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# Promising olive varieties for extra virgin oil production in Mendoza, Argentina



Adriana P. Banco<sup>a,\*</sup>, Carlos M. Puertas<sup>a</sup>, Eduardo R. Trentacoste<sup>a</sup>, Norberto F. Gariglio<sup>c</sup>, Viviana P. Jofré<sup>b</sup>

<sup>a</sup> National Institute of Agricultural Technology Junín Agricultural Experiment Station, Isidoro Busquets s/n La Colonia, Junín, Mendoza, Argentina

<sup>b</sup> National Institute of Agricultural Technology Mendoza Agricultural Experiment Station, San Martín 3853, Mayor Drummond, Mendoza, Argentina

<sup>c</sup> ICIagro Litoral, UNL, CONICET, FCA, R. P. Kreder 2805, Esperanza, Santa Fe, Argentina

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## ABSTRACT

There are more than 2000 varieties of olives grown worldwide, of which only a few (2 %) are cultivated in Argentina. Mendoza is one of the main oil-producing provinces in the country due to its adequate agro-ecological conditions. In addition, Mendoza has an olive germplasm collection with over 70 accessions. This work aimed to characterize olive oil from 18 preselected varieties in the collection for qualitative characteristics that are important to the olive industry (i.e., industrial yield, acidity, oxidative stability, total phenolic compounds and phenolic profile). As a result, all evaluated characteristics were significantly different among varieties ( $p < 0.001$ ) and allowed identifying excellent qualities in varieties not currently cultivated. Five scarcely cultivated varieties ('Villalonga', 'Nebbio', 'Nevadillo Blanco', 'Canino', and 'Piangente') were highlighted above the most widespread cultivars in Mendoza, Argentina. Featuring on average of 14 % of industrial yield, 15 h of oxidative stability and 373 mg kg<sup>-1</sup> of total phenolic compounds.

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## 1. Introduction

The olive oil industry represents an important worldwide activity because of its economic value and its social and agro-ecological effects. Currently, olive oil has ridden a wave of increasing popularity due to its health benefits (Aguilera et al., 2004; Kiritsakis, 2020). According to the Statista (2022a,b), olive oil consumption worldwide is very low in comparison with traditional seed oils such as palm, soybean, rapeseed, sunflower, palm kernel, peanut oil, cottonseed and coconut oil. Nevertheless, in the last six years, olive oil consumption has increased globally, exceeding 3 million tons since the 2019/2020 growing season (Statista, 2022a,b). Along the same lines, the average olive oil production worldwide has surpassed 3 million tons in the last 5 years (2017/2021) (European Commission, 2022). Furthermore, the forecast seems to be more

favorable for non-European Union (EU) countries than for EU member countries (European Commission, 2022). In Argentina, olive crops add up to 110,000 ha between oil and table cultivars, and the annual fruit production is around 14,000 t. After grape, olive is the most important fruit crop in the central-western region and both activities promote employment and rural development (Benencia et al., 2014).

Virgin olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical procedures that do not alter the quality parameters of oil, such as acidity or oxidative stability (IOC, 2015a,b). Furthermore, virgin olive oil is the only one that does not need to be refined before consumption (Kiritsakis, 2020). As a result, it can retain the characteristics of the variety, as well as environmental and crop management conditions that are unique to each region (Kiritsakis, 2020). In contrast, seed oils are altered during the refining process, affecting most of their original sensory and nutraceutical attributes (El-Mallah and El-Shami, 2011).

Olive oil is made up of a major fraction (saponifiable) and a minor fraction (unsaponifiable). The effects of cultivar and environment have been examined in different compounds of the saponifiable fraction, including fatty acids (Oğraş et al., 2016), triglycerides (Giuffrè, 2014), waxes (Giuffrè, 2013), and sterols (Kycyk et al., 2016), among others.

\* Corresponding author.

E-mail address: [banco.adriana@inta.gob.ar](mailto:banco.adriana@inta.gob.ar) (A.P. Banco).

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The phenolic profile represents an unsaponifiable fraction of olive oil and has been studied by numerous authors for its beneficial health attributes (Oliveras-Lopez et al., 2007; Visioli et al., 2002; Visioli and Galli, 1998), which are associated with its antioxidant properties and oxidative stability (Beltrán et al., 2000). The phenolic profile is also used to characterize the origins and genuinity of oils geographically (Bajoub et al., 2016). On the other hand, oil organoleptic characteristics are influenced by the phenolic profile, which depends on the olive variety used in the industrial process (Ceci et al., 2017; Del Monaco et al., 2015; Franco et al., 2014).

Oleuropein is a phenolic compound exclusive to the *Oleaceae* family and classified in the secoiridoid group. Furthermore, phenolic alcohols such as tyrosol and hydroxytyrosol are derived from secoiridoid compounds (Sánchez et al., 2019), which have even more antioxidant capacity than oleuropein (Giovannini et al., 1999; Gómez-Rico, 2008). In addition, hydroxytyrosol and oleuropein have antimicrobial activity (Tuck and Hayball, 2002). Flavonoids such as rutin, catechin, apigenin, and quercetin are recognized to remove free radicals, prevent coronary heart disease, and present antitumor properties (Yao et al., 2004). Some phenolic acid compounds such as cinnamic, syringic, *p*-coumaric, vanillic, and gallic acids are associated with fruit color and sensory attributes (Bendini et al., 2007).

According to the worldwide catalog, more than 2000 olive varieties are known nowadays. Nevertheless, Argentine farmers cultivate <30 varieties, especially those that immigrants brought from Europe. In comparison to the rest of the existing varieties, these varieties had demonstrated good oil qualities in their origin regions but had not previously been evaluated in Argentine environments. Thus, the evaluation of the phenolic composition of olive oil from Argentina has focused on the main commercial varieties such as Arauco, Arbequina, Picual, Farga, Frantoio, Empeltre, Manzanilla, Changlot, Coratina, Koroneiki, Barnea, Ascolano, and Nevadillo, among others (Bodoira et al., 2015; Ceci et al., 2017; Lémole et al., 2018; Monasterio et al., 2017; Silva et al., 2014; Torres and Maestri, 2006).

The Olive Germplasm Collection at Estación Experimental Agropecuaria Junín-INTA (EEA Junín) was established in the late 1940s in the province of Mendoza (Argentina). It has 74 accessions from Spain, Italy, France, Algeria, the United States, Tunisia, and Argentina, but most of them are unknown to farmers and are not cultivated in the country. According to Beltrán et al. (2004) water content is the major olive fruit component, and dry matter content can be considered as a variety-dependent trait. Varieties with high moisture have a negative influence on the oil extraction process (for example: Picual and Hojiblanca, known as “difficult pastes” (Cruz et al., 2007)). Trentacoste and Puertas (2011) evaluated the agronomic and morphological traits of accessions from the collection of Junín. These authors selected varieties based on their high oil yield and low moisture. However, the oil characteristics and quality attributes of these non-cultivated varieties have not yet been evaluated.

This work aimed to characterize the industrial yield, free acidity, oxidative stability, total phenolic compounds, and phenolic profile of the extra virgin oils of 18 pre-selected varieties from the olive germplasm collection of INTA Junín (Mendoza, Argentina), and thus detect the varieties with better performance.

## 2. Materials and methods

### 2.1. Plant material and location

The experiment was carried out during the 2013/2014 and 2014/2015 growing seasons using over 70-year-old olive plants (*Olea europaea* L.), vase-formed and grafted from rootstock seed.

Olive trees were surface irrigated according to the schedule of zone (every 15 days), avoiding water deficit, and planted 12 × 12 m apart at the Olive Germplasm Collection of EEA Junín (Mendoza, Argentina; 33°06'S, 68°29'W, 653 m.a.s.l.). The soil is clay-loam. It belongs to the order Entisols and is classified as Typic Torrifluvent, pH = 7.5 (Regairaz, 1996). Fertilizers and insecticides were applied on a calendar basis. The mean annual temperature during experimental period (from 2013 to 2015) was 16.5 °C, with a mean annual rainfall of 238.4 mm, mostly concentrated in the summer (Table 2), and a frost-free period of 150 days from October to April. The 18 preselected varieties are shown in Table 1 for the high oil and low humidity content of the fruits (Trentacoste and Puertas, 2011).

### 2.2. Standards and reagents

Standards of tyrosol (≥99.5 %), hydroxytyrosol (≥99.5 %), gallic acid (≥97.0 %), vanillic acid (≥97.0 %) (Fluka, Buchs, Switzerland), apigenin (≥95.0 %), quercetin (≥90.0 %), catechin (≥98.0 %), syringic acid (≥95.0 %), oleuropein (≥80.0 %), *p*-coumaric acid (≥98.0 %), cinnamic acid (≥99.0 %), and rutin (≥94.0 %) (Sigma-Aldrich, St. Louis, MO, USA) were used. The phenolic compound extraction was carried out with HPLC-grade methanol (Sintorgan, Buenos Aires, Argentina), and n-hexane (Biopack, Buenos Aires, Argentina). Folin-ciocalteu (Biopack, Buenos Aires, Argentina) and sodium carbonate (99.0 %) (Cicarelli, Santa Fe, Argentina).

### 2.3. Maturity index

One hundred fresh fruits per tree were randomly selected to determine the maturity index (MI) according to Beltrán et al. (2004). Fruits were classified from 0 to 7 according to skin and flesh color at veraison (MI ≈ 2.5). The harvest day for each variety was previously determined by a visual fortnightly test classifying maturity index, according to Banco et al. (2021) (Table 1). The fruit harvest was advanced to avoid damage in the event of a strong frost occurrence.

### 2.4. Olive oil extraction and industrial yield

A fruit sample of 20 kg from each tree was harvested manually around the tree canopy at the veraison stage and was carried in

**Table 1**  
Origin, purpose and harvest day of evaluated varieties.

Varieties	Purpose	Harvest day	
		Season 2013–2014	Season 2014–2015
Arauco (A)	table and oil	21-may.	13-may.
Blanqueta (S)	Oil	16-may.	27-apr.
Canino (I)	Oil	20-may.	14-may.
Criolla Salvarredi (A)	Oil	21-may.	30-apr.
Cucci (I)	Oil	12-may.	26-may.
Dritta (I)	Oil	22-may.	23-apr.
Dulzal (S)	Oil	15-may.	29-apr.
Empeltre (S)	Oil	9-may.	22-apr.
Farga (S)	Oil	8-may.	22-apr.
Frantoio (I)	Oil	23-may.	29-apr.
Genovesa (S)	Oil	9-may.	21-apr.
Jabaluno (S)	table and oil	14-may.	30-apr.
Morchiaio (I)	Oil	10-may.	20-apr.
Nebbio (I)	Oil	9-may.	23-apr.
Nevadillo Blanco (S)	Oil	7-may.	24-apr.
Piangente (I)	Oil	8-may.	21-apr.
Selección N°1 (A)	Oil	13-may.	27-apr.
Villalonga (S)	Oil	13-may.	28-apr.

(A) origin Argentina (S) origin Spain (I) origin Italy.

plastic crates to the EEA-Junín oil mill for oil extraction. For each variety, three samples were harvested, and an extra sample was harvested from several trees to regulate the oil-extracting machine and remove waste from the previously processed variety.

The olive oil was extracted immediately after harvest, to ensure best quality oil, in a two-phase, cold (without added heat), and continuous system (20 kg h<sup>-1</sup>) (Alfa-Laval SPREMOLIVE NEW Single Phase. MF-Toscana Enologica Mori-Italia). Fifteen kilograms of fruits were selected for their excellent phytosanitary status and were crushed with a hammer mill. The paste produced from milling was malaxed for 40 min. Then, the paste was centrifuged to obtain an oily juice that was collected in drums. After a week, the oil was filtered through cotton and stored in dark amber glass bottles (50 mL) at -20 °C until it was analyzed. An amber glass bottle was opened for each analysis.

The industrial yield (IY) was calculated by equation (1).

$$IY(\%) = \frac{\text{olive oil extracted (Kg)}}{\text{crushed fruit (Kg)}} \times 100 \quad (1)$$

### 2.5. Free acidity and oxidative stability

Free fatty acid content (expressed as a percentage of oleic acid) was determined according to the International Olive Council (IOC) method (2015). The induction time to oil oxidation was determined using the “Rancimat” technique. The method is based on the decomposition of hydroperoxides and the formation of short-chain fatty acids, which change the electrical conductivity of water (Banco, 2017). Briefly, an olive oil sample (3 g) was heated (110 °C) and submitted to air-flow (10 L h<sup>-1</sup>) to force oxidation. The volatile compounds were collected in a beaker with 50 mL of distilled water with a conductivity of  $\leq 2.5 \mu\text{S cm}^{-1}$ . The curve inflection point showed the time (in hours) necessary to oxidize the oil sample.

### 2.6. Total phenolic content

The phenolic content was determined by the Folin-Ciocalteu method (Folin and Ciocalteu, 1927). In short, 40 g of olive oil was added to a solution of 20 mL of methanol and distilled water (80:20 % v/v); afterward, the solution was shaken and centrifuged for 10 min at 5000 rpm (2800 G-force). The supernatant was transferred to a 250 mL flask, and the extraction process was repeated three times. The final supernatant was homogenized, and an aliquot of 200  $\mu\text{L}$  was transferred to a 250 mL flask for phenolic compound determination. Then, 1.8 mL of distilled water, 10 mL of Folin-Ciocalteu (10 %), and 8 mL Na<sub>2</sub>CO<sub>3</sub> (75 g l<sup>-1</sup>) were added. The solution rested for 2 h in darkness. Total phenolic content was measured at 725 nm using a UV-Vis spectrophotometer model Lambda 25 (Perkin-Elmer Instruments, Hartford, CT). The calibration curve was obtained using caffeic acid solutions (50, 150, 250, 450, 650, and 800 mg kg<sup>-1</sup>), thus total phenolic content was expressed as mg of caffeic acid equivalents (CAE).

### 2.7. Phenolic profile

Solid Phase Extraction (SPE) was used to obtain phenolic compounds with Sep-Pak® Vac Diol 1 cc cartridges (Waters Corporation, Milford, Massachusetts, USA). The cartridges were preconditioned with 5 mL of methanol and 5 mL of n-hexane. A sample (1.6 g) of olive oil was diluted with 1 mL of n-hexane, and a non-polar oil fraction was extracted with 5 mL of n-hexane. The phenolic compound extraction was carried out with 1.5 mL of methanol at a constant dripping rate (2 mL min<sup>-1</sup>). The samples were kept in the dark at -20 °C until they were analyzed.

Identification and quantification of phenolic compounds were done by CZE (Capillary Zone Electrophoresis with a CAPEL-105 M UV detector Lumex Ltd., St. Petersburg, Russia). Equipment use conditions were the following: 20 kV, 20 mbar, injection time 2 s, 240 nm, column: 75  $\mu\text{m}$ , temperature 25 °C and wash and analysis buffer: sodium borate 30 mM and pH 9.5. The elution time of each compound was determined using a solution prepared with 12 phenolic compounds (final concentration of 76.9 mg kg<sup>-1</sup>). The calibration curves were made with a solution containing 12 phenolic compounds in known concentrations from 0.75 to 30 mg kg<sup>-1</sup> (Table 3).

### 2.8. Statistical analysis

A completely randomized experimental design of three replications (trees) for each variety (n = 3) was used. Analysis of variance was used to test oil characteristics and phenolic profiles of the 18 varieties during two seasons (2013/2014 and 2014/2015), and means were separated using the least significant differences (LSD) test for a level of significance  $\alpha = 0.05$ . Statistical analyses were performed using the InfoStat version 1.5 program (InfoStat, 2003). Linear regression was employed for correlation analysis between oil characteristics and phenolic profile using Pearson coefficients. Biplot analysis was used to select varieties with the best performance among three evaluated traits (total phenolic compounds, industrial yield, and oxidative stability).

## 3. Results and discussion

### 3.1. Maturity index and industrial yield

The maturity index was significantly different among varieties ( $p < 0.001$ ) (Table 4). On average for the two seasons, the maturity index ranged from 4.25 to 1.55, with ‘Empeltre’ and ‘Nebbio’ as the earliest and latest harvested varieties, respectively. The harvest period is under the threat of early frosts in Mendoza, Argentina (Trentacoste et al., 2019). Morelló et al. (2003) evaluating effects of frost damage in olive oils, determined lower stability oils and sensory changes, such as sweet oils and absent of bitterness. As a consequence, the harvesting period was as short as possible to avoid frost damage, totaling 16 and 24 days in 2014 (from 7 may to 23 may) and 2015 (from 20 april to 14 may), respectively. This agroecological characteristic explains the variability in the maturity index among the varieties. Traditionally, it was thought that at a low maturity index, fruits had not yet reached their maximum oil concentration. However, Bodoira et al. (2015) evaluated olive var. ‘Arauco’ and determined the best relationship between oil concentration and oil quality at a maturity index below 1. Diraman and Dibeklioglu (2009), obtained comparable results when evaluating early harvest on seven olive varieties, from seven regions of Turkey over a six-year period (2001/2007).

The industrial yield was significantly different among varieties and years ( $p \leq 0.023$ ) (Table 4). In general, our results were low because they were measured by an experimental machine. These results are useful to compare varieties under the same conditions, but not with results obtained by industrial machines. On average for the two seasons, the industrial yield ranged from 17.6 % in ‘Villalonga’ to 4.1 % in ‘Empeltre’. The percentage of industrial yield is one of the most important traits evaluated by oil makers and is related to variety, fruit moisture percentage, and maturity index. Fruit moisture decreases as fruit matures, while oil content increases (Beltrán et al., 2004). In our results, ‘Empeltre’ presented a high maturity index (4.25) but a very low industrial yield percentage (4.1 %), which shows that ‘Empeltre’ cannot express its whole potential in Mendoza as in other regions of the world. After

**Table 2**  
Monthly rainfall data taken during the trial.

Seasons	Max (°C)	Mean (°C)	Min (°C)	Rainfall (mm)
2013/2014	24.6	16.4	8.2	259.2
2014/2015	24.5	16.6	7.3	217.6
Average	24.6	16.5	7.8	238.4

Max: temperature maxima, Mean: mean temperature and Min: temperature minima.

**Table 3**  
Calibration curves equations of 12 evaluated phenolic compounds.

Phenolic compounds	Equation	R <sup>2</sup>
Tyrosol	A* = 0.66050 + 0.12068 × C*	73.71
Oleuropein	A = 0.33284 + 0.43684 × C	97.75
Hydroxytyrosol	A = 0.06749 + 0.29454 × C	97.93
Rutin	A = -0.01583 + 0.69230 × C	98.21
Catechin	A = -0.21302 + 0.56459 × C	98.84
Cinnamic acid	A = -1.04913 + 1.37278 × C	98.11
Syringic acid	A = 1.85485 + 1.50876 × C	97.75
Apigenin	A = -0.17989 + 1.82233 × C	98.61
Quercetin	A = -0.56490 + 1.32736 × C	82.21
p-Coumaric acid	A = -1.12702 + 1.07971 × C	96.94
Vanillic acid	A = -0.58340 + 0.92077 × C	92.72
Gallic acid	A = -1.81204 + 2.89314 × C	98.41

A\* area of the electrophoretic peak, C\* phenolic compounds concentration.

evaluating the agronomical and commercial behavior of five varieties grown in Tarragona, Spain, [Tous et al. \(1998\)](#) described 'Empeltre' as a variety with high fruit production and oil yield.

### 3.2. Oil characteristics

Free acidity was significantly different among varieties ( $p < 0.001$ ) ([Table 4](#)). 'Canino' showed the highest acidity (0.66 %), but was not significantly different from 'Morchiaio' (0.55 %). Thus, all the samples were within the International Olive Council legal limit (0.8 % oleic acid), which determines that oil is extra virgin quality ([IOC, 2015a,b](#)). However, free acidity is more closely

**Table 4**  
Evaluation of the oil characteristics from 18 olive varieties cultivated at the Olive Germplasm Collection of the 'Estación Experimental Agropecuaria del INTA Junín', located at the Mendoza province, Argentina. Data are the means of two growing seasons (2013/2014 and 2014/2015).

Variety	Maturity index	Industrial yield (%)	Acidity (% oleic acid)	Oxidative stability (h)	Total phenols (mg kg <sup>-1</sup> )
Arauco	1.57 g	10.8 def	0.25 fg	9.3 ghi	413.63 ab
Blanqueta	2.72 de	12.3 cde	0.45 bcd	11.2 fgh	324.77 cde
Canino	2.93 cd	14.5 bc	0.66 a	9.6 ghi	440.64 a
Criolla Salvarredi	1.98 fg	9.7 ef	0.21 g	11.4 fg	371.16 bc
Cucci	3.27 bcd	8.5 f	0.24 g	9.1 hi	105.45 k
Dritta	2.71 de	11.8 cde	0.35 def	7.5 i	241.52 ghij
Dulzal	3.63 b	12.2 cde	0.43 cd	14.9 cd	250.22 ghij
Empeltre	4.25 a	4.1 g	0.28 efg	16.9 bc	217.03 ij
Farga	3.52 bc	11.0 def	0.24 g	12.6 ef	268.93 fgh
Frantoio	3.42 bc	13.0 bcd	0.38 de	9.3 ghi	227.34 hij
Genovesa	3.48 bc	13.5 bcd	0.25 fg	14.5 de	236.74 ghij
Jabaluno	2.22 ef	8.5 f	0.24 g	20.3 a	231.58 hij
Morchiaio	3.64 ab	15.8 ab	0.55 ab	12.7 def	285.92 efg
Nebbio	1.55 g	14.4 bc	0.51 bc	11.4 fg	416.06 ab
Nevadillo Blanco	2.98 cd	11.4 def	0.53 bc	20.7 a	345.92 cd
Piangente	3.68 ab	11.4 def	0.23 g	19.1 ab	304.96 def
Selección N°1	2.97 cd	10.0 ef	0.18 g	9.6 ghi	190.81 j
Villalonga	2.15 efg	17.6 a	0.23 g	14.3 de	357.14 c
Variety	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year	0.0865	0.0234	0.751	0.592	<0.0001

Values with the same letter in the column are not significantly different according the LSD-test  $p \leq 0.05$ .

related to the sanitary state of raw materials and the storage conditions of oils than to varietal characteristics ([Nierat, 2014](#)). Therefore, this parameter does not seem to be useful for classifying among varieties.

The oxidative stability of the oil was significantly different among varieties ( $p \leq 0.0001$ ) ([Table 4](#)). The highest values were measured in oils from 'Nevadillo Blanco', 'Jabaluno', and 'Piangente' varieties (20.7 h, 20.3 h, and 19.1 h, respectively), whereas the lowest value was detected in 'Dritta' (7.5 h) which did not differ from 'Cucci', 'Frantoio', 'Arauco', 'Selección N° 1' and 'Canino'. Oil production is a seasonal output that lasts only a few months (two or three), whereas oil sales can take up to two years. Oxidative stability represents the shelf life of an oil ([Velasco, 2002](#)). Therefore, identifying varieties with higher oxidative stability allows a longer time for storage and sale.

The main importance of phenols is given by the nutritional attributes and health properties related to their antioxidant capacity when are regularly consumed ([Aguilera et al., 2004; Menendez et al., 2008; Moreno et al., 2001; Visioli, 1998; Visioli et al., 2002](#)). In the present work, the total phenolic content was significantly different among varieties and years ( $p < 0.0001$ ) ([Table 4](#)). On average for the 18 varieties and two years, total phenolic content was 290.5 mg kg<sup>-1</sup> and ranged from 105.5 to 440.6 mg kg<sup>-1</sup>. The highest values were observed in oils from 'Canino', 'Nebbio', and 'Arauco' varieties (440 mg kg<sup>-1</sup>, 416 mg kg<sup>-1</sup>, and 413 mg kg<sup>-1</sup>, respectively), while 'Cucci' showed the lowest phenolic content (105 mg kg<sup>-1</sup>). [El Riachy et al. \(2012\)](#) found similar phenol ranges when analyzing three cultivars and their cross-over, as well as [Juhaimi et al. \(2017\)](#) who reported similar phenol ranges in five different varieties from Turkey and Saudi Arabia. The narrow range of total phenolic content of this study, grouped the majority of the varieties as "medium" total phenolic content, except for 'Selección N°1', which was considered "low" total phenolic content, according to the classification of [Montedoro et al. \(1992\)](#). In their evaluation of the antioxidant capacity of many compounds, [Bouayed and Bohn \(2010\)](#) mentioned two factors that appear to influence the positive attributes of plant-food: (1) low concentrations of compounds and (2) additive or synergistic interactions between compounds. Similarly, [Lambert de Malezieu et al. \(2021\)](#) found that phenol mixtures had higher antioxidant effects than separate phenols. These finding

underline the importance of continuing to research the varieties in the collection based on their oil phenolic profiles and the synergism between those compounds.

The total phenolic content was not correlated with oxidative stability ( $r = 0.12$ ) (data not shown). Unlike what was observed by Aparicio et al. (1999) and Velasco (2002), most of the assays have been done over a small number of varieties, while in this work, 18 varieties were used. Probably, the high genetic variability and the narrow range observed among total phenolic compounds can explain the lack of correlation.

The olive tree normally has an “on-year” followed by an “off-year”, which represents a high and a low crop load year, respectively. Olive trees with low crop load advance their maturity, while olive trees with high crop load delay it (Trentacoste et al., 2010). The differences observed in total phenolic compounds between years could be explained by the lower maturity index in 2014 (2.85) compared with 2015 (3.02) due to a higher crop load during 2014.

### 3.3. Oil phenolic profile

The phenolic profile was significantly different among varieties and years ( $p < 0.0001$ ) (Table 5). The average value for all phenols and varieties for the two growing seasons was  $38.5 \text{ mg kg}^{-1}$ . The mean values of tyrosol, hydroxytyrosol, catechin, cinnamic acid, syringic acid, quercetin, and *p*-coumaric acid concentration for all varieties were significantly higher during 2015 than in 2014. In contrast, the content of oleuropein, rutin, apigenin, vanillic acid, and gallic acid concentration was higher during the year 2014. On average, tyrosol and quercetin showed the highest concentration, each reaching around 26 % of the total phenolic compounds. Oleuropein and apigenin were other phenolic compounds that showed high relative concentrations of 14 % and 8 %, respectively. In contrast, gallic and vanillic acids were the phenolic compounds with the lowest concentration (Table 5).

Concerning the sum of individual phenols, ‘Canino’ and ‘Morchiaio’ varieties presented the highest and lowest concentration ( $106.37$  and  $15.38 \text{ mg kg}^{-1}$ , respectively). As shown in Fig. 1, the total phenolic content was much higher than the sum of individual ones evaluated in the present work, which indicates two aspects to

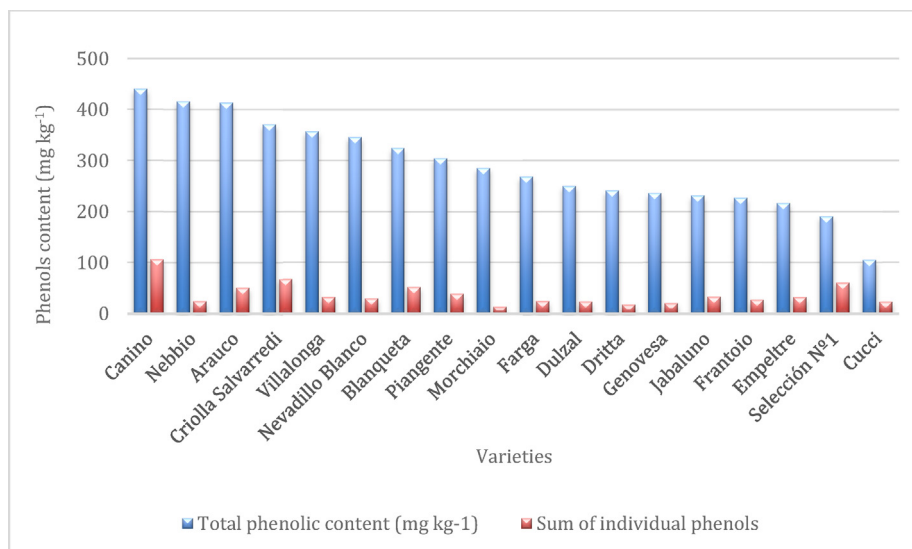
consider: (1) as Everett et al., 2010 determined, the Folin method reacts over many compounds, particularly phenols; and (2) that much of the total phenolic content has not been evaluated in terms of phenolic profile. Guodong et al. (2019) determined that between 44 % and 70 % of the total phenolic concentration corresponded to maslinic acid (not evaluated in the present study) when evaluating putative genes for phenol biosynthesis in different fruit growth stages and leaf aging degrees. As a result, and without sacrificing the goal of evaluating the varieties under identical conditions, it was decided to classify them according to their total phenolic content.

Regarding the concentration of each phenol by variety, ‘Dulzal’ and ‘Cucci’ presented the highest concentrations of oleuropein ( $9.67$  and  $9.46 \text{ mg kg}^{-1}$  respectively) without significant differences between them, while ‘Frantoio’ presented the lowest value ( $2.18 \text{ mg kg}^{-1}$ ). The highest phenolic alcohol concentration was found in the ‘Canino’ variety (tyrosol:  $69.7 \text{ mg kg}^{-1}$  and hydroxytyrosol:  $5.13 \text{ mg kg}^{-1}$ ), whereas the lowest was detected in ‘Morchiaio’ (tyrosol:  $1.75 \text{ mg kg}^{-1}$  and hydroxytyrosol:  $0.02 \text{ mg kg}^{-1}$ ). The hydroxytyrosol and tyrosol contents were also reported in Sarulak ( $14.36$  and  $9.39 \text{ mg kg}^{-1}$ ), Savrani ( $2.56$  and  $3.65 \text{ mg kg}^{-1}$ ), Al-Joif ( $2.29$  and  $3.94 \text{ mg kg}^{-1}$ ), Gemlik ( $14.42$  and  $21.47 \text{ mg kg}^{-1}$ ), and Ayvalık ( $1.23$  and  $7.15 \text{ mg kg}^{-1}$ ) olive varieties by Juhaimi et al. (2017). Both phenolic alcohols show antioxidant potential (Owen, 2000). Furthermore, hydroxytyrosol has been linked to antimicrobial properties as well as the induction of cell apoptosis (Ferran-Font, 2015). According to Brenes et al. (2001), the hydrolysis of oleuropein gives rise to an increase in phenolic alcohols. Hydroxytyrosol production from oleuropein inside the seed could be related to an oil protection mechanism (reserve substance for the embryo), and simultaneously, could be a senescence beginning signal. That could explain why in 2015, with a slightly higher maturity index, there was a higher concentration of both alcohols and the absence of oleuropein. However, this behavior could also be explained by the evolution of oleuropein to oleuropein aglycone, a compound that was not evaluated in the present work (Carrasco-Pancorbo et al., 2006). In addition, for many years, it has been known that there is a decrease in oleuropein during fruit maturation (Amiot et al., 1986, Dağdelen et al., 2013).

**Table 5**  
Phenolic profile from 18 olive varieties analyzed during two growing seasons (2013/2014 and 2014/2015) in Junín, Mendoza, Argentina.

Varieties	Tyrosol	Oleuropein	Hydroxytyrosol	Rutin	Catechin	Cinnamic acid	Syringic acid	Apigenin	Quercetin	<i>p</i> -Coumaric acid	Vanillic acid	Gallic acid
Arauco	11.37 c	4.81 e	0.66 cde	nd	5.39 a	nd	nd	1.86 g	12.67 cd	14.26 b	nd	nd
Blanqueta	34.05 b	3.18 fg	nd	nd	1.27 de	nd	1.52 defg	2.09 fg	10.04 def	nd	nd	nd
Canino	69.7 a	5.61 de	5.13 a	nd	4.62 a	nd	1.09 fgh	4.56 c	15.66 bc	nd	nd	nd
Criolla Salvarredi	5.51 def	5.31 de	0.53 de	1.16 b	5.04 a	2.12 abcd	1.15 efg	5.78 b	13.03 cd	28.47 a	nd	nd
Cucci	1.84 gh	9.46 a	1.27 cd	nd	nd	nd	1.74 cdef	1.89 g	6.17 fgh	nd	2.42	nd
Dritta	5.08 defg	3.2 fg	1.25 cd	nd	1.80 cde	0.48 d	2.23 bcde	2.29 fg	7.56 efgh	nd	nd	nd
Dulzal	3.38 efgh	9.67 a	nd	nd	4.00 ab	2.81 abc	nd	0.77 h	3.82 hi	nd	nd	nd
Empeltre	2.38 fgh	3.28 f	1.61 c	nd	nd	1.26 bcd	2.12 bcdef	2.10 fg	19.62 b	nd	nd	nd
Farga	4.12 defgh	2.81 fg	nd	nd	1.87 cde	0.96 cd	3.47 a	2.61 ef	9.21 defg	nd	nd	nd
Frantoio	6.36 de	2.18 g	0.99 cde	nd	4.26 ab	0.63 d	0.48 gh	2.94 e	9.67 defg	nd	nd	nd
Genovesa	2.42 fgh	7.36 b	3.66 b	nd	nd	nd	nd	2.18 fg	4.76 h	nd	nd	nd
Jabaluno	4.67 defg	5.72 de	0.41 de	nd	3.58 abc	2.11 abcd	3.09 ab	1.80 g	10.97 de	0.69 d	nd	0.34
Morchiaio	1.75 h	6.93 bc	0.02 e	nd	3.67 abc	nd	nd	3.01 e	nd	nd	nd	nd
Nebbio	5.56 def	5.37 de	nd	nd	4.27 ab	3.26 ab	2.73 abc	3.81 d	nd	nd	nd	nd
Nevadillo Blanco	4.89 defg	6.30 cd	nd	nd	5.26 a	3.88 a	1.47 efg	2.38 efg	5.82 gh	nd	nd	nd
Piangente	4.85 defg	2.81 fg	1.30 cd	1.49 a	1.53 de	1.86 abcd	2.57 abcd	4.84 c	11.75 cde	6.73 c	nd	nd
Selección N°1	7.16 d	7.42 b	1.21 cd	nd	2.69 bcd	nd	2.11 bcdef	8.15 a	31.72 a	nd	nd	nd
Villalonga	6.01 de	7.51 b	0.47 de	nd	4.72 a	1.92 abcd	1.35 efg	2.97 e	7.79 efgh	nd	nd	nd
Variety	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values with the same letter in the column are not significantly different according to the LSD-test at  $p \leq 0.05$ . nd: not detected. Values were expressed in  $\text{mg CAE Kg}^{-1}$ .

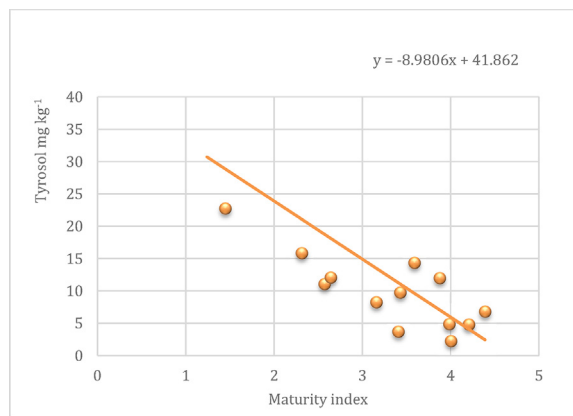


**Fig. 1.** Total phenolic content and sum of all phenolic compounds analyzed in the oil of 18 olive varieties from the germplasm collection of the INTA Junín. Mendoza, Argentina.

Flavonoids were the most abundant among the phenols with an average of 16.27 mg kg<sup>-1</sup> for all varieties and the two years of study. Rutin was present only in two varieties, ‘Nevadillo Blanco’ (1.49 mg kg<sup>-1</sup>) and ‘Criolla Salvarredi’ (1.16 mg kg<sup>-1</sup>) during the first year. This behavior can be explained because rutin is a precursor of luteolin, a compound not evaluated here. The highest concentration of catechin was observed in ‘Arauco’ (5.39 mg kg<sup>-1</sup>) whose content was not significantly different from ‘Canino’, ‘Criolla Salvarredi’, ‘Dulzal’, ‘Frantoio’, ‘Jabaluno’, ‘Morchiaio’, ‘Nebbio’, ‘Nevadillo Blanco’, and ‘Villalonga’. The lowest concentration was observed in ‘Blanqueta’ (1.27 mg kg<sup>-1</sup>) not different from ‘Dritta’, ‘Farga’, ‘Piangente’, and ‘Selección N°1’. Catechin had an important genotypic effect, not observed in ‘Cucci’ and ‘Genovesa’ in the study years. In addition, catechin has been related to a higher oil-oxidation tolerance up to a threshold of 250 μM (Di Mattia et al., 2009). This observation could explain why varieties with low total phenol concentration such as ‘Empeltre’ (217 mg kg<sup>-1</sup>) or ‘Jabaluno’ (232 mg kg<sup>-1</sup>) showed high oxidative stability (16 and 20 h, respectively). The highest concentrations of apigenin and quercetin were measured in ‘Selección N°1’ (8.15 and 31.72 mg kg<sup>-1</sup> respectively), while the lowest values were observed in ‘Dulzal’ (0.77 and 3.82 mg kg<sup>-1</sup>, respectively). Apigenin

presented a significantly higher concentration during the first year (2014), differently from quercetin and catechin. Quercetin and rutin showed higher environmental variability because they were observed only in 2015. Similar results were observed by Dell’Agli et al. (2008). The apigenin, quercetin and rutin contents were also reported in ‘Leccino’ variety (0.38, 1.48 and 483.33 mg kg<sup>-1</sup>) by Guodong et al. (2019).

Except for ‘Genovesa’ and ‘Morchiaio’, the rest of the varieties presented at least one phenolic acid. Among all evaluated phenols, acids showed the lowest concentration (1.11 mg kg<sup>-1</sup>); however, they were higher than the concentration observed by Montedoro et al. (1992). Cinnamic, syringic, and *p*-coumaric acids had a higher concentration during the second year (2015). Cinnamic acid ranged from 0.48 mg kg<sup>-1</sup> in ‘Dritta’ to 3.88 mg kg<sup>-1</sup> in ‘Nevadillo Blanco’, while syringic acid ranged from 3.47 mg kg<sup>-1</sup> in ‘Farga’ to 0.48 mg kg<sup>-1</sup> in ‘Frantoio’. The *p*-coumaric acid was present only in four varieties: ‘Criolla Salvarredi’, ‘Arauco’, ‘Piangente’, and ‘Jabaluno’. The *p*-coumaric acids content was also reported in ‘Arbequina’ (0.17 mg kg<sup>-1</sup>), ‘Picual’ (0.02 mg kg<sup>-1</sup>) ‘Frantoio’ (2.93 mg kg<sup>-1</sup>), ‘Arauco’ (0.18 mg kg<sup>-1</sup>) and ‘Nevadillo’ (1.68 mg kg<sup>-1</sup>) varieties by Monasterio et al., 2013. Lastly, vanillic and gallic acids were detected only during the first year and in two



**Fig. 2A.** Relationship between maturity index and tyrosol content in the oil from 18 olive varieties from the germplasm collection of the INTA Junín. Mendoza, Argentina.



**Fig. 2B.** Relationship between maturity index and hydroxytyrosol content in the oil from 18 olive varieties from the germplasm collection of the INTA Junín. Mendoza, Argentina.

varieties, 'Cucci' and 'Jabaluno'. In this respect, [Morelló et al. \(2003\)](#) discovered a slight increase in vanillic acid in oils made with freeze-damaged fruits. In April 2014, the minimum temperature registered was  $-1^{\circ}\text{C}$ . Even though the olive is a frost-resistant tree, that event could have triggered the vanillic acid production.

Interestingly, many relationships among parameters from both years were evaluated, but only the total phenolic content and maturity index showed an acceptable correlation coefficient ( $r = -0.46$ ;  $p = 6.3 \text{ E}-0.7$ ). Relationships among the parameters in separate years were also evaluated. Tyrosol concentration decreased while the maturity index increased ( $r = -0.63$ ,  $p = 0.0155$ ) ([Fig. 2A](#)). In contrast, hydroxytyrosol concentration increased as maturity increased ( $r = 0.67$ ;  $p = 0.0337$ ) ([Fig. 2B](#)). Similar results were obtained by [Bonoli et al. \(2003\)](#), who evaluated the number of phenolic compounds in oil from several Italian olive varieties.

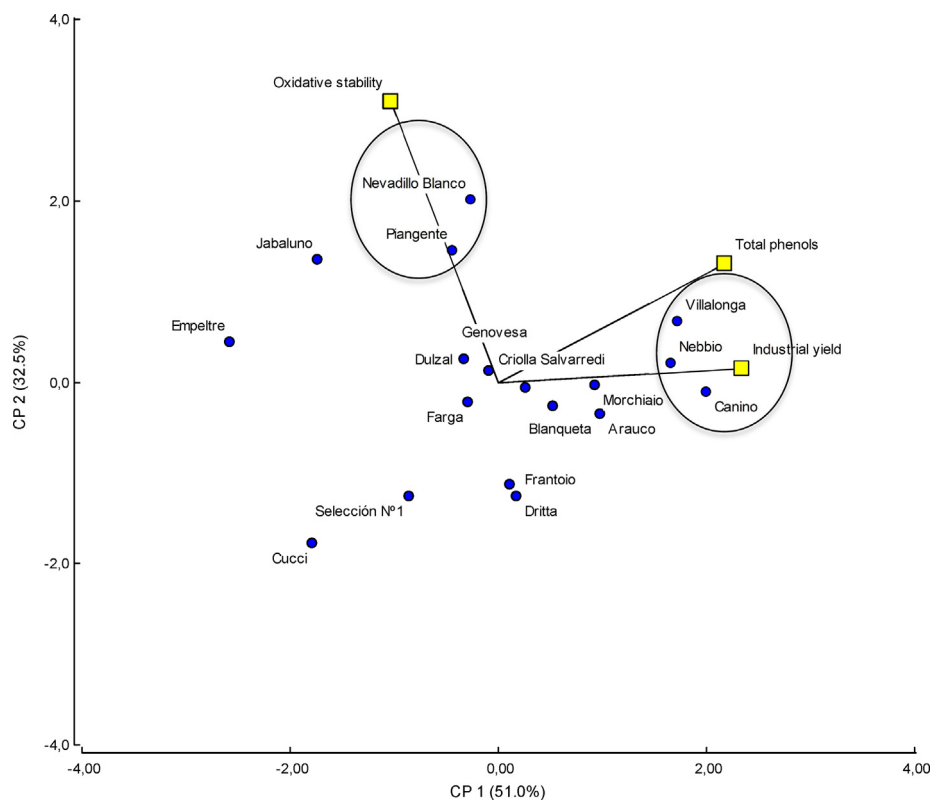
### 3.4. Selection of varieties

Using biplot analysis, five out of eighteen varieties were highlighted in terms of total phenolic content, industrial yield, and oxidative stability (see [Fig. 3](#)). The five selected varieties are not currently cultivated in Argentina, and they presented major characteristics compared to the traditional commercial varieties such as 'Empeltre', 'Frantoio', 'Farga' and 'Arauco'. Varieties were classified taking into account the maturity index: one variety of late maturation ('Nebbio'), three of medium maturation ('Villalonga', 'Nevadillo Blanco', and 'Canino'), and one of early maturation ('Piangente').

In these varieties, the sum of individual phenols (from  $106 \text{ mg kg}^{-1}$  to  $13 \text{ mg kg}^{-1}$ ) was quite lower than the total pheno-

lic content (from  $441 \text{ mg kg}^{-1}$  to  $106 \text{ mg kg}^{-1}$ ). This sum of phenols only represented a meager proportion of the total phenolic content ( $<32\%$ ) ([Fig. 1](#)). [Guodong et al. \(2019\)](#) determined that 75 % of total phenols were represented by maslinic acid (not evaluated in the present study). Olive oil's phenolic profile varies widely according to varieties and environments. [Yorulmaz et al. \(2012\)](#) proposed using trans cinnamic acid to differentiate Turkish varieties grown in Anatolia but could not identify hydroxytyrosol in any of one hundred samples analyzed. [Esti et al. \(1998\)](#), proposed using demethyloleuropein as a varietal marker from Italian varieties (Coratina and Leccino). In contrast, [Franco et al. \(2014\)](#) found quantitative but not qualitative differences among Spanish varieties. For these reasons, we selected total phenolic content as a parameter of selection to avoid rejecting varieties because of the limited phenolic profile used. However, the olive phenolic profile in unknown commercial varieties, together with the knowledge of specific properties of phenolic compounds, is a useful tool to select varieties based on specific targets ([Hu et al., 2014](#)). Thus, a biplot analysis was conducted taking into account total phenolic content, industrial yield, and oxidative stability. The scatter, comparison, and ranking biplot explained 83.5% of the total variation (51 % and 32.5 % from PC1 and PC2, respectively). 'Villalonga' was highlighted for its total phenolic content, 'Nebbio' and 'Canino' for their industrial yield, and 'Nevadillo Blanco' and 'Piangente' for their oxidative stability.

The five selected varieties are not cultivated in Argentina and have been classified above commercially cultivated varieties such as 'Frantoio', 'Farga' and 'Empeltre', in terms of total phenolic content, industrial yield, and oxidative stability. Regarding 'Arauco', this variety, so widely cultivated in the country, also showed a better performance than 'Frantoio', 'Farga', and 'Empeltre'.



**Fig. 3.** Biplot model discriminating varieties according three evaluated traits (Oxidative stability, total phenolic content and industrial yield).

#### 4. Conclusions

The characterization of the olive oil of 18 varieties not cultivated in the country and preselected from the Olive Germplasm Collection of the EEA Junín (Mendoza, Argentina) allows identifying five promising varieties ('Villalonga', 'Nebbio', 'Nevadillo Blanco', 'Canino', and 'Piangente') according to their industrial yield, acidity, oxidative stability, total phenolic compounds, and phenolic profile. These varieties were highlighted far above the most widespread cultivars in Mendoza, Argentina (such as 'Empeltre', 'Frantoio', and 'Farga').

Continuing evaluations at the experimental site and more work elsewhere are needed to ratify these results, especially as regards to improve oils by looking for higher yields, longer shelf-life, better quality and healthy attributes. Evaluating many accessions from a local olive germplasm collection, varieties with better characteristics than the ones currently cultivated can be selected. They can even be re-selected according to new knowledge about properties of their phenolic content.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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