Elsevier Editorial System(tm) for Food Chemistry

Manuscript Draft

Manuscript Number: FOODCHEM-D-16-00134R3

Title: Characterization of virgin olive oils produced with autochthonous Galician varieties

Article Type: Research Article (max 7,500 words)

Keywords: characterization, Galician virgin olive oils, phenolic compounds, volatile compounds, sensory analysis.

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Abstract: The interest of Galician oil producers (NW Spain) in recovering the ancient autochthonous olive varieties Brava and Mansa has increased substantially in recent years. Virgin olive oils produced by co-crushing both varieties in two different proportions, reflecting the usual and most common practice adopted in this region, have gradually emerged for the production of virgin olive oils. Herein, the sensory and chemical characteristics of such oils were characterized by quality and genuineness-related parameters. In particular, minor components such as phenolic and volatile compounds were investigated. The results of chemical analysis are also discussed in terms of their effective contribution to the sensory profile, which suggests useful recommendations for olive oil producers to improve the quality of oils. A major content of phenolic compounds as well as a predominance of trans-2-hexen-1-al and cis-3-hexen-1-ol, responsible for bitter and green-leaf sensory notes, were seen when co-crushing with a higher proportion of Brava olives.

# Characterization of virgin olive oils produced with autochthonous Galician varieties

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## Abstract

The interest of Galician oil producers (NW Spain) in recovering the ancient autochthonous olive varieties *Brava* and *Mansa* has increased substantially in recent years. Virgin olive oils produced by co-crushing both varieties in two different proportions, reflecting the usual and most common practice adopted in this region, have gradually emerged for the production of virgin olive oils. Herein, the sensory and chemical characteristics of such oils were characterized by quality and genuineness-related parameters. The results of chemical analysis are discussed in terms of their effective contribution to the sensory profile, which suggests useful recommendations for olive oil producers to improve the quality of oils. Antioxidant compounds, together with aromas and coloured pigments were determined, and their contribution in determining the functional value and the sensory properties of oils was investigated. In general, given the high levels of phenolic compounds (ranging between 254 and 375 mg/kg oil), tocopherols (about 165 mg/kg oil) and carotenoids (10-12 mg/kg oil); these are oils with long stability, especially under dark storage conditions, because stability is reinforced with the contribution of chlorophylls (15-22 mg/kg oil). A major content of phenolic compounds, as well as a predominance of *trans*-2-hexen-1-al within odour-active compounds (from 897 to 1645 µg/kg oil), responsible for bitter sensory notes. This characterization allows to developing new antioxidant-rich and flavour-rich VOOs, when co-crushing with a higher proportion of *Brava* olives, satisfying the consumers' demand in having access to more healthy dishes and new sensorial sensations.



***Keywords:*** *characterization, Galician virgin olive oils, phenolic compounds, volatile compounds, sensory analysis.*

## 1. Introduction

Olive oil is a valuable product that is traditionally produced in Mediterranean countries. Olive groves are present in 34 of the 50 Spanish provinces and occupy an area of 2,584,564 ha (AICA, 2015). Spain is the largest olive oil producer worldwide. In the last few years, half of Spanish olive oil production is consumed domestically and the other half is exported (Morales, Aparicio-Ruíz and Aparicio, 2013). Within the Spanish territory, Galicia (NW Spain) has gradually emerged as a new olive-growing zone producing virgin olive oils (VOOs) with autochthonous cultivars growing in particular environmental and pedoclimatic conditions that characterize this area. Although traditional Spanish varieties, such as Arbequina and Picual cv., are predominant in the new Galician plantations, the interest of oil producers in ancient autochthonous varieties (known by producers as *Brava* and *Mansa*) has increased substantially in recent years due to their suitable edafo-climatic adaptation.

Two previous studies have evaluated the potential of these ancient cultivars (Reboredo-Rodríguez *et al*, 2015a, 2015b). In the first, with the aim of providing extra value to the final VOOs, Galician Arbequina or Picual fruits, separately, were co-crushed with low proportions of a mixture of *Brava* and *Mansa* varieties (such a mixture is known by producers as *Local*). The experimental results showed that the effect of co-crushing on minor compounds, phenolics, and $C_6$ volatiles, both responsible for the sensory profile, cannot be easily modulated because of a complex, non-progressive, and non-predictable change in their composition, in contrast to most quality indices (*viz.* free acidity, peroxides, and UV extinction coefficients) and fatty acid composition, which change linearly in strict correlation with the fruit mass ratio. On the other hand, blending *Local* VOOs (also in low percentages) with Arbequina or Picual monovarietal VOOs might be another strategy to produce high quality VOOs with pre-established characteristics. In

this case, previous knowledge of the quality-related indices and fatty acid composition as well as the concentrations of minor compounds of monovarietal VOOs make it possible to obtain oils "*à la carte*".

Nowadays, the current trend of the VOO market is production of high quality products from traditional minor olive varieties with a specific designation of origin and characteristic, well-defined sensory, nutritional, and health promoting properties (especially with respect to the aromatic and phenolic composition) (Del Monaco *et al*, 2015; Bajoub *et al*, 2015). Up to now, no investigations have been carried out on the chemical and sensory characterization of VOOs produced exclusively from *Brava* and *Mansa* autochthonous Galician cultivars. Nevertheless, since the local Galician producers traditionally co-crush different proportions of fruits from *Brava* and *Mansa* cultivars, the aim of this work was to characterize VOOs produced by mixing fruits of these varieties in different proportions, similar to the ones adopted by producers. Towards this aim, chemical parameters and sensory analysis were first investigated to classify olive oils according to EU Regulation 2568/91 and subsequent amendments. Moreover, antioxidant compounds, together with aromas and coloured pigments were determined, and their contribution in determining the functional value and the sensory properties of oils was investigated. This characterization allows to developing new VOOs by mixing these new varieties with other high-yield varieties with two main purposes: the search for new antioxidant-rich and flavour-rich oils for dressings. These are the two major trends driving the market sells today, since the consumers demand more healthy dishes and new sensorial sensations. Food chemists have the goal to satisfy consumers' demands and help food companies to increase the level of sales with this kind of innovations regarding olive oil-derived products and dishes.

## 2. Materials and Methods

### 2.1. Olive oil samples

Olives were harvested in the 2013/2014 crop season (specifically, between November 2013 and January 2014) in a cultivation area under organic agricultural practices located between two municipalities, Ribas do Sil (42º 27´59.8´´ N 7º 17´15.8´´W) and Quiroga (42º 29´04.8´´ N 7º 12´33.4´´W) in the Lugo province (NW Spain). **Table 1** shows the climatic conditions for the study area over the crop year 2013.

Four VOOs (coded as VOO1, VOO2, VOO3, VOO4) were produced by co-crushing of different proportions of two varieties known by the local producers as *Brava* and *Mansa*. Neither of the autochthonous varieties are included in the database of the World Olive Germplasm Bank of Córdoba, Spain (WOGBC), which is one of the world's largest collections of olive germplasm (Trujillo *et al*, 2014).

It should be noted that obtaining monovarietal oils of *Brava* and *Mansa* at a semi-industrial scale in this area is economically unprofitable due to the low production. In particular, VOO1 and VOO2, crushed on 27 November and 9 December, respectively, were the result of mixing 70% *Brava* and 30% *Mansa*. On the other hand, VOO3 and VOO4 were both obtained by co-crushing 90-100% *Mansa* and 0-10% *Brava* olives, but on 9 and 17 January, respectively. To summarize data from oils obtained by the same percentages of the two varieties, despite their different maturation indexes, all analyses individually performed on VOO1 and VOO2 were averaged, resulting in VOOa (n=2). The same was performed with the other oils (VOO3 and VOO4), resulting in VOOb (n=2). These percentages are among the most common that it is possible to find in the cultivation area under study.

To suppress variability due to the extraction procedure, oils were obtained under identical conditions at a semi-industrial scale in a local two phase mill. Oils were allowed to settle and racked several times for about 4 months before sampling, since this is the procedure typically used by local producers before marketing their oil. Three replicates of each of the four oils were sampled and analyzed. Once in the laboratory, samples were kept at a constant temperature of 10±2°C in amber bottles without headspace until analysis.

## 2.2. Analytical plan

### *2.2.1. Chemical and sensory parameters for classification of olive oils in different commercial classes*

In conjunction with sensory analysis, the following chemical parameters, valid for establishing quality and authenticity criteria of VOOs, were determined by using the analytical methods proposed in the different Annexes of EU Regulation 2568/91 and subsequent amendments. The acceptable values for these parameters in olive oil are regulated by the European Union (EU Regulation 1348/2013).

***Free acidity (FA).*** FA, expressed as a percentage of oleic acid, was determined by a simple acid-base titration with 0.1 M KOH of free fatty acids in an oil sample previously dissolved in ethanol/ether 2:1 (v/v) (EU Regulation 702/2007).

***Peroxide value (PV).*** PV, expressed as milliequivalents of active oxygen per kg of oil (meq $O_2$/kg) was determined by titration with 0.01 N $Na_2S_2O_3$ of an oil sample previously dissolved in acetic acid/chloroform 3:2 (v/v) in the presence of KI (EU Regulation 2568/91).

***Specific UV extinction coefficients ($K_{232}$ and $K_{270}$).*** $K_{232}$ and $K_{270}$ extinction coefficients were calculated from absorption values at 232 and 270 nm, respectively, and measured with an UV-Vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan), previously calibrated with an optical glass filter containing holmium oxide (Type 666-F1, Hellma GmbH & Co., Müllheim, Germany) by analyzing a 1% solution of the oil in cyclohexane and quartz cuvette with a path length of 1 cm (EU Regulation 299/2013).

***Sensory analysis.*** Sensory evaluation of olive oil samples was carried out by nine fully trained judges of the panel of the Department of Food Science (University of Bologna, Italy) recognized in 2006 by the Italian Ministry (MIPAAF, Ministry of Agricultural Policies, Food and Forestry), and in accordance with the official method of International Olive Oil Council (IOC/T.20/Doc.Nº15/Rev.7/2015) within the framework of EU Regulations 1348/2013.

The trained tasters evaluated positive gustatory (bitter), olfactory-gustatory(fruity), and tactile/kinaesthetic attributes (pungent), as well as negative attributes, namely fusty/muddy sediment, musty-humid-earthy, winey-vinegary-acid-sour, frostbitten olives, rancid, and others (heated, burnt, hay-wood, rough, greasy, vegetable water, brine, metallic, esparto, grubby, cucumber). In addition, the tasters had the possibility to describe each oil with positive olfactory descriptors (i.e. greenly fruity, ripely fruity, red fruits, exotic fruits, apple, almond, grass, green-leaf, floral, aromatic herbs, thistle, tomato), according to the list of descriptors reported in IOC/T.20/Doc. Nº 22 November 2005. All the attributes were assessed on an oriented 10 cm linear scale and their intensities were quantified by measuring the location (in cm) of the mark from the origin of the scale. The data obtained for the five descriptors that finally characterized the selected oils (*viz.* fruity, bitter, pungent, green-leaf, ripely fruity) were used to define the sensory profile of each sample.

***Total sterols, erythrodiol, and uvaol.*** The sterol fraction was separated by preparative chromatography. Next, a silanization reaction was performed to analyze the sterol composition by gas chromatography-flame ionization detection (GC-FID) (EU Regulation 1348/2013).

***ΔECN42.*** The percentage difference between the theoretical and actual content of triglycerides with ECN42 (ECN42 is corresponded with trilinolein) provides the ΔECN42 parameter (EU Regulation 2568/91). Equivalent Chain Number ECN= CN-2*n*, where CN is the actual acylcarbon number and *n* the number of double bonds of fatty acids constituting the triacylglycerols.

***Waxes.*** Waxes were determined by GC-FID after passing the oil sample through a preconditioned packed silica gel column with *n*-hexane and then eluted with *n*-hexane/ethyl ether 99:1 (v/v) (EU Regulation 2568/91).

**Tocopherols.** Their content was determined according to the IUPAC 2.432 method. A sample of 1.5 g of VOO was dissolved in 10 mL hexane and injected into the HPLC system. Tocopherols were quantified by external α-, β-, γ- and δ- tocopherol standards from Sigma Chemical Co.

**Squalene.** Squalene was obtained by adsorption chromatography following the method described by Lanzón, Albi, Cert and Gracián (1994). GC was performed by using an Agilent 6890A chromatograph equipped with a cold on-column injector with oven-track system and a flame-ionization detector. Concentration of squalene was obtained comparing the total area and the squalene internal standard area.

**Pigment content.** Carotenoids and chlorophylls were determined as described by Minguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sanchez-Gomez, and Garrido-Fernandez (1991). 3 g of VOO was accurately weighed and dissolved in cyclohexane up

188 to a final volume of 10 mL; the carotenoid and chlorophyll content were determined by
189 measuring the absorbance at 470 and 670 nm, respectively. The results were expressed
190 as mg $kg^{-1}$ and calculated using the following equations:

191 $$[\text{Chlorophylls}]\ \text{mg kg}^{-1} = (A_{670} \times 10^6) / (E_1 \times 100 \times d)$$

192 $$[\text{Carotenoids}]\ \text{mg kg}^{-1} = (A_{470} \times 10^6) / (E2 \times 100 \times d)$$

193 where A is the absorbance, d is the spectrophotometer cell thickness (1 cm) and $E_1$ and
194 $E_2$ are, respectively, the values of the specific extinction coefficients: $E_1$ = 613 for
195 pheophytin (as major component in the olive oil chlorophyll fraction), and $E_2$ = 2000 for
196 lutein (as major component in the olive oil carotenoid fraction).

197

198 ***2.2.2. Phenolic compounds***

199 ***Determination of phenolic compounds by HPLC.*** Phenolic compounds in the VOOs
200 samples were also determined following the procedure developed by Mateos *et al*
201 (2001). Briefly, a solution of the internal standard (250 mL of 15 mg/kg of syringic acid
202 in methanol) was added to a sample of VOO (2.5 g) and the solvent was evaporated
203 with a rotary evaporator at 35 ºC under vacuum. The oil was then dissolved in 6 mL of
204 hexane and a diol-bonded phase cartridge (Supelco Co., Bellefonte, USA) was used to
205 extract the phenolic fraction. Phenols were eluted with methanol (15 mL) and the
206 solvent was removed with a rotary evaporator at 35 ºC under vacuum until dryness. The
207 phenolic residue was dissolved in methanol/water (1:1 v/v; 250 µL) and analysed by
208 HPLC using an Agilent Technologies 1100 series system equipped with an automatic
209 injector, a column oven and a diode array UV detector. A ZORBAX SB-C18 column
210 (250 x 4.6 i.d. mm, 5 µm particle size) (Agilent Technologies, USA) was used,
211 maintained at 30 ºC, with an injection volume of 20 µL and a flow rate of 1.0 mL/min.

Mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B) and acetonitrile (C): from 95% (A) – 2.5% (B) – 2.5% (C) to 34% (A) – 33% (B) – 33% (C) in 50 min. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard.

***Determination of phenolic compounds by spectrophotometric assays***

***Extraction of phenolic compounds***

The phenolic fraction was extracted from olive oils following the International Olive Council method (IOC/T.20/Doc Nº 29), with some modifications. Briefly, 2.0 g of olive oil were weighed in a 15 mL screw-cap tube, 6 mL of MeOH/$H_2O$ mixture (80/20, v/v) was added and shaken for 1min. The extraction was performed usingan ultrasonic bath for 15 min at room temperature. The tube was then centrifuged at 4000 rpm for 25min. The MeOH/$H_2O$ phase was collected and was filtered through a 0.2 μm filter with a nylon membrane.

***Determination of the total phenolic content using the Folin-Ciocalteu (FC) assay.*** FC was applied according to Mateos, Espartero, Trujillo and Rios (2001). A volume of 0.1 mL of the phenolic extract was added to 0.5 mL FC reagent and 2 mL of $Na_2CO_3$ (15% w/v) in a 10 mL volumetric flask reaching the final volume with purified water. Each sample was stored for at least 2h at room temperature before reading the absorbance; total phenolic compounds were detected at 750 nm and quantified using both gallic acid (GA) ($r^2$=0.9970) and hydroxytyrosol (HTyr) ($r^2$=0.999) calibration curves. Data were expressed as mg GA/g of oil and mg HTyr/g of oil, respectively.

***Determination of total o-diphenolic content.*** An aliquot of 4 mL of a solution prepared by mixing 0.5 mL of phenolic extract and 5 mL of MeOH/$H_2O$ (1:1 v/v) were added to 1 mL of a 5% solution of sodium molybdate dihydrate in EtOH/$H_2O$ (1:1 v/v) and

vortexed for 1 min. After 10 min at room temperature, the mixture was centrifuged for 5 min at 3000 rpm; *o*-diphenolic compounds were detected at 370 nm and quantified using GA calibration curves ($r^2$=0.9985). Data were expressed as mg GA/g of oil (Cert, Romero and Cert, 2007).

### *2.2.3. Volatile compounds*

The analysis of volatile compounds was performed by automated SPME extraction coupled with GC/qMS. SPME fibers were coated with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) phase (50/30 µm, 2 cm long from Supelco Ltd., Bellefonte, PA, USA). In particular, a GCMS-QP2010 Ultra (Shimadzu) provided with an auto sampler AOC-5000 plus (Shimadzu) was used. For preparation of the sample, 4-methyl-2-pentanone (Fluka, New York, USA) dissolved in refined peanut oil (0.1 g at a concentration of 5 ppm) was added as an internal standard to 1 g of oil. After pre-incubation (2 min), volatile compounds were extracted for 30 min at 40 °C, and then desorbed in the injector for 5 min at 240 °C.

Desorption time was 5 min. Analytes were separated on a ZB WAX column 30 m, 0.25 mm i.d., 1.00 µmf.t. (Phenomenex, Torrence, CA, USA) coated with polyethylene glycol phase. The carrier gas, helium, was circulated at 1 mL/min in constant flow mode. A split/splitless injector in the split mode was used (split ratio, 1:10). Column temperature was as follows: 40ºC for 10 min; 3ºC/min ramp to 200ºC (held for 3 min) and 10ºC/min ramp to 240ºC (held for 5 min). The ion source and the transfer line were set at 200ºC and 240ºC, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 30-250 amu mass range.

Volatile identification was tentatively obtained by comparison of mass spectral data with information from the NIST library (2005 version), MS literature data, and available standards. A calibration curve was made using mixtures prepared by

dissolving the internal standard in refined peanut oil at different concentrations; volatile compounds were quantified and expressed as μg of internal standard per kg of oil.

## 2.3. Statistical analysis

All chemical analyses were performed in triplicate. Data were subjected to Student´s *t*-test using the statistical software package Statgraphics Centurion XV for Windows v. 15.2.06 from Statistical Graphics Corp. (Herndon, VA,USA).

## 3. Results and discussion

### 3.1. Classification of olive oils according to European Regulations

EU Regulation 2568/91 and subsequent amendments establishes limit values for several chemical parameters, in conjunction with organoleptic analysis (Panel test) of olive oils, with the purpose of assessing their quality, checking their genuineness, and classifying them within the different categories established by the European Commission (EU Regulation 1348/2013).

#### *3.1.1. Quality-related parameters*

Olive oils are classified by the European law (EU Regulation 1348/2013) according to quality, which is established on the basis of set parameters that depend on the quality of olives, accuracy in milling technologies, and mode of preservation of the oils.

***FA:*** FA percentage is a direct measure of the quality of the oil and represents the extent of hydrolytic activities reflecting the care taken from blossoming and fruit set to eventual sale and consumption of the oil. Both olive oil samples had FA values that were much lower than 0.8% **(Table 2)**, which is the maximum value established by EU Regulation for extra virgin olive oils (EVOOs).

***PV:*** Peroxides are the primary products of oxidation of olive oil. They may adversely affect the nutritional value of the oil and originate secondary oxidation products that also have an unpleasant flavour. A limit value of 20 meq$O_2$/kg has been established for VOOs in EU Regulation. The PVs for all olive oils were under this limit **(Table 2)**.

***Absorbance in ultraviolet region:*** Specific absorbances are measured in the region at the wavelengths corresponding to the maximum absorption of the conjugated dienes and trienes, at 232 and 270 nm, respectively. They are formed during autoxidation from the

293 hydroperoxides of unsaturated fatty acids and their fragmentation products. For both
294 oils, $K_{232}$ and $K_{270}$ values were lower than the limits established for EVOOs (2.50 and
295 0.22, respectively) by EU Regulation 299/2013**(Table 2)**.

296 ***Sensory evaluation:*** Sensory analysis is an important tool for classifying oils obtained
297 from olives in different commercial categories (extra virgin, virgin, lampante) and to
298 differentiate them within the same category of extra virgin. Sensory analysis of the two
299 *Local* olive oils was performed by defining the main attributes, both positive (such as
300 fruity, bitter, and pungent) and negative (fusty/muddysediment, musty-humid-earthy,
301 winey-vinegary-acid-sour, metallic, and rancid). The intensity of the attribute values
302 perceived by tasters, expressed as cm, were statistically assessed to calculate the median
303 of each positive and negative sensory characteristic, as seen in **Table 3**. According to
304 the results, VOOa can be classified as "extra virgin" due to the median of defects equal
305 to 0 and the median of the fruity attribute more than 0. On the contrary, VOOb must be
306 classified as "virgin" because the defect of fusty/muddy sediment (the most perceived
307 one) was found with a median above 0, but not higher than 3.5 (EU Regulation
308 1348/2013). The intensity of both defects found -fusty/muddy sedimentand winey- was
309 low, namely 1.0 and 0.8, respectively. Fusty/muddy sediment is the characteristic flavor
310 of oils obtained from olives in an advanced stage of anaerobic fermentation or of oils
311 which have been left in contact with the sediment that settles in underground tanks and
312 vats, which has also undergone a process of anaerobic fermentation. On the other hand,
313 winey-vinegary is a sensory note due to high concentrations of acetic acid, ethyl acetate,
314 and ethanol (EU Regulation 1348/2013). It seems that olives of the cultivars *Mansa* (90-
315 100%) and *Brava* (0-10%) harvested in January had undergone some type of
316 preservation under inadequate conditions before olive oil processing, even if they were
317 processed on the same day of harvest.

According to the intensity of perceptions, as specified by EU legislation (EU Regulation 1348/2013), both oils were "well-balanced". Nevertheless, there was a clear decline in the positive attributes of VOOa with respect to VOOb, as confirmed by the lower values in fruity, bitter, and pungent seen in VOOb compared with VOOa **(Table 3)**. Actually, VOOb was characterized by a low intensity for all their positive attributes (i.e. the median of attributes is less than 3). Its score was close to the "mild" olive oils (for which the median of the bitter and pungent attributes is 2 or less) (EU Regulation1348/2013).

### *3.1.2. Olive Oil genuineness-related parameters*

The following parameters established by the EU Regulation 2568/91 and subsequent amendments help to guarantee the authenticity of the oil.

***Fatty acid composition:*** Fatty acid composition of olive oils is affected by several factors including production area, latitude, climate, and fruit ripeness, but is dependent primarily on genetic factors. Fatty acid composition of the oils are shown in **Table 2** and fell within the recommended ranges for EVOOs set by EU Regulation 1348/2013. As can be seen, there are no statistically significant differences between oils, except for the minor *trans*-oleic isomers (C18:1 T), behenic (C22:0), and margaroleic (C17:1) acids. As expected, the major fatty acid was oleic acid (C18:1; 70.77% on average), followed by palmitic acid (C16:0, 13.49% on average), linoleic acid (C18:2; 10.34% on average), stearic acid (C18:0; 2.03% on average), palmitoleic acid (C16:1, 1.19% on average), and linolenic acid (C18:3; 1.00% on average). Finally, *trans*-oleic isomers were lower than 0.05 and the sum of *trans*-linoleic and *trans*-linoleic isomers were not detected. Saturated fatty acids comprised about 16% of the total fatty acids, whereas

monounsaturated and polyunsaturated fatty acids represented 72% and 11%, respectively.

Several studies performed in Spain on cultivar collection have concluded that Arbequina oils are characterized by a high C18:2 (12.57-13.06%) content and a low C18:1 content (68.20-65.83%), whereas Picual oil shows an opposite trend (4.43-4.44% and 78.28-78.34%, respectively) (Uceda, Beltrán and Jiménez, 2005). The main fatty acid composition of Galician *Local* oils (i.e. C16:0, C18:0, C18:1 and C18:2) was intermediate between the above mentioned varieties. It is also worth noting that there was a high concentration of linolenic acid, near 1%. Some genuine Moroccan olive oils can be also characterized by 1% of this acid (Angerosa, Campestre and Giasante, 2006).

***Sterol and triterpene dialcohol composition:*** Sterols are present in the unsaponifiable fraction of olive oil, reaching between 20-23%of its total amount in olive oil. The sterol profile is highly species-specific (EU Regulation 1348/2013). **Table 2** shows the sterol content of the samples analyzed. The predominant sterol in olive oils is β-sitosterol (sum of β-sitosterol and some of its degradation products such as $\Delta^{5,23}$- and $\Delta^{5,24}$-stigmastadienols, cholerosterol, sitostanol and $\Delta^{5}$-avenasterol) followed by campesterol. All levels complied with the legislation established for EVOOs (EU Regulation 1348/2013). Statistically significant differences were detected for all individual sterols between the two samples, except for $\Delta^{7}$-stigmastenol. However, no difference was found in total sterol content. The sum of the triterpene diols could not differentiate the oils, although none had levels above the established limit (4.5%) for EVOOs.

***ΔECN42:*** ΔECN42 is a very useful and effective tool in detecting adulteration of olive oils (naturally lacking in linolenic acid) with other oils rich in linolenic acid (seed oils). In both *Local* olive oils, ΔECN42 was within the range considered appropriate for EVOOs and listed in EU Regulation 796/2002.

***Wax content:*** The determination of the sum of $C_{40-46}$ aliphatic waxes can be considered to be a reliable parameter to detect olive-residue oil (e.g. pomace olive oil) in VOO; in this case, their sum was lower than 150 mg/kg, the maximum limit established for EVOOs (EU Regulation 183/1993).

***Tocopherols and tocotrienols:*** Tocopherols are the main lipid-soluble antioxidants present in olive oil. Four isomers can be found in olive oil (α, β, γ and δ). α-tocopherol (vitamin E) is the most abundant (90-95%). The concentration of these compounds in olive oil range from 150 to 250 mg/Kg (Amelio, 2003). **Table 2** shows the content of the four tocopherol isomers determined in the present oils, as well as, the total tocopherol and tocotrienol content. As can be seen in such table, no significant differences are observed in both cultivars.

***Squalene:*** Squalene exhibits antitumor activity against different cancer types (Rao, Newqmark and Reddy, 1998; Smith, 2000; Murakoshi et al, 1992, Ohkuma, Otagiri, Tanaka and Ikekawa, 1983) and may also be useful for the treatment of cardiovascular diseases (Banks et al, 2004). In olive oil, squalene achieves a concentration of 700 mg/100 g (Newmark, 1999) with a range variation comprised between 90 and 870 mg/100g (De Leonardis, Maccionala and De Felice, 1998). Squalene levels varied from 7436 to 6518 mg/Kg for VOOa and VOOb, respectively **(Table 2)**. VOOa was quite rich in squalene content. Squalene content of *Local* olive oils was in the range as those showed in Beltrán *et al* 2015. According to this paper, our oils can be included in the category IV (high squalene content: 600-750 mg/100 g).

**Pigment content:** The evaluation of chlorophylls and carotenoids pigments profile in olive oil is of great importance, given their antioxidant properties, as well as their crucial role as colorful substances responsible of greenness (chlorophylls) and yellowness (carotenoids) of olive oil (Giuliani, Cerretani, & Cichelli, 2011). As can be

seen in **Table 2**, significant differences in the levels of the analysed oils were observed in the chlorophyll and in the β-carotene content. Chlorophyll levels varied from 22 to 15 mg/Kg for VOOa and VOOb, respectively. The content of β-carotene was quite similar with levels of about 10 mg/Kg for both oils.

## 3.2. Other quality-related parameters not included in current European Regulations

### *3.2.1. Phenolic composition*

Phenols are considered natural antioxidants that are responsible for oil stability against oxidation, and also contribute to the characteristic bitter, pungent, and astringent sensory notes of olive oils. They have been shown to have a wide range of beneficial effects from healing sunburn to lowering cholesterol, blood pressure, and risk of coronary disease. VOOs contains five different classes of phenols clustered into phenolic acids, simple phenols, complex oleuropein derivatives, flavonoids, lignans, and hydroxy-isocromans (Servili, 2014).

**Table 4** shows the concentration of the individual phenolic compounds (mg/kg oil) of the studied VOOa and VOOb. VOOa -obtained co-crushing 70% *Brava* cv. and 30% *Mansa* cv.- shows 433 mg/kg oil and VOOb -obtained co-crushing 90-100% *Mansa* cv. and 0-10% *Brava* cv.- shows 325 mg/kg oil. The content of hydroxytyrosol derivatives for VOOa and VOOb oils (174 and 120 mg/kg, respectively) were lower than tyrosol derivatives (257 and 201 mg/kg). The concentrations obtained were in accordance with those previously determined (Reboredo-Rodríguez *et al*, 2015a, 2015b).

Moreover, **Table 4** shows the total *o*-diphenolic and phenolic content of the VOOs. Total *o*-diphenolics are expressed as mg of GA/kg of oil, whereas total phenolics can be

expressed equally as mg of GA/kg of oil or mg of HTyr/kg of oil. VOOb was significantly less rich in phenols than VOOa: they could be considered as low and medium content oils, respectively, in accordance with the normal ranges established by Servili (2014) for total phenols in VOOs (high content > 500 mg GA/kg oil; medium content from 250 to 500 mg GA /kg oil; low content < 250 mg GA /kg oil).

It should be emphasized that the phenolic content was higher for VOOa than for VOOb, which suggests that, in this investigation, the sample produced by co-crushing 70% *Brava*/30% *Mansa* olives was an oil richer in these healthy compounds: this finding could be due to the different varieties or to the earlier harvesting date of olives processed to obtain the sample VOOa.

### *3.2.3. Volatile composition*

$C_5$ and $C_6$ volatile compounds, responsible for the positive green sensory perceptions in olive oil, were the main compounds identified in the Galician *Local* olive oils **(Table 5)**. $C_6$ compounds are mainly produced by endogenous olive enzymes through the lipoxygenase (LOX) pathway, which consists of a cascade of oxidative reactions that give rise to a variety of metabolites with different functions from polyunsaturated fatty acids (either linoleic and linolenicacids as the initial substrates) (Feussner and Wasternack, 2002). $C_5$ compounds are generated through an additional branch of the LOX pathway that involves the production of a 13-alkoxyl radical by LOX. Many factors influence the presence of these compounds: variety of olives, weather and location of the orchard, care that went into growing, maturity of olives at harvest, oil extraction, storage conditions, and age of the oil (Kalua *et al*, 2007).

***$C_6$ compounds.*** The most abundant volatiles in all samples were those in the $C_6$ alcohol fraction (primarily *cis*-3-hexen-1-ol), followed by aldehydes (*trans*-2-hexen-1-al was the most abundant) and esters (*cis*-3-hexenyl acetate) (see **Table 5**). This is consistent with the results of a previous study on oils obtained by olives of the same varieties, where the process of sedimentation and further racking of oil samples for a minimum of 2 months was found to promote the formation of $C_6$ alcohols in most samples (Reboredo-Rodríguez, González-Barreiro, Cancho-Grande and Simal-Gándara,2013).

VOOa, as a result of 70% *Brava* and 30% *Mansa* co-crushing, had a markedly higher content in all relevant $C_6$ compounds than VOOb (0-10% *Brava* and 90-100% *Mansa*), except for *trans*-3-hexen-1-ol and 1-hexanol.

***$C_5$ compounds.*** $C_5$ compounds were detected at low levels compared with the $C_6$ volatile fraction **(Table 5)**. Pentene dimers was the most abundant class, and 3-pentanone (without significant differences between the two samples) followed by 1-penten-3-ol (with statistically significant different concentrations for both oils) were the major single components of the $C_5$ fraction. On the other hand, 1-penten-3-one and *trans*-2-pentenal were minor compounds in both samples.

***Minor volatile compounds***

Several terpenic hydrocarbons, such as 3-carene, limonene, and α-farnesene were found **(Table 5)** in minor concentrations with respect $C_6$ and $C_5$ compounds. Carene was the only compound present in both samples ranging from 11.0 to 20.5 µg IS/kg oil. Several authors (Vichi, Guadayol, Caixach, López-Tamames and Buxaderas, 2006) consider these compounds as genetic orgeographic markers of VOO.

462 Only ethylbenzene as a mono-ring aromatic hydrocarbon (MAH) was observed in
463 VOOa at low levels (9.6 µg IS/kg oil). Aromatic hydrocarbons in olive oils may arise
464 from both exogenous contamination and endogenous pathways (Sabatini, Perriand
465 Marsilio, 2009).

466 Other minor volatile compounds derived from autoxidation reactions and related to oil
467 rancidity, such as octane, nonanal, *trans*-2-heptenal, and *trans, trans*-2,4-hexadienal,
468 were also found in both samples.

469 Some products deriving from amino acid transformation were also detected. The highest
470 concentrations were 2-methyl-1-butanol and 3-methyl-1-butanol (221.8 and 319.3 µg
471 IS/kg oil in samples VOOa and VOOb, respectively), while 2-methyl-1-butanal, even if
472 present at a lower concentration (19.9 and 39.0 µg IS/kg oil in samples VOOa and
473 VOOb, respectively), exceeded its odor threshold (5.2 µg/kg oil). This latter compound
474 may contribute to the fusty defect (Morales *et al*, 2005) that was detected by tasters in
475 VOOb.

476 Ethyl acetate and acetic acid are metabolites normally associated with ethanol to
477 undesired fermentation occurring in the olive fruits before olive oil extraction. VOOb
478 showed higher levels of these compounds, and especially acetic acid (805.1 µg IS/kg
479 oil). Such aconsiderable amount of acetic acid, mainly originated by the fermentation
480 metabolism of *Acetobacter* in olives (Angerosa, 2002) and in amounts higher than its
481 sensory threshold, could explain the winey defect scored by judges. It is likely that
482 incorrect preservation of olives in terms of bad storage conditions was the cause of
483 alcoholic fermentation. Ethanol was also detected in olive oils: it should be noted the
484 low processing capacity of small mill plants located in this area may have produced a
485 conservation of the piling olives at room temperature for a few days before processing,
486 thus inducing yeast and bacteria growth that increased sugar fermentation and amino

487 acid degradation. Moreover, in the case of VOOb, 0-10% of olives of the cv. *Brava*
488 were probably over-ripe when collected (at a date after their optimal maturation stage),
489 thus promoting degradation.

490 The aroma perceived by smelling can rarely be ascribed to a single, specific compound,
491 but rather to a mixture of several volatile molecules. However, not all compounds
492 contribute to oil aroma to the same extent; in fact, the contribution of a given compound
493 depends, among other factors, on its odor threshold (Delahunty, Eyres, and Dufour,
494 2006). For this reason, the odor activity value (OAV), which represents the
495 concentration of a volatile compound divided by its odor threshold value, allows an
496 estimation of the contribution of each individual compounds to the oil overall aroma
497 (Reboredo-Rodríguez *et al*, 2015a). However, considering each single OAV to
498 tentatively establish the aromatic profile of anoil can give rise to a model complex and
499 difficult to interpret. Thus, volatile compounds with similar odor descriptors were
500 grouped into five odorant series (**Table 5**; *viz.* sweet odor notes, pungent odor notes,
501 bitter odor notes, green-leaf, and ripely fruit). The total OAV of each odorant series was
502 calculated by summing the OAV calculated for each volatile compound belonging to a
503 particular series. This procedure, borrowed from the characterization of white wines
504 (Sánchez-Palomo, Gómez García-Carpintero, Alonso-Villegas and González-Viñas,
505 2010), can be useful in finding a relation between the quantitative information obtained
506 by the chemical analysis of the volatiles and the sensory perceptions in the form of a
507 tentative aroma profile. It should be noted that some volatile compounds, such as 1-
508 penten-3-one, could contribute to the bitter and pungent sensations perceived, even if it
509 is well known that these two attributes are basically due, respectively, to stimulation of
510 gustatory cells and the trigeminal nerve byphenolic soluble and not volatile compounds,
511 such as secoiridoids (Angerosa *et al*, 2000).

512 **Figure 1** shows the contribution of each odorant series to the aroma profile of VOOa (a)
513 and VOOb (b) according to instrumental data (OAV calculation) and Panel results,
514 respectively. Taking into account only sensory evaluation, pungent and bitter were
515 highlighted as the main attributes in VOOa compared to VOOb, while sweet, ripely
516 fruity, and green leaf descriptors showed a similar organoleptic score in both oils.

517 As reflected in **Figure 1**, considering the results of the two samples characterized by
518 different chemical composition, a good relation was found between sensory (mean of
519 the attributes) and instrumental (OAV values) data. Actually, the intensities of the
520 perceived sensory attributes (Panel test results) were substantially in accord with the
521 instrumental data (OAV) and the Panel test results, except for "pungent odor notes"
522 series in the case of VOOb.

523 The pungent sensation perceived by the tasters is not mainly produced by volatile
524 substances grouped in **Table 5** as "pungent", but is instead caused by trigeminal/tactile
525 stimulation of the mucosa by secoiridoids. Volatiles grouped in **Table 5** as "pungent"
526 can reinforce this action but can not alone be predictive. In addition, acetic acid present
527 in VOOa and VOOb could help to boost the perception of "pungent odor notes". This
528 compound was not considered because only $C_5$ and $C_6$ compounds were used to
529 construct the odorant series (Reiners and Grosch, 1998), but if the contribution of acetic
530 acid was included in that series, the OAV for this series would be 18.3 and 1.8for VOOa
531 and VOOb, increasing by 0.6 and 1.6 units, respectively. Consequently both criteria,
532 sensory and instrumental data, are better suited. This synergistic effect of acetic acid in
533 the perception of "pungent odor notes" that here has been hypothesized requires,
534 however, additional tests to be confirmed.

535

## 4. Conclusions

The chemical and sensory characterization of VOOs obtained from different proportions of autochthonous varieties from a new Spanish olive-growing zone has been investigated herein. The fatty acid composition of the Galician *Local* oils (i.e. C16:0, C18:0, C18:1 and C18:2) was intermediate between usual Arbequina and Picual oils. The Galician *Local* oils showed a medium content in total phenolic compounds, but samples produced with higher proportion of *Brava* variety had more abundant phenolic content than those obtained using a high percentage of Mansa. In general, given the high levels of phenolic compounds, tocopherols and carotenoids, these are oils with long stability, especially under dark storage conditions, because stability is reinforced with the contribution of chlorophylls.

Concerning volatile compounds, the most abundant volatiles in all samples were those in the $C_6$ alcohol fraction, typical of good quality products. Oils obtained by co-crushing 70% *Brava* and 30% *Mansa* olives had a markedly higher content in all relevant $C_6$ compounds than oils with 90-100% of *Mansa* olives, except for *trans*-2-hexen-1-ol.

Pungent and bitter were highlighted as the main sensory attributes in oils with a higher percentage of *Brava* cv., whereas oils with a higher percentage of *Mansa* cv. were characterized by a low intensity for all positive attributes. Moreover, the sensory quality of the oil obtained with the highest percentage of *Mansa* cv. was penalized by decreasing its commercial category to VOO because of the presence of fusty/muddy sediment and winey defects, as confirmed by the presence of some volatile compounds deriving from amino acid transformation (2-methyl-1-butanol and 3-methyl-1-butanol) and acetic acid. This is probably due to the fact that these oils are traditionally produced in local, small size mills by non-expert producers who are not yet fully aware of the optimal procedures to store olives before processing; of course, with the aim to improve

561 the quality of the product, better conditions in the preservation of olives, providing air
562 circulation and avoiding accumulation, should be pointed out to local producers in the
563 future. Moreover, it is also important to take into account the optimal degree of
564 maturation of olives when harvesting with the aim to reach the quality standard of
565 EVOOs.

566

## Acknowledgements

This work was granted by European Regional Development Funds (FEDER). The authors are grateful to Miguel Rodríguez González (Aceites Figueiredo) for providing oil samples. Patricia Reboredo-Rodríguez acknowledges pre-doctoral and short-stay fellowships from the University of Vigo.

## Figure legends

**Figure 1.** Sensory profile and OAV values calculated for the odorant series (see **Table 5**) of VOOs. For this calculation, the sums of the OAV evaluated for specific compounds were made: **sweet odor notes series**: hexanal, hexyl acetate, *cis*-2-penten-1-ol, 3-pentanone; **pungent odor notes series:** 1-penten-3-ol, 1-penten-3-one, 1-pentanol; **bitter odor notes series:** *trans*-2-hexen-1-al, *trans*-3-hexen-1-ol, 1-penten-3-one, pentanal, *trans*-2-pentenal; **green-leaf series:** *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, 1-penten-3-ol, 1-penten-3-one; **ripely fruit series:** *cis*-3-hexenyl acetate, 1-hexanol, hexyl acetate, 1-penten-3-ol, *cis*-2-penten-1-ol, 1-pentanol, 3-pentanone, as reported in **Table 5**.

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**Table 1.** Climatic conditions of the growing area in the crop year season 2013.

| Climatic conditions | | | | | |
|---|---|---|---|---|---|
| **Year** | **R (L/m$^2$)** | **T (ºC)** | **TCT$_7$ (days)** | **RH (%)** | **MBP (hPa)** |
| 2013 | 1044.5 | 13.3 | 6.8 | 76.9 | 987.9 |

**R**, total rainfall; **T**, mean air temperature; **TCT$_7$**, total cold time (T<7ºC); **RH**, mean air relative humidity; **MBP**, mean barometric pressure.

**Table 2.** Quality and genuineness-related parameters of the studied VOOs.

| **Quality-related indices** | **VOOa** | **VOOb** |
|---|---|---|
| ***FA (% oleic acid)*** | 0.2±0.0[a] | 0.2±0.0[a] |
| ***PV (meq $O_2$/kg oil)*** | 9.2±0.7[a] | 10.4±0.6[b] |
| ***$K_{232}$*** | 2.01±0.08[b] | 1.89±0.09[a] |
| ***$K_{270}$*** | 0.15±0.01[a] | 0.14±0.04[a] |
| ***ΔK*** | 0.0020±0.0003[a] | 0.0013±0.0002[a] |

| **Genuineness-related indices** | **VOOa** | **VOOb** |
|---|---|---|
| ***Fatty acid composition (% m/m methyl esters)*** | | |
| Myristic C14:0 | 0.01±0.00[a] | 0.01±0.00[a] |
| Palmitic C16:0 | 13.42±0.54[a] | 13.57±0.61[a] |
| Palmitoleic C16:1 | 1.19±0.09[a] | 1.19±0.02[a] |
| Margaric C17:0 | 0.11±0.02[a] | 0.13±0.01[a] |
| Margaroleic C17:1 | 0.26±0.03[a] | 0.32±0.03[b] |
| Stearic C18:0 | 2.07±0.18[a] | 1.99±0.13[a] |
| Oleic C18:1 | 71.19±1.20[a] | 70.36±0.36[a] |
| Linoleic C18:2 | 10.06±0.59[a] | 10.63±0.23[a] |
| Linolenic C18:3 | 0.97±0.09[a] | 1.04±0.04[a] |
| Arachidic C20:0 | 0.30±0.01[a] | 0.30±0.03[a] |
| Eicosenoic C20:1 | 0.25±0.01[a] | 0.27±0.01[a] |
| Behenic C22:0 | 0.08±0.01[a] | 0.12±0.02[b] |
| Lignoceric C24:0 | 0.04±0.01[a] | 0.05±0.01[a] |
| *trans*-OleicisomersC18:1 T | 0.02±0.00[a] | 0.04±0.00[b] |
| *trans*-Linoleic + *trans*-Linolenic | n.d. | n.d. |
| ***Sterol relative amounts (%)*** | | |
| Cholesterol | 0.03±0.01[a] | 0.06±0.00[b] |
| Brassicasterol | Tc | Tc |
| Campesterol | 2.39±0.08[a] | 2.54±0.11[b] |
| Stigmasterol | 0.75±0.01[a] | 0.96±0.18[b] |
| Apparent β-sitosterol | 96.37±0.18[b] | 95.96±0.33[a] |
| $\Delta^7$-Stigmastenol | 0.08±0.01[a] | 0.11±0.02[a] |
| Total sterols (µg/g) | 1582.07±160.64[a] | 1686.08±190.51[a] |
| Erythrodiol + Uvaol | 1.12±0.12[a] | 1.22±0.19[a] |
| ***ΔECN42*** | 0.08±0.01[a] | 0.10±0.02[a] |
| ***Waxes(µg/g)*** | 32.32±4.35[a] | 39.77±6.14[a] |
| ***Tocopherols (mg/Kg)*** | | |
| *α-tocopherol* | 160±8[a] | 157±8[a] |
| *β-tocopherol* | 2±0.1[a] | 2±0.1[a] |
| *γ-tocopherol* | 3±0.1[a] | 5±0.1[a] |
| *δ-tocopherol* | <2±0.1[a] | <2±0.1[a] |
| *Total tocopherols* | 165±8[a] | 164±8[a] |
| *Squalene (mg/Kg)* | 7436±372[b] | 6518±326[a] |
| *Chlorophyll (mg/Kg)* | 22±1.1[b] | 15±0.7[a] |
| *β-carotene (mg/Kg)* | 12±0.6[b] | 10±0.5[a] |

**VOOa** is the average of results obtained for VOO1 and VOO2, elaborated by co-crushing 70% *Brava* cv. and 30% *Mansa* cv. (n=2).**VOOb** is average of results obtained for VOO3 and VOO4, elaborated by co-crushing 90-100% *Mansa* cv. and 0-10% *Brava* cv. (n=2).

Values are mean ± standard deviation. All analyses were performed in triplicate. Different letters within rows indicate significantdifferences by a Student's *t*-test ($p < 0.05$).

n.d.: not detected (<LOD).

Tc: Traces (<LOQ).

**Table 3.** Sensory analysis of VOOs.

| **Attributes** | **VOOa** | **VOOb** |
|---|---|---|
| ***Positive*** | | |
| Fruity | 3.3 [3.6-3.0] | 2.5 [2.9-2.1] |
| Bitter | 4.7 [5.2-4.2] | 2.5 [3.2-1.8] |
| Pungent | 4.1 [4.6-3.7] | 2.3 [2.8-1.8] |
| ***Negative*** | | |
| Fusty/muddy sediment | n.p. | 1.0 [1.2-0.8] |
| Winey-vinegary-acid-sour | n.p. | 0.8 [1.3-0.4] |
| **Panel test classification** | EXTRA VIRGIN | VIRGIN |

**VOOa** is the average of results obtained for VOO1 and VOO2, elaborated by co-crushing 70% *Brava* cv. and 30% *Mansa* cv. (n=2). **VOOb** is the average of results obtained for VOO3 and VOO4, elaborated by co-crushing 90-100% *Mansa* cv. and 0-10% *Brava* cv. (n=2).

Values are medians; confidence intervals of the median at 95%are reported in brackets.

s: significant.

n.p.: not perceived.

**Table 4.** Concentrations of the main phenolic compounds (mg/kg oil) of the studied VOOs and concentration of *o*-diphenolics and total phenolics of VOOs.

| | Concentration ± SD (mg/Kg) | |
|---|---|---|
| | **VOOa** | **VOOb** |
| Hydroxytyrosol (3,4-DHPEA) | 11.23±0.21[b] | 9.18±0.37[a] |
| Tyrosol (*p*-HPEA) | 24.11±0.03[b] | 27.50±1.16[a] |
| 3,4-DHPEA-EDA | 112.38±8.80[b] | 64.19±0.21[a] |
| *p*-HPEA-EDA | 195.27±20.67[b] | 142.56±2.26[a] |
| 3,4-DHPEA-EA | 50.34±4.38[a] | 46.69±3.19[a] |
| *p*-HPEA-EA | 37.32±6.28[a] | 31.49±7.62[a] |
| *Σ Hydroxytyrosol derivatives* | *174* | *120* |
| *Σ Tyrosol derivatives* | *257* | *201* |
| Vanillic acid | 0.23±0.02[a] | 0.26±0.03[a] |
| Vainillina | 0.25±0.002[a] | 0.27±0.01[b] |
| *p*-Coumaric acid | 0.19±0.07[a] | 0.18±0.001[a] |
| Pinoresinol | 2.08±0.36[a] | 2.37±0.11[a] |
| ***Total phenols*** | ***433.41±39.43***[b] | ***324.69±7.09***[a] |
| ***o*-Diphenolics** | | |
| Expressed as GA | 132.6±10.7[b] | 83.9±12.0[a] |
| **Total phenolics** | | |
| Expressed as GA | 340.4±24.1[b] | 215.6±24.7[a] |
| Expressed as HTyr | 374.8±24.1[b] | 253.9±22.1[a] |

**VOOa** is the average of results obtained for VOO1 and VOO2, elaborated by co-crushing 70% *Brava* cv. and 30% *Mansa* cv. (n=2). **VOOb** is the average of results obtained for VOO3 and VOO4, elaborated by co-crushing 90-100% *Mansa* cv. and 0-10% *Brava* cv. (n=2).

Values are mean ± standard deviation. All analyses were performed in triplicate. Different letters within rows indicate significant differences using a Student's *t*-test ($p < 0.05$).

**Table 5.** Concentrations of $C_6$ and $C_5$volatile compounds of VOOs.

| **$C_6$, $C_5$volatile compounds** | **Odor threshold* (µg/kg oil)** | **Odorant series** | **Concentration ± SD (µg IS/kg oil)** | |
|---|---|---|---|---|
| | | | **VOOa** | **VOOb** |
| *trans*-2-Hexen-1-al | 420 | Bitter | 1645.0±17.9[b] | 897.1±258.9[a] |
| ***Σ C6/LnA-Aldehydes*** | | | ***1645***±18 | ***897***±259 |
| *cis*-3-Hexen-1-ol | 1100 | Green-leaf | 1694.3±144.9[b] | 1196.9±47.5[a] |
| *trans*-3-Hexen-1-ol | 1500 | Bitter | 40.9±14.0[a] | 24.9±4.1[a] |
| *trans*-2-Hexen-1-ol | 5000 | Green-leaf | 482.2±47.3[a] | 887.4±162.0[b] |
| ***Σ C6/LnA-Alcohols*** | | | ***2217±153*** | ***2109±169*** |
| *cis*-3-Hexenyl acetate | 200 | Ripely fruity | 275.5±6.4[b] | 56.2±16.6[a] |
| ***Σ C6/LnA-Esters*** | | | ***276±6*** | ***56±17*** |
| Hexanal | 75 | Sweet | 173.8±1.6[b] | 85.9±4.3[a] |
| ***Σ C6/LA-Aldehydes*** | | | ***174±2*** | ***86±4*** |
| 1-Hexanol | 400 | Ripely fruity | 561.1±32.8[a] | 582.5±84.4[a] |
| ***Σ C6/LA-Alcohols*** | | | ***561±33*** | ***583±84*** |
| Hexyl acetate | 1040 | Ripely fruity, sweet | 35.3±9.2[b] | 12.9±1.9[a] |
| ***Σ C6/LA-Esters*** | | | ***35±9*** | ***13±2*** |
| 1-Penten-3-ol | 400 | Ripely fruity, green-leaf, pungent | 56.2±10.8[a] | 72.8±8.4[b] |
| *cis*-2-Penten-1-ol | 250 | Ripely fruity, sweet | 33.5±2.9[a] | 50.7±8.4[b] |
| ***Σ C5/LnA-Alcohols*** | | | ***90±11*** | ***124±12*** |
| 1-Penten-3-one | 50; 0.73 | Green-leaf; bitter, pungent | 12.8±1.3 | n.d. |
| ***Σ C5/LnA-Ketones*** | | | ***13±1*** | **-** |
| Pentanal | 240 | Bitter | 44.8±12.3[a] | 26.7±0.4[a] |
| *trans*-2-Pentenal | 300 | Bitter | 4.7±2.0 | n.d. |
| ***Σ C5/LnA-Aldehydes*** | | | ***49±13*** | ***27±0.4*** |
| 1-Pentanol | 470; 3000 | Ripely fruity, pungent | 25.6±1.3[a] | 31.4±1.7[b] |
| ***Σ C5/LA-Alcohols*** | | | ***26±1*** | ***31±2*** |
| 3-Pentanone | 70000 | Ripely fruity, sweet | 96.2±12.0[a] | 112.1±14.4[a] |

| | | | |
|---|---|---|---|
| ***Σ C5/LA-Ketones*** | | ***96±12*** | ***112±14*** |
| 3-Ethyl-1,5-octadiene (1) | | 50.6±7.7[b] | 31.5±2.5[a] |
| 3-Ethyl-1,5-octadiene (2) | | 50.7±3.6[b] | 28.0±2.5[a] |
| 3-Ethyl-1,5-octadiene (3) | | 27.4±3.1[a] | 23.3±3.2[a] |
| 3-Ethyl-1,5-octadiene (4) | | 34.5±1.3[b] | 20.4±1.3[a] |
| ***Σ Pentene dimers*** | | ***163±9*** | ***103±5*** |
| **Total compounds** | | **5345±160** | **4141±321** |
| **Minor volatile compounds** | **Odor threshold* (µg/kg oil)** | **Concentration ± SD (µg IS/kg oil)** | |
| | | **VOOa** | **VOOb** |
| Ethylbenzene | - | 9.6±1.6 | n.d. |
| Limonene | - | 34.4±2.7 | n.d. |
| 3-Carene | - | 20.5±5.1[b] | 11.0±3.9[a] |
| α-Farnesene | - | 8.2±0.9 | n.d. |
| ***Hydrocarbons*** | | ***73±6*** | ***11±4*** |
| Octane | 940 | 21.9±1.8[a] | 59.0±8.6[b] |
| Nonanal | 150 | 9.1±2.3 | n.d. |
| *trans*-2-Heptenal | 5 | n.d. | 12.4±2.2 |
| *trans, trans*-2,4-Hexadienal | 2000 | 46.8±13.0 | n.d. |
| ***Autooxidation*** | | ***78±13*** | ***71±9*** |
| 2-Methyl-1-butanal | 5.2 | 19.9±6.0[a] | 39.0±5.4[b] |
| 2-Methyl-1-propanol | - | 51.3±11.2[a] | 80.9±0.4[b] |
| 2-Methyl-1-butanol+3-methyl-1-butanol | 480;100 | 221.8±66.4[a] | 319.3±35.7[b] |
| ***Aminoacids metabolites*** | | ***293±68*** | ***439±36*** |
| Methyl acetate | - | 68.1±17.2[a] | 80.4±14.9[a] |
| Ethyl acetate | 940 | 146.7±13.0[a] | 205.3±9.2[b] |
| Methanol | 33000 | 378.1±20.7[a] | 402.8±93.7[a] |
| Ethanol | 30000 | 3594.0±841.0[b] | 2030.3±504.0[a] |

| | | | |
|---|---|---|---|
| Acetic acid | 500 | 279.1±17.3[a] | 805.1±96.8[b] |
| Acetone | - | 29.1±6.7[a] | 35.2±10.6[a] |
| ***Sugar fermentation*** | | ***4495±842*** | ***3559±522*** |
| **Total compounds** | | **4939±845** | **4080±523** |

* As reported in Aparicio and Morales, (1998); Reiners and Grosch, (1998); Aparicio and Luna, (2002); Burdock, (2002); Morales, Luna, and Aparicio (2005); Cayuela, Gómez-Coca, Moreda, & Pérez-Camino, (2015).

**VOOa** is the average of results obtained for VOO1 and VOO2, elaborated by co-crushing 70% *Brava* cv. and 30% *Mansa* cv. (n=2). **VOOb** is the average of results obtained for VOO3 and VOO4, elaborated by co-crushing 90-100% *Mansa* cv. and 0-10% *Brava* cv. (n=2).

All analyses were performed in triplicate. Different letters within rows indicate significantdifferences using a Student's *t*-test ($p < 0.05$).

n.d.: not detected (< LOD).

Figure(s)

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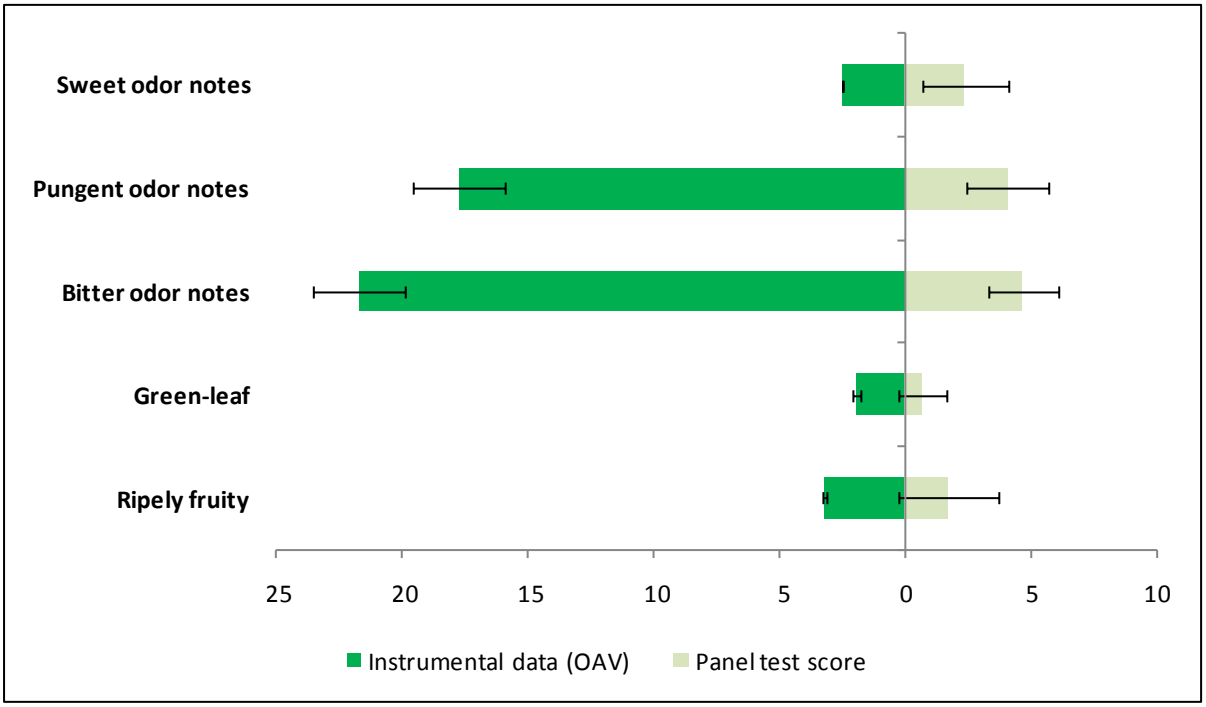
**VOOa**




**VOOb**

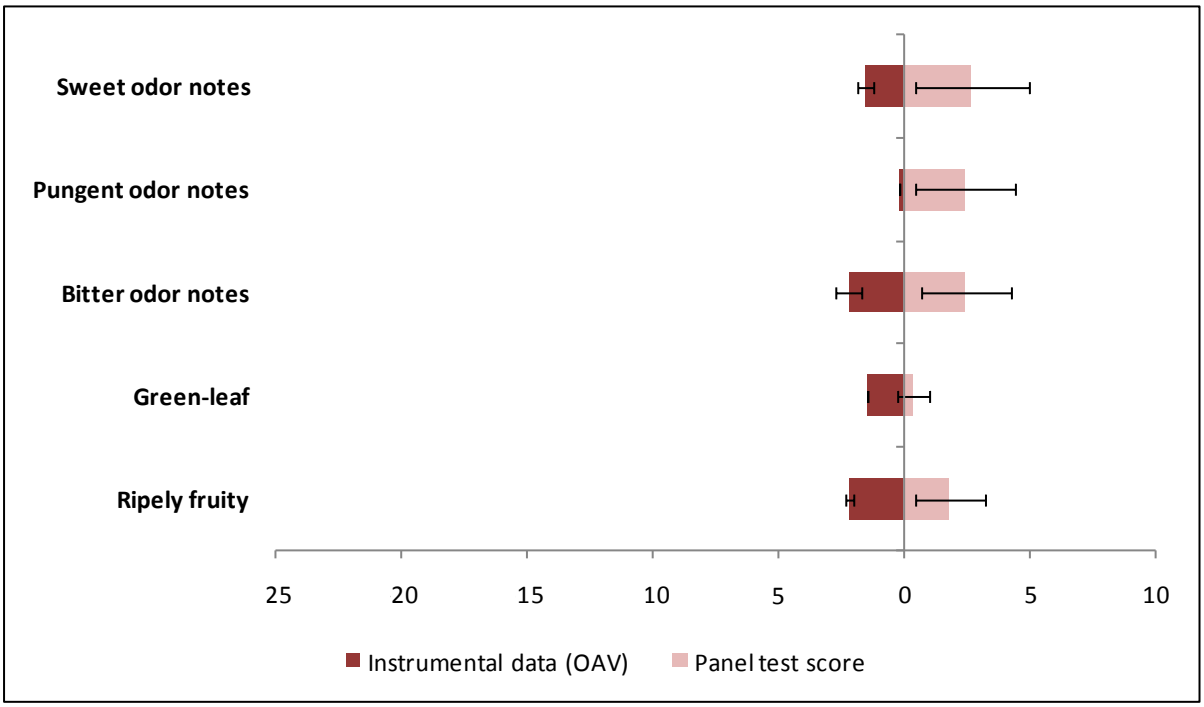



**Figure 1.**

**Supplementary Material**

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