious in the three cultivars in 2013 by enhancing the complexity with a wide range of green descriptors. The oxidative stability indices of the oils were also higher in the three cultivars in 2013. The multiple linear regression model suggested that the aldehyde and hydroxylic form of oleuropein aglycone (3,4-DHPEA-EA) was the main contributor (65.5%) to bitterness, while the dialdehyde form of decarboxymethyl ligstroside aglycone (*p*-HPEA-EDA), besides reducing bitterness, was the major contributor to pungency (52.2%). Among simple phenols, *o*-coumaric acid contributed to bitterness (2.3%), and tyrosol (6.3%) and tyrosyl acetate (3.3%) were related to pungency.

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Keywords Biophenols · Olive oil · Bitterness · Pungency · Secoiridoids

Introduction

In the last 10 years, the olive oil production of Argentina has notably increased (25,000 t in 2015) and a large proportion of the oil (17,500 t) has been directed to exportation [1]. In the digital crop survey of 2015, there were 18,200 ha cultivated with olive in the oasis of San Juan province [2]. In the 2000/2009 decade, the volume of olive oil exported from San Juan increased by 227%, and during that period, the participation of the province represented about 15% of total Argentine olive oil exports [3]. Owing to its particular soil and climate characteristics and the excellent quality of its irrigation water originating in the thawing of the high mountains of the Cordillera de los Andes, the olive oils from San Juan province are characterized by their excellent quality. A compositional data review done in 2010 revealed a small number of characterization studies performed in monovarietal Argentinian olive oils [4]. Therefore, there is great interest in increasing studies to typify, characterize, and optimize the quality of these oils that are commercialized not only regionally but also towards member countries and non-member states that adopt the directives of the International Olive Council (IOC). Further, studies on the contents and composition of biophenols in virgin olive oils (VOO) as well as their sensory profile will contribute to the knowledge of the quality of oils that are available on the international market.

A distinctive feature of VOO over other vegetable oils is that the former contains biophenols, which are considered nutraceuticals. Moreover, it is well known that the content of phenolic compounds is an important parameter in the

evaluation of VOO quality because phenols largely contribute to oil flavor and aroma, protect it from oxidation, and confer health properties [5–7]. Nowadays, there is a lack of studies to establish the phenolic composition in monovarietal Argentinian olive oils, and specifically in those from San Juan province.

Phenolic compounds of VOO belong to different classes: simple phenols and derivatives, secoiridoids and derivatives, lignans and flavonoids [5]. Simple phenols and derivatives in VOO include phenyl alcohols, namely 3,4-dihydroxyphenylethanol (3,4-DHPEA or hydroxytyrosol) and *p*-4-hydroxyphenylethanol (*p*-HPEA or tyrosol) as well as phenolic acids. Secoiridoids are coumarin-like compounds related to the iridoids (precursors of indole alkaloids). The addition of water to olive paste and the effect of endogenous enzymes (glycosidases and esterases) during crushing and malaxation result in glycoside degradation with the subsequent formation of various secoiridoid derivatives [6]. The major secoiridoid compounds of VOO are derivatives of 3,4-DHPEA, in particular the dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), an isomer of oleuropein aglycone (3,4-DHPEA-EA), the dialdehydic form of elenolic acid linked to *p*-HPEA (*p*-HPEA-EDA) and the ligstroside aglycone (*p*-HPEA-EA). The lignans pinoresinol and 1-acetoxypinoresinol were also found in VOO [8]. Lignans are not present in the pericarp of the olive drupes or in the leaves and twigs, but they are in the olive pits—pinoresinol being their major component [8]. Flavonoids are secondary metabolites widespread in the vegetable kingdom, and their aglycones can be subdivided into flavones, flavonols, flavanones, and flavanols. Two flavones, luteolin and apigenin, are present in VOO [5]. In addition, the polar phenol content in olive oils varies in quantity and quality, being closely related to the cultivar, agricultural techniques used, soil composition, climate, ripening stage of the olive drupes at harvest, processing techniques, and storage [9].

The objective of this work was to contribute to a deeper knowledge of extra virgin olive oils (EVOO) from San Juan province of Argentina by evaluating their total and individual contents of biophenols and their sensory profile. In addition, the influence of the cultivar, the anticipation of the harvest dates based on the biophenol profiles and organoleptic characteristics, as well as the contribution of the different biophenols to the bitterness and pungency were considered.

Experimental Procedures

Materials

Thirty samples of olive fruits from Arbequina, Changlot Real, and Coratina cultivars were harvested in four departments (25 de Mayo, Sarmiento, Zonda, and Ullum) located in San Juan province (Argentina). The coordinates of these departments range from 31 to 32°S and from 68 to 69°W and their altitudes above sea level are 555–785 m. In 2012, the fruits were harvested at the beginning of the harvest period (late April–early May) and at the end of the season (late May–early June). The maturity index of the fruits was 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 (Table 1). In 2013, to reduce the maturity indices and avoid processing overripened olives, the harvest time was brought forward by 15–17 days, reaping the fruits in early April and early May. The maturity indices were Arbequina (5 samples) = 1.62–3.27, Changlot Real (6) = 2.53–3.33, and Coratina (4) = 0.33–1.36 (Table 1). About 100 kg of fruits was processed using OLIOMIO two-phase equipment to extract the oil (temperatures and beating times = 20.0–27.5 °C and 40 min, respectively).

Methods

Chemical Analysis

Classical quality indices such as free acidity, peroxide value, and specific extinctions in the UV were determined according to IOC regulations [10]. The oxidative stability index (OSI) was determined using a Metrohm 679 Rancimat apparatus (110 °C, airflow = 20 L/h). Total polyphenols (milligrams of caffeic acid per kilogram of oil) were determined spectrophotometrically at 725 nm according to the Folin–Ciocalteu (F–C) method [11].

Biophenols were extracted and quantified by HPLC with the aid of a UV detector at 280 nm according to the IOC method (COI/T.20/Doc. No. 29, 2009) [12]. The individual biophenols were identified by their relative retention times (IOC method) and by comparison with those from available commercial standards. Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid, ≥95%) and tyrosol [2-(4-hydroxyphenyl)ethanol, 98%] were provided by Sigma-Aldrich (Gillingham, England). The following standards were used for peak identification: caffeic acid (≥99%), vanillin (99%), *p*-coumaric acid (≥98%), *trans*-ferulic acid (99%), *trans*-cinnamic acid (97%), pinoresinol (≥95%), luteolin (≥98%), and apigenin (≥95%) from Sigma–Aldrich, and vanillic acid (≥97%) from Fluka. The peak numbers with the names of the corresponding compounds (footnotes, Tables 1, 2) are the same as those given in the IOC method. All the results are expressed as milligrams of tyrosol equivalents per kilogram of oil and are averages of two replicates injected twice into the liquid chromatograph. Luteo lin and apigenin were also quantified at 335 nm by using

Table 1 Total polyphenols by Folin–Ciocalteu method, total biophenols and simple phenols and derivatives by HPLC–UV at 280 nm for the EVOO samples

	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**
Total polyphenols (mg caffeic acid/kg)									
	70–158a	129–194b**	A	39–157a	125–364b**	B	362–657a	394–487a	C**
Total biophenols (mg tyrosol eq./kg)									
	109–232a	170–313b**	A	64–207a	154–530b**	B	458–611a	519–712b**	C**
Peak No.	Simple phenols and derivatives (mg tyrosol eq./kg)								
P1	0.2–2.8a	1.7–5.0b**	A	0.4–15.7a	3.9–14.9b*	B	3.6–7.5a	10.2–22.2b**	C**
P2	1.5–3.8a	1.4–2.7a	A	0.5–23.2b	3.2–10.5a**	B	4.5–12.3a	6.2–11.1b*	B**
P3	2.1–4.7a	1.8–4.5a	C	0.4–1.7a	0.5–2.1a	A	1.1–2.5a	0.8–2.2a	B**
P4	0.1–0.6b	TR–0.2a**	C	0.1–0.2a	TR–0.2a	B	0.04–0.1a	TR–0.2b*	A**
P6	0.3–2.6a	0.7–1.4a	A	0.6–3.3b	0.5–2.5a*	B	0.4–3.7a	0.6–1.2a	A*
P7	1.7–6.4a	2.2–6.3a	B	0.1–1.2a	0.9–4.9b**	A	0.8–2.2a	1.0–3.0a	A**
P8	2.0–9.7a	2.9–16.5a	B	0.2–1.5b	0.2–0.4a**	A	0.6–0.9b	0.1–1.0a**	A**
P9	1.1–2.7a	0.7–4.2a	A	1.2–3.3a	2.5–4.2b**	A	2.9–11.8b	1.0–3.1a**	B**
P10	0.2–0.5a	0.2–0.9a	A	0.5–17.6b	0.2–0.7a**	B	0.1–2.9b	0.1–0.9a*	A*
P15	0.1–0.8a	0.6–1.3b**	A	0.2–0.5a	0.7–1.7b**	A	0.3–0.8a	0.8–1.0b**	A
P19	0.6–1.2a	0.6–1.4a	A	2.1–9.3a	5.2–20.7b**	B	16.0–33.0b	15.1–28.4a**	C**
Total	12–32a	21–33b*	A	9–55a	19–51a	B	38–65a	41–70a	C**

Ranges of average values of two replicates injected twice into the liquid chromatograph. 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, $p \leq 0.05$)

TR traces (≤0.04), *P1* 3,4-DHPEA, hydroxytyrosol, *P2* *p*-HPEA, tyrosol, *P3* vanillic acid, *P4* caffeic acid, *P5* syringic acid (internal standard), *P6* vanillin, *P7* *p*-coumaric acid, *P8* hydroxytyrosyl acetate, *P9* ferulic acid, *P10* *o*-coumaric acid, *P15* tyrosyl acetate, *P19* cinnamic acid

* $p \leq 0.05$; ** $p \leq 0.01$

their respective 5-point external calibration curves, and the results are expressed as milligrams of analyte per kilogram of oil.

Sensory Analysis

A panel of 12 tasters recognized by the IOC evaluated the organoleptic attributes of the oils according to COI/T.20/Doc. No. 15/Rev. 4 (2011) method [12]. The panelists were suitably selected and trained to identify and evaluate the intensities of positive (fruity, bitterness, and pungency) and negative (e.g., fusty or muddy sediment, winey-vinegary, frostbitten olives, or wet wood) sensory perceptions. Samples were randomly presented and tasters were requested to mark their perceptions on a profile sheet and to evaluate their intensity on an unstructured scale ranked from 0 to 10 [12]. The median of each attribute was estimated by the software package accompanying the method. In addition, olfactory, gustatory, tactile, and kinesthetic characteristics were assess following COI/T.20/Doc. No. 22 (2005) method [12].

Statistical Analysis

Simple ANOVA (software: INFOSTAT 2014, Universidad Nacional de Córdoba, Argentina) was used to assay the total and individual biophenols, and the Duncan test ($p \leq 0.05$) was performed to estimate significant differences between cultivars and harvest years. SYSTAT for Windows software (version 12, Systat Inc.) was used to perform multiple linear regression analyses. One model for bitterness and another for pungency were built using as dependent variables the bitter and pungency indices (BI and PI) determined by sensory analysis ($n = 30$). First, the regression of BI and PI with the total biophenol content as independent variable was studied, and then the regression with the content of each family of compounds (simple phenols, secoiridoids, lignans, and flavonoids) was evaluated. Finally, the content of each individual compound was

Table 2 Secoiridoids and derivatives, lignans, and flavonoids in the EVOO samples

Peak No.	Arbequina			Changlot Real			Coratina		
	2012	2013		2012	2013		2012	2013	
Secoiridoids and derivatives at 280 nm (mg tyrosol eq./kg)									
P(11 + 11a)	0.5–4.6a	1.0–5.1a	A	0.7–2.6a	1.6–35.5b**	B	9.6–34.7a	10.8–57.8b**	C**
P12	3.0–35.4a	27.2–118.4b**	B	0.1–0.4a	3.6–21.0b**	A	32.8–90.4a	57.3–89.3b**	C**
P13	1.4–5.1a	2.7–6.0b*	A	0.4–5.2a	11.5–54.7b**	B	16.0–38.4a	15.4–72.0b**	C**
P14	0.6–1.5b	0.8–1.0a*	A	0.3–1.8a	1.4–15.9b**	B	8.5–17.7a	2.4–16.5a	C**
P(16 + 16a)	7.6–11.3a	9.0–12.2a	A	7.3–14.4a	12.0–49.1b**	B	25.0–61.7a	38.4–61.7a	C**
P17	8.1–28.7a	17.5–27.3b**	B	2.3–7.0a	6.5–16.7b**	A	43.5–73.5a	38.1–87.6a	C**
P20	2.1–4.8b	1.2–3.8a*	C	1.0–1.6b	0.2–2.4a*	A	2.3–5.4b	0.2–0.4a**	B**
P(21 + 21a)	2.0–4.8a	2.4–5.4a	A	0.7–3.9a	2.3–8.5b**	B	5.6–11.0b	4.5–7.0a**	C**
P21b	2.5–11.1a	5.9–13.4b**	A	0.7–25.3a	24.3–133.9b**	B	18.5–82.0a	74.9–135.8b**	C**
P23	1.5–4.0b	0.5–1.0a**	A	0.8–14.1a	4.2–13.3a	B	22.1–33.8b	9.0–15.4a**	C**
P(24 + 24a + 24b)	1.0–2.0a	2.6–3.5b**	A	2.0–14.4a	1.9–12.7a	B	10.2–38.6b	9.4–14.9a**	C**
P27	2.3–6.7a	2.9–4.5a	B	0.3–7.2a	0.8–2.5a	A	1.2–9.3b	1.0–3.5a**	B**
Total	34–105a	82–194b**	A	19–74a	76–355b**	B	276–369a	343–465b**	C**
Lignans at 280 nm (mg tyrosol eq./kg)									
P18	36.7–53.7a	37.0–50.2a	B	28.5–44.3a	28.3–72.1b*	A	56.9–85.7a	64.5–86.9a	C**
Flavonoids at 280 nm (mg tyrosol eq./kg)									
P22	7.3–15.3a	10.6–30.0b**	B	1.9–7.7a	3.1–16.1b**	A	9.1–51.6a	18.5–25.7a	C**
P25	2.6–16.7b	1.9–7.6a**	A	1.1–18.0a	2.3–11.3a	A	3.8–20.5a	9.5–33.5b**	B**
P26	4.0–9.9b	0.6–1.8a**	A	0.8–14.9a	3.9–25.0b**	B	6.3–44.5a	20.4–33.0a	C**
Total	16–35a	13–39a	A	4–38a	20–53b**	A	25–86a	56–79a	B**
Flavonoids at 335 nm (mg analyte/kg)									
P22	2.2–5.4a	3.3–10.9b**	B	0.7–1.4a	2.1–4.2b**	A	1.1–2.7a	2.4–3.6b**	A**
P25	0.3–1.3a	0.5–2.3b*	B	0.2–0.9a	0.3–0.8a	A	0.7–1.1b	0.5–0.9a*	B**

Ranges of average values of two replicates injected twice into the liquid chromatograph. 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, $p \leq 0.05$)

P(11 + 11a) decarboxymethyl oleuropein aglycone (oxidized dialdehyde form), *P12* 3,4-DHPEA-EDA, decarboxymethyl oleuropein aglycone (dialdehyde form), *P13* oleuropein, *P14* oleuropein aglycone (dialdehyde form), *P(16 + 16a)* decarboxymethyl ligstroside aglycone (oxidized dialdehyde form), *P17* *p*-HPEA-EDA, decarboxymethyl ligstroside aglycone (dialdehyde form), *P18* pinoresinol + 1-acetoxypinoresinol, *P20* ligstroside aglycone (dialdehyde form), *P(21 + 21a + 21b)* oleuropein aglycone (oxidized aldehyde and hydroxylic form), *P22* luteolin, *P23* 3,4-DHPEA-EA, oleuropein aglycone (aldehyde and hydroxylic form), *P(24 + 24a + 24b)* ligstroside aglycone (oxidized aldehyde and hydroxylic form), *P25* apigenin, *P26* methyl–luteolin, *P27* *p*-HPEA-EA, ligstroside aglycone (aldehyde and hydroxylic form)

* $p \leq 0.05$; ** $p \leq 0.01$

fitted to the models as independent variable. The p values for entering and removing new variables into the statistical models were $p \leq 0.05$ and $p > 0.05$, respectively.

Results and Discussion

All the samples were classified as EVOO according to the trade standard of IOC [10] by chemical analysis: free acidity ≤0.8% m/m as oleic acid, peroxide value ≤20 mEq/kg, extinction coefficients (K) at 268 nm ≤0.22, K at 232 nm ≤2.50, ΔK at 268 nm ≤0.01 (results not shown). The sensory analysis also categorized the samples as EVOO: median of defects = 0 and median of the fruity attribute >0.

Total Polyphenol Content and Biophenol Composition

Cultivar Influence

The ranges of average contents of polyphenols and total and individual and biophenols are shown in Tables 1 and 2. The F–C method is the most commonly used analytical methodology to quantify phenolic compounds in VOO owing to its simplicity. It is based on the spectrophotometric measurement of the polyphenols in a

hydroalcoholic extract of olive oil dissolved in hexane. Five categories have been proposed to classify olive oils by their polyphenol content determined with the F–C reagent in milligrams of caffeic acid per kilogram: very high (>600), high (450–600), medium (300–450), low (150–300), and very low (<150). According to this classification, the oils analyzed in this work were classified into the categories of polyphenol contents: very low or low for Arbequina (70–194 mg caffeic acid/kg); very low, low, or medium for Changlot (39–364 mg caffeic acid/kg); and medium, high, or very high for Coratina (362–657 mg caffeic acid/kg). The polyphenol contents obtained by the F–C method are different from those determined by HPLC (Table 1). The HPLC–UV method determines "biophenols", a newer term that includes minor polar phenolic compounds in olive oils, such as the natural and oxidized derivatives of oleuropein and ligstroside, lignans, flavonoids, and phenolic acids. Differences in the total content of phenolic compounds between F–C and HPLC methods come from differences in the molar absorptivity per reactive group between phenols.

According to the HPLC method, the Coratina oils had the highest contents of total biophenols (458–712 mg/kg), whereas the Arbequina oils exhibited the lowest contents (109–313 mg/kg), with a wider range being observed for the Changlot Real oils (64–530 mg/kg), as can be seen in Table 1. Cultivar and harvest year are the determinant factors for the content of phenolic compounds in olive oils [13].

The highest level of total biophenols in the Coratina oils was based on their higher content of secoiridoids and simple phenols and their derivatives, of up to 465 and 70 mg/kg, respectively (Tables 1, 2). The Arbequina oils had less than 194 mg of total secoiridoids/kg and 33 mg of total simple phenols/kg. The oleuropein contents stood out among the secoiridoids being Coratina > Changlot > Arbequina (Table 2). According to Amiot *et al*. oleuropein contents of olive fruits have exhibited very considerable quantitative differences between cultivars, and there was an inverse correlation between the size of the fruits and their oleuropein contents [14]. This relationship first observed at the beginning of maturation remained valid until full maturity of the fruit. The smaller size of the Coratina olives could be explain the larger oleuropein content observed in the oils of this cultivar studied in this work (Table 2).

Total flavonoid contents were also the highest for the Coratina cultivar ($p \leq 0.01$); however, no significant differences were detected between Arbequina and Changlot Real (Table 2). The Coratina oils also exhibited the highest luteolin and apigenin concentrations ($p \leq 0.01$) when these flavonoids were measured at 280 nm (Table 2). However, when the two flavones were measured at 335 nm, the luteolin concentrations were higher for Arbequina oils and the apigenin concentrations were higher for the Coratina and Arbequina oils (Table 2). The chromatogram region at 280 nm corresponding to flavonoids shows overlapping peaks whose integration is too complex. The co-elution of luteolin and apigenin with other flavonoids is also possible. The results suggest that the method applied at 280 nm is not adequate to measure luteolin and apigenin concentrations in EVOO. The higher flavonoid contents in Coratina oils would be mainly associated with higher concentrations of other non-identified flavonoids. In Arbequina oils, the luteolin and apigenin concentrations at 335 nm were 2.2–10.9 and 0.3–2.3 mg analyte/kg oil, respectively (Table 2). In Coratina oils, luteolin and apigenin ranged from 1.1 to 3.6 mg/kg and from 0.5 to 1.1 mg/kg, respectively (Table 2). Values of total flavonoids of 2.3 and 5.5 mg/kg were observed in two Coratina oils [15].

The lignan contents (pinoresinol + 1-acetoxypinoresinol) differed among cultivars as follows: Coratina > Arbequina > Changlot Real (Table 2, $p \leq 0.01$). Lignans may represent the major phenolic compounds in some Arbequina and Empeltre oils [16]. In olive fruits, the pulp/pit ratio is largely influenced (72–75%) by the cultivar [13]. Arbequina and Changlot Real olives have higher pulp/pit ratios in the ranges of 4.6–6.6 and 3.6–5.0, respectively [13, 17]. However, Coratina olives have smaller pulp/pit ratio, with values ranging from 2.22 to 3.21 [18]. This lower pulp/pit ratio of the Coratina olives could strengthen the contribution of the pit (rich in lignans) to the output stream of the mill; and given their lipid solubility, these lignans could easily pass to the oil.

The Arbequina oils were characterized by higher contents of vanillic and caffeic acids (P3 and P4, respectively) among simple phenols and of the dialdehyde form of ligstroside aglycone (P20, precursor of *p*-HPEA-EDA) among the secoiridoids (Tables 1, 2). With the exception of P20, the levels of all the individual components of the secoiridoid fraction were the highest for Coratina oils (Table 2, $p \leq 0.01$). Hydroxytyrosol (3,4-DHPEA, P1) and cinnamic acid (P19) were prominent among simple phenols in the Coratina oils (Table 1, $p \leq 0.01$). Low or medium contents of individual components in the fractions of secoiridoids and simple phenols were registered in the Changlot oils (Tables 1, 2). The predominance of secoiridoid compounds in Coratina oils can be clearly observed in Fig. 1. Moreover, in both harvests, higher relative percentage of simple phenols and their derivatives characterized the Changlot oils in comparison with the Coratina oils.

Harvest Year Influence

Bringing forward the harvest time in 2013 with respect to 2012 produced a highly significant increase ($p \leq 0.01$) in the total biophenol contents of the oils of the three cultivars

(Table 1). This increase could be due to the significant reduction observed in the maturity indices of the three olive cultivars in the 2013 harvest with respect to the 2012 harvest (Table 1). The average maturity index was reduced from 3.85 in 2012 to 2.41 in 2013 in Arbequina olives, from 4.82 to 2.99 in Changlot olives, and from 1.95 to 0.79 in Coratina olives. It is well known that, during olive fruit maturation, the concentration of phenolic compounds progressively increases until it reaches a maximum after which it decreases [6, 19]. In addition, the cell wall of very green olives is more rigid and resistant to breakage, hindering the transfer of biophenols to the oil phase. Thus, in the early stages of fruit maturity, the water-soluble phenolic compounds are not entirely transferred to the oil phase during processing. As maturity progresses and the water content in the fruits decreases, the concentration of phenolic compounds increases in the oil phase until it reaches a maximum [20, 21]. Moreover, there is a generalized consensus that hydrophilic phenols show their lowest content in oils obtained from overripe olives [6, 21–24]. The lower total biophenol contents observed for the oils from the three cultivars harvested in 2012 suggest that overripe olives were processed. Clearly, bringing forward the harvest time in 2013 optimized the maturity indices of the processed olives, improving the biophenol contents in the extracted olive oils.

The higher total biophenol contents obtained for the 2013 samples were mainly due to higher contents of secoiridoids and derivatives in the oils from the three cultivars (Table 2, $p \leq 0.01$). The higher contribution of the secoiridoid family and derivatives to the average relative profiles of the oils from the three cultivars in 2013 can also be observed in Fig. 1. The secoiridoid levels in the oils behave similarly to total phenols in the course of olive ripening: they increase during the maturation process until the maximum is reached, and then there is a very rapid fall [20, 21, 24]. This fact agrees with the results of this paper because the advancement of the harvest date significantly reduced the maturity indices and therefore increased the contents and relative percentages of secoiridoids (Table 1).

The contents of simple phenols and derivatives showed no significant differences between years for the Changlot and Coratina oils, while they slightly increased for the Arbequina oils in the 2013 harvest (Table 1). The concentrations of simple phenols in the oils from three Italian cultivars remained similar during fruit ripening [22]. However, a decrease in the levels of these compounds was observed for the oils obtained from olives of two Tunisian cultivars in advanced maturity [20]. These results suggest that the trend observed in the levels of these phenolic compounds in the oils during the maturation of the olives is dependent on the cultivar. The secoiridoid contents significantly increased in the Changlot oils of 2013 without any significant change

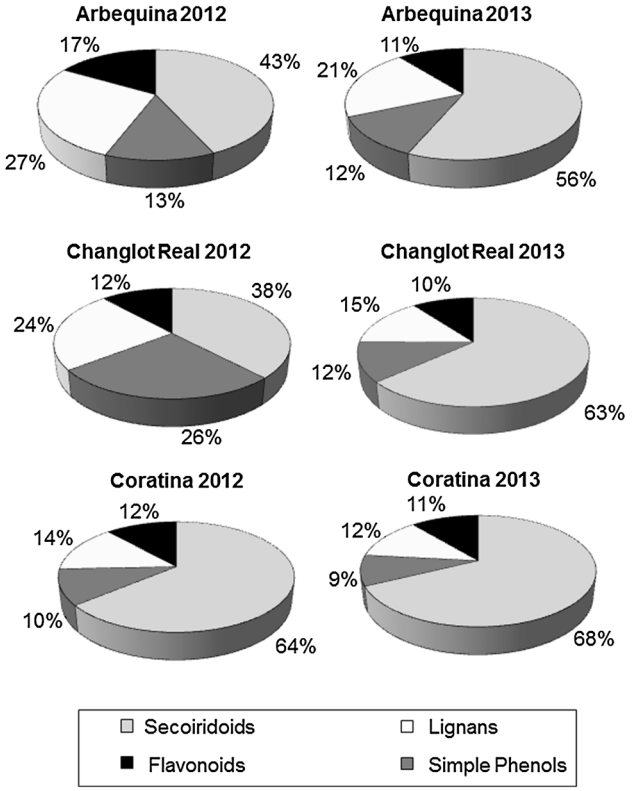

Fig. 1 Average relative percentages of the biophenol families grouped by cultivar and harvest year

in the simple phenol contents. This is reflected in the decrease of the relative percentage of simple phenols and the increase observed for the secoiridoids (Fig. 1). On the other hand, only the Changlot oils had higher contents of total flavonoids in 2013 (Table 2, $p \leq 0.01$). The lignan contents in Arbequina and Coratina oils were not significantly different between the two harvests. Other authors have found that lignan concentrations in the oils were not significantly different at different maturity stages [22]. However, bringing forward the harvest time in 2013 significantly improved the lignan levels in the oils from the Changlot cultivar (Table 2). A fall in the lignan contents was also observed in oils obtained from overripe olives [21, 23]. For the Changlot cultivar, the decrease in the maturity indices of the olives in 2013 with respect to 2012 had a higher significance level than for the other two cultivars (Table 1). Thus, the oils from this cultivar show deeper changes in the contents of all biophenol families.

The decrease in secoiridoid concentrations in the oils from the three cultivars and in flavonoids and lignans in the Changlot oils, observed during the final maturation stages, could be mainly attributed to the lower activity of endogenous hydrolytic enzymes in the olives during this mature stage [25]. The earlier harvest in 2013 could maintain the activities of the endogenous enzymes at a higher level. In

the case of lignans, the influence of the pulp/pit ratio of the processed olives should also be taken into account. Increases in the pulp/pit ratio with time were observed for some olive varieties characterized by late ripening [26]. Changlot Real is a late-ripening cultivar, and a lower pulp/pit ratio could strengthen the contribution of pits in the olive pastes, thus increasing the lignan concentrations in the oils obtained in 2013.

The earlier harvest time in 2013 produced complex changes in the contents of individual components of the secoiridoid fraction (Table 2). Higher levels of oleuropein compounds were observed in the oils from the three cultivars in 2013, such as 3,4-DHPEA-EDA (P12), oleuropein (P13), and an oxidized derivative of oleuropein aglycone (P21b), as well as lower contents of a derivative from ligstroside (P20). Other individual secoiridoids showed increases or decreases in their contents bringing forward the harvest time in 2013 depending on the cultivar, and the Changlot oils had the largest number of compounds affected. Evidently, the deeper changes in the contents of the individual biophenols observed in Changlot oils in 2013 with respect to 2012 are a consequence of more significant changes in the maturity indices of the olives from this cultivar (Table 1).

The oleuropein content in the olives increases rapidly during fruit growth; the maximum obtained depends of the variety [14]. Later, during the two successive phases characterizing olive maturation, a very rapid decrease is first observed and then a much slower decline after the change in color of the fruit. Moreover, the changes in glycosidase activities in the olives show the same trend as the corresponding changes in oleuropein concentrations, reaching a maximum when the oleuropein level is highest and decreasing when oleuropein levels fall [25]. The earlier harvest time in 2013 increased the oleuropein levels in the olive fruits, and surely also the activities of its hydrolytic enzymes acting during the oil extraction process. 3,4-DHPEA-EDA is one of the main products of oleuropein degradation in the proposed biochemical mechanism for the formation of secoiridoid derivatives [6]. Thus, 3,4-DHPEA-EDA levels were higher in the oils obtained in 2013.

On the other hand, the primary product of ligstroside degradation is *p*-HPEA-EDA (P17), and P20 is an intermediate in its synthesis [6]. The lower contents of P20 in the oils from the three cultivars and the higher concentrations of *p*-HPEA-EDA in the oils from the Arbequina and Changlot cultivars in 2013 are indicative of higher endogenous enzyme activities. 3,4-DHPEA-EA (P23) and *p*-HPEA-EA (P27) are isomers of oleuropein and ligstroside aglycones, respectively, that contribute to the formation of other secoiridoid derivatives [6]. In 2013, lower concentrations of 3,4-DHPEA-EA were observed in the Arbequina and Coratina oils and lower levels of *p*-HPEA-EA were detected in the Coratina oils (Table 2). These findings suggest the involvement of these compounds in the enzymatic biosynthesis of other secoiridoid derivatives.

The simple phenols and derivatives showed less complex changes with the harvest year than the secoiridoids (Table 1). In 2013, hydroxytyrosol (P1) was significantly higher in the oils from the three cultivars. Tyrosol (P2) was higher only in the Coratina oils, while tyrosyl acetate (P15) was higher in the oils from the three cultivars. Hydroxytyrosol and tyrosol derive from the hydrolysis of oleuropein and ligstroside aglycones, respectively [21]. The results suggest that the degradation reactions of the aglycone secoiridoids involve interconversion reactions between the phenyl alcohols and their acetates, whose rates depend on cultivar and maturity stage. Tyrosyl acetate is also recognized as one of the phenolic compounds responsible for the bitter taste of olive oils [27]. Several works in the literature support an increase in these phenyl alcohols during maturity of the olive fruits [9, 28]; but other authors have reported contradictory results [25]. A rapid decrease of the phenyl alcohol levels in oils obtained from overripe olives was observed [21, 23]. Bringing forward the harvest time in 2013 ensured a higher content of hydroxytyrosol and tyrosyl acetate in the oils obtained from the three cultivars.

The Arbequina oils reflected minor changes in the components of the fraction of simple phenols between harvest years. Eight out of 11 components of this fraction did not vary with the earlier harvest time in 2013 (Table 1). Among phenolic acids, vanillic acid (P3) did not change significantly with harvest year for any of the three cultivars (Table 1). The *o*-coumaric acid concentration (P10) in the Changlot and Coratina oils was higher in the 2012 harvest (Table 1). In 2012, the concentration of ferulic acid (P9) was also higher in the Coratina oils.

By bringing the harvest time forward in 2013 it was possible to obtain oils with significantly higher contents of luteolin (P22), measured at 335 nm for the three cultivars (Table 2, $p \leq 0.01$). However, no significant differences were detected in luteolin concentrations between harvest years for the Coratina cultivar when the measurement was made at 280 nm (Table 2). Luteolin concentrations were higher for the Arbequina and Changlot oils in 2013 by both measurement methods. The co-eluting of other flavonoids that also absorb at 280 nm together with the luteolin, as suggested above, would render the measurement of luteolin concentration at this wavelength inadequate. The lower luteolin levels for all the 2012 oils, in addition to the lower levels of oleuropein and secoiridoid derivatives, could be explained by the lower concentrations of glycosides and lower activity of the hydrolytic enzymes in the overripe olives. A strong decrease in luteolin levels was detected in Roggianella oils obtained from olives in an advanced

maturity stage [23]. In the present work, the apigenin concentrations measured at 335 nm were lower than those of luteolin and exhibited a different behavior between harvest years for the oils from the different cultivars (Table 2). The apigenin concentration did not show any distinct trend in the VOO with fruit maturation [29].

Sensory Profiles

In general, according to the intensity of fruity perception, the oils from the three cultivars could be classified as "medium-intensity oils" (median or fruity index, FI = 3–6) [12]. Two Arbequina samples and one Changlot in the 2012 harvest had FI a little below 3. No significant differences were observed between cultivars ($p = 0.125$) and the average FI was 3.76 for Arbequina, 4.58 for Changlot, and 4.22 for Coratina. The Arbequina oils had a lower bitterness index in average (BI = 1.40, $p < 0.0001$). However, no significant differences were observed between Changlot (average BI = 3.86) and Coratina (average BI = 4.11). The Coratina oils were characterized by the highest pungency index (average PI = 4.93), being 3.72 for Changlot and 2.63 for Arbequina ($p < 0.0001$).

The sensory profiles of the oils improved markedly with the earlier harvest time and the lower maturity indices of the olives in 2013 (Fig. 2). In the Arbequina oils, the three positive attributes significantly increased their average intensities: FI (2012 = 3.07, 2013 = 4.59, $p = 0.0016$), BI (2012 = 1.11, 2013 = 1.76, $p = 0.0375$), and PI (2012 = 2.19, 2013 = 3.16, $p = 0.0208$). The average FI also improved significantly for the Changlot oils (2012 = 3.50, 2013 = 5.30, $p = 0.0006$) and bitterness and pungency increased on average, although no significant differences were detected between years. No significant change was observed regarding FI for the Coratina oils between harvest years. However, bitterness and pungency significantly decreased with the earlier harvest time of 2013: BI (2012 = 4.66, 2013 = 3.43, $p = 0.0100$) and PI (2012 = 5.17, 2013 = 4.64, $p = 0.0230$).

An extremely bitter olive oil may not be acceptable to the majority of consumers, despite its health benefits and high resistance to oxidation [29]. The earlier harvest time proposed in this work ensured an average BI lower 5 in the oils from the three cultivars, thus enhancing the content and profile of biophenols. Certain consumers prefer "sweet oils" or oils characterized by a less bitter-pungent taste and velvety tactile sensation [25]. In the case of the Arbequina oils studied in this work, the sweet taste was lower on average when the harvest time was brought forward in 2013 (2.30) compared to 2012 (2.65) (Fig. 2). However, no significant differences were detected between harvest years ($p = 0.349$) with respect to sweetness. In the Coratina oils, the earlier harvest in 2013 reduced the bitter-pungent taste without

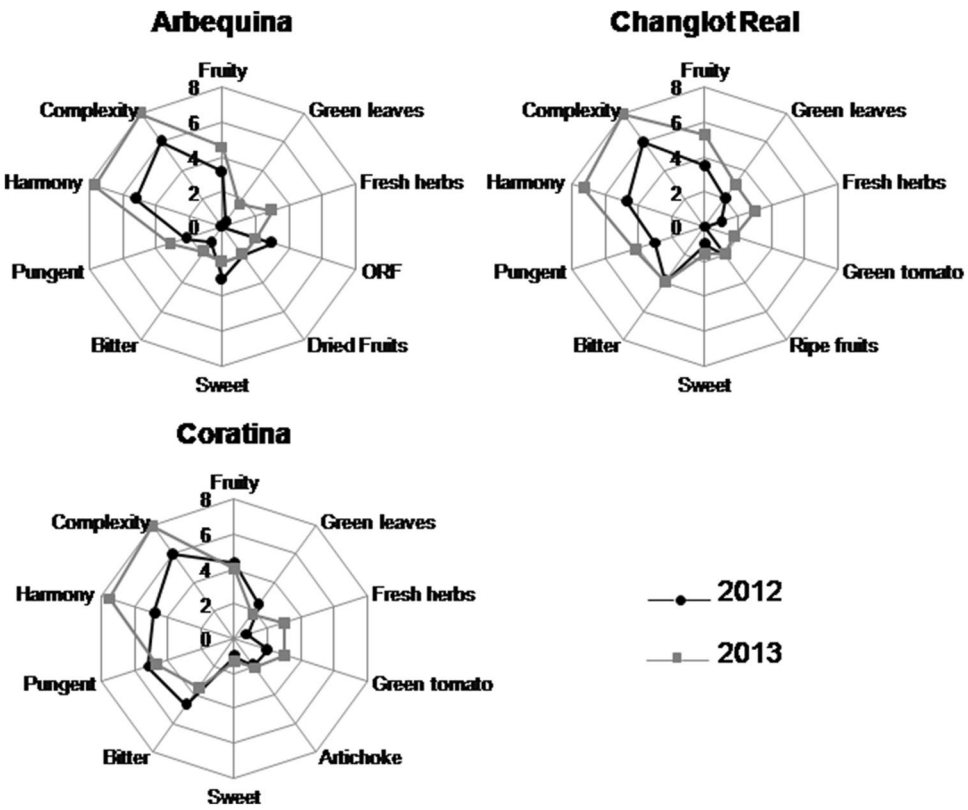

Fig. 2 Average sensory profiles by cultivar and harvest year. *ORF* other ripe fruits

significantly changing the sweetness (2012 = 0.88, 2013 = 1.30, $p = 0.273$). Clearly bringing forward the harvest time in 2013 provided a tool to enhance the bitter-pungent taste without significantly reducing the sweetness.

By lowering the maturity indices of the olives, it was possible to obtain oils with better harmony between the positive attributes (Fig. 2). Moreover, a wider range of descriptors for fruitiness were observed, from green, green with ripe notes, ripe with green notes up to ripe, and the green notes, such as herbs, leaves, and green tomatoes, were intensified. The oils from the 2013 harvest also evidenced a higher complexity, essentially based on their larger number of descriptors (Fig. 2). A delay in olive harvesting for cultivars yielding bitter to pungent oils (e.g., Coratina) has been proposed to enhance oil preservation [24]. However, the results obtained in this study suggest that bringing forward the harvest time by 15 days contributes to obtaining not too bitter and pungent oils, thus avoiding consumer rejection and producing more harmonious and complex oils. On the other hand, the average OSI for oils from Arbequina cultivar was 9.6 h in 2012, increasing significantly up to 13.7 h in 2013 ($p = 0.0003$). The same occurred with Changlot oils whose OSI in average was 9.8 h in 2012 and 23.7 h in 2013 ($p < 0.0001$). Coratina oils also showed significant changes in their OSI with harvest year (2012 = 23.5 h, 2013 = 34.6 h, $p < 0.0001$). The oxidative stability of the oils depends mainly on the fatty acids profiles and total polyphenol content [7]. The polyphenol contents were higher in the Arbequina and Changlot oils of 2013, and were not significantly different in the Coratina oils (Table 1); besides, improved the fatty acids profiles in 2013 (results not shown).

Relationship Between Biophenol Contents and Bitterness Indices

A highly significant linear regression between BI as dependent variable and the total content of biophenols as independent variable was observed ($R = 0.646$, $p = 2.023 \times 10^{-4}$). The total content of secoiridoids and derivatives had the most significant regression with BI ($R = 0.633$, $p = 3.038 \times 10^{-4}$). The other compound families (simple phenols and derivatives, lignans, and flavonoids) presented regressions with less statistical significance. Therefore, the statistical model for bitterness was fitted first to the individual contents of each one of the secoiridoids and derivatives. Highly significant positive contributions of one derivative of the oleuropein (P23, 3,4-DHPEA-EA) and an oxidized derivative from ligstroside, P(24 + 24a + 24b) were observed (Table 3).

The contents of the ligstroside aglycone dialdehyde form (P20) and its predominant decarboxylated form (P17, *p*-HPEA-EDA) contributed negatively to the bitterness of the oils (Table 3). The phenolic compound *o*-coumaric acid (P10) was the only non-secoiridoid compound whose regression with bitterness was significant (Table 3). Lignans (P18) and flavonoids (P22, P25, and P26) had no significant input to the statistical model for bitterness of the oils.

The values of the adjusted multiple *R*-squared (Table 3) were used to estimate the following contributions to the bitterness model: 65.5% for 3,4-DHPEA-EA (P23), 6.4% for P20, 6.0% for oxidized ligstroside aglycone [P(24 + 24a + 24b)], 3.9% for *p*-HPEA-EDA (P17), 2.3% for *o*-coumaric acid (P10), and 15.9% for other compounds. A highly significant linear correlation was observed between the bitterness values (BI) estimated by the

Table 3 Multiple linear regression analyses for bitter indices

Dependent variable[a] = BI $n = 28$[c] Multiple $R = 0.933$		Standard error of estimate = 0.554 ANOVA, $p = 4.7 \times 10^{-9}$**		
Independent variable[b]	Coefficient	Standard error	Adjusted R^2[d]	Significance (two-tailed p)[e]
Constant	2.145	0.228		3.574×10^{-9}**
P23	0.087	0.018	0.655	7.356×10^{-5}**
P(24 + 24a + 24b)	0.091	0.019	0.715	1.005×10^{-4}**
P20	−0.271	0.080	0.779	0.003**
P17	−0.014	0.007	0.818	0.040*
P10	0.069	0.033	0.841	0.049*

[a] *BI* bitter index

[b] See the footnotes of Tables 1 and 2 for peak identification

[c] Two samples from Changlot 2013 were discarded as outliers

[d] Refers to progressive addition of each component into the model

[e] ** Highly significant regression ($p \leq 0.01$), * significant regression ($p \leq 0.05$)

proposed model and the values determined by the sensory panel (Fig. 3, estimated bitterness index = 0.980 × sensory bitterness index, $R = 0.924$).

Several authors have suggested that secoiridoid derivatives of oleuropein and demethyl oleuropein such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA are the main contributors to VOO bitterness [6, 24]. Moreover, a strong correlation between bitter and pungent sensory notes and ligstroside derivatives such as *p*-HPEA-EDA has been observed [6]. For the EVOO analyzed in this work, the relationship between bitterness and total biophenol content and the strong contribution of 3,4-DHPEA-EA to the bitter taste were confirmed. However, 3,4-DHPEA-EDA did not contribute significantly. On the other hand, the bitter taste was to a lesser extent related to the oxidized derivatives from ligstroside aglycone, and the dialdehydic form of ligstroside aglycone and its demethylated form (*p*-HPEA-EDA) negatively contributed to the bitterness intensity. The pure phenolic acids extracted from VOO and dissolved in lipophylic solutions did not show any relationship with the bitter sensory note [6]. However, the results obtained in this study demonstrate a contribution of *o*-coumaric acid to the oil bitterness, suggesting that the bitter sensation is more complex than just the profile of secoiridoid derivatives.

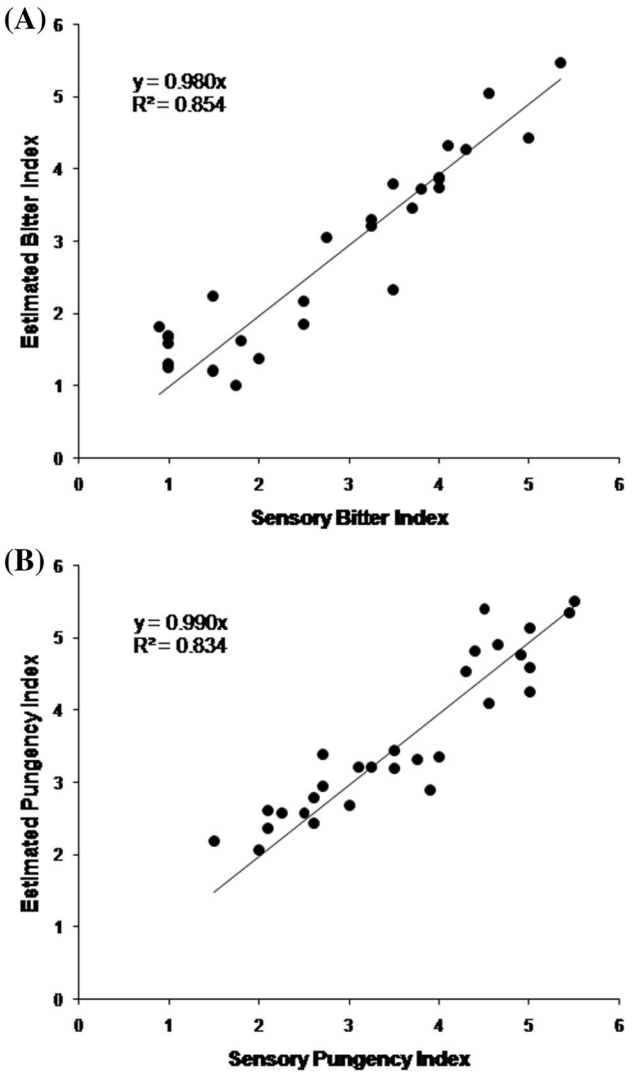

Fig. 3 Linear correlations between the attributes estimated by the statistical model and evaluated by a sensory panel. **a** Bitterness, **b** pungency

Relationship Between Biophenol Contents and Pungency Indices

The pungency index (PI) estimated by the sensory panel had a highly significant linear regression with total biophenol content ($R = 0.844$, $p = 9.024 \times 10^{-9}$). The secoiridoids and derivatives presented the most significant regression ($R = 0.841$, $p = 1.152 \times 10^{-8}$), although the contribution of the family of simple phenols was also important ($R = 0.826$, $p = 3.460 \times 10^{-8}$). Lignans showed a less significant contribution while flavonoids did not exhibit any significant contribution to pungency.

As observed in Table 4, a highly significant positive linear regression was observed between pungency of the oils and the contents of two secoiridoids (P17 and P14) and of two simple phenols, P2 (*p*-HPEA, tyrosol) and P15 (tyrosyl acetate). P17 (*p*-HPEA-EDA) negatively contributed to the bitterness of the oils (Table 3). *p*-HPEA-EDA is the phenolic compound responsible for the majority of the burning pungent sensory notes in VOO [30]. The results obtained in this study show that the increase in the concentration of this derivative not only makes the oil more pungent but also reduces the perception of its

Table 4 Multiple linear regression analyses for pungency indices

Dependent variable[a] = PI $n = 29$[c] Multiple $R = 0.925$		Standard error of estimate = 0.477 ANOVA, $p = 9.4 \times 10^{-10}$**		
Independent variable[b]	Coefficient	Standard error	Adjusted R^2[d]	Significance (two-tailed p)[e]
Constant	1.582	0.278		7.4×10^{-6}**
P17	0.022	0.004	0.522	5.9×10^{-6}**
P14	0.085	0.018	0.735	1.2×10^{-8}**
P2	0.063	0.017	0.798	1.8×10^{-9}**
P15	0.764	0.315	0.831	9.4×10^{-10}**

[a] *PI* pungency index

[b] See the footnotes of Tables 1 and 2 for peak identification

[c] One sample from Changlot 2013 was discarded as an outlier

[d] Refers to progressive addition of each component into the model

[e] **Highly significant regression ($p \leq 0.01$)

bitter taste. The dialdehyde form of oleuropein aglycone (P14) is the minority non-demethylated form of P12 (3,4-DHPEA-EDA). Lignans (P18) and flavonoids (P22, P25, and P26) did not make a significant contribution to the pungency model.

As in the case of bitterness, tyrosol and its acetyl derivative show the contribution of simple phenols to the pungency of the oils and the complexity of this sensory attribute. The following contributions can be estimated by the progressive addition of each component into the pungency model, taking into account the adjusted R^2 values (Table 4): P17 = 52.2%, P14 = 21.3%, P2 = 6.3%, P15 = 3.3%, and others = 16.9%. The pungency indices determined by the sensory panel correlated with those estimated by the proposed model (Fig. 3, estimated PI = 0.990 × sensory PI, $R = 0.995$).

Conclusions

The results presented in this paper provide information about biophenol content and profile and their relationship with flavor in EVOO from San Juan, Argentina. The contents of total biophenols, secoiridoids, and simple phenols strongly depended on the cultivar with the highest levels being observed in the Coratina oils, variable levels in the Changlot Real oils, and the lowest levels in the Arbequina oils. The proposed models for bitterness and pungency of the oils demonstrated a strong contribution of secoiridoid derivatives of oleuropein and ligstroside, as well as of some simple phenols and derivatives. The main contribution to bitterness oils was given by 3,4-DHPEA-EA. *p*-HPEA-EDA mostly contributed to the pungency of the oils, negatively influencing their bitterness together with its precursor, ligstroside aglycone (dialdehyde form). These results are important for the local olive oil industry. With an earlier harvest, regional producers could obtain oils with enhanced biophenol profiles in their quality and concentration, more harmonious and complex from a sensory point of view, and more stable against oxidation.

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

Compliance with Ethical Standards

Conflict of interest The authors have declared no conflict of interest.

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