Chemical and sensory characterization of Brazilian virgin olive oils

Lilia Zago[a,*], Giacomo Squeo[b], Edna Ivani Bertoncini[c], Graziana Difonzo[b], Francesco Caponio[b,*]

[a] Department of Experimental and Basic Nutrition, Institute of Nutrition, University of the State of Rio de Janeiro, São Francisco Xavier Street, 524, Rio de Janeiro 20550-090, Brazil.

[b] Department of Soil, Plant and Food Sciences, Food Science and Technology Unit, University of Bari Aldo Moro, Via Amendola, 165/A, Bari I-70126, Italy.

[c] Oliva São Paulo Group, São Paulo Agency of Agribusiness and Technology - APTA, Highway SP 127, km 30, Piracicaba 13400-970, Brazil.

Authors to whom correspondence should be addressed:

L. Zago. University of the State of Rio de Janeiro, São Francisco Xavier Street, 524, Rio de Janeiro 20550-090, Brazil. E-mail address: lilia.zago@gmail.com and lilia.zago@uerj.br

F. Caponio. University of Bari Aldo Moro, Via Amendola, 165/A, Bari I-70126, Italy. E-mail address: francesco.caponio@uniba.it

Abstract

Brazil is an emerging virgin olive oil producer country whose oils have been scarcely studied till now and, in particular, no data are present in literature about their volatile profiles and organoleptic characteristics as well as the Pearson's correlations between chemical and sensory parameters. Hence, giving insights about these aspects was the aim of the current investigation. The volatile profiles showed the presence of the aldehydes responsible for the positive attribute of VOOs (i.e. *trans*-2-hexenal and hexanal) although, in some cases, volatiles from anaerobic and aerobic fermentation were also detected. The panel test showed low values of fruity and pungent notes (mean values of 1.1 and 0.6, respectively) whereas the bitter taste was detected only in one sample, probably due to the low amount of total phenolic compounds (ranging from 40 to 280 mg kg^{-1}). Accordingly to the volatiles analysis, slight defects of the oils were evidenced by the panelists. Tocopherols ranged from 123 to 222 mg kg^{-1}; carotenoids from 10.69 to 26.18 mg kg^{-1}, chlorophylls from 14.06 to 54.90 mg kg^{-1}, antioxidant activity from 976 to 1790 μmol TE g^{-1}, and fatty acid ethyl esters from 2.56 to 19.22 mg kg^{-1}. Positive Pearson's correlations were highlighted between hydroxytyrosol derivatives and antioxidant activity ($r=0.9601$, $p<0.0001$), *trans*-2-hexenal and fruity median ($r=0.6526$, $p<0.05$), acetic acid and vinegary defect ($r=0.7854$, $p<0.0001$), and fatty acid ethyl esters and vinegary defect ($r=0.8418$, $p<0.0001$). Our findings give first insights about sensory characteristics of Brazilian virgin olive oils and their association with chemical quality parameters. Finally, based on the obtained data, an improvement of preliminary operations (harvesting, storage) of the extraction process is recommended.

Keywords: VOO quality; Brazilian VOO; Phenolic compounds; Antioxidants; Volatile compounds; Ethyl esters; Panel test

Introduction

Virgin olive oil (VOO) is obtained from the fruit of the olive tree only by mechanical or physical processes in conditions which do not affect olive oil quality (Official Journal of the European Communities, 2001). VOO is widely consumed due to its beneficial health properties and unique sensory characteristics, which, in turn, are the result of its physicochemical features in terms of quantity and quality of its constituents (Bernardini & Visioli, 2017; Martín-Peláez, Covas, Fitó, Kusar, & Pravst, 2013; Nocella et al., 2018; Parkinson & Cicerale, 2016). VOO quality is defined according to the results of the chemical and sensory assessments (Bajoub, Bendini, Fernández-gutiérrez, & Carrasco-Pancorbo, 2018) and in this framework International Olive Council (IOC), European Union (EU), and Codex Alimentarius Commission (CODEX STAN) provide the international regulation for olive oil classification. According to IOC (International Olive Council, 2015b) 28 physicochemical parameters, including those classic and innovative, should be measured in order to evaluate the genuineness and quality of VOO. However, according to EC regulation 2568/91 (Official Journal of the European Communities, 1991) and subsequent modification and additions, free acidity, peroxide value, absorbance in ultra-violet, ethyl esters of fatty acids, which are parameters of quality, besides sensory evaluation must be taken into account to classify VOOs.

Sensory evaluation is an important instrument to analyze the quality and to classify virgin olive oils in commercial category. This analysis should be performed by a group called "panel", constituted by 8 to 12 tasters trained and qualified by regulatory bodies. The group is coordinated by a leader (Panel Leader), who collects the scores given to the positive (fruity, bitter, and pungent) and negative (sensory defects) sensory attributes. According to the median values of fruity and sensory defects, the virgin olive oil receives its classification:

73 extra virgin, virgin, or *lampante* (Bertoncini & Testa, 2014; International Olive Council,
74 2015a).

75 VOO sensory attributes arise from the stimulation of the gustative and olfactive receptors
76 through a great number of volatiles and some non volatile compounds, such as phenolic
77 substances (Campestre, Angelini, Gasbarri, & Angerosa, 2017; Cerretani, Salvador, Bendini,
78 & Fregapane, 2008; Procida, Cichelli, Lagazio, & Conte, 2015). Phenolic compounds are
79 responsible for the taste perception of bitterness and chemesthetic perceptions of pungency
80 (Dierkes et al., 2012). The other sensory perceptions of VOO are attributed to the stimulation
81 of the olfactory epithelium by the volatile fraction (Angerosa, 2002; Angerosa et al., 2004;
82 Kalua et al., 2007).

83 Olive is a millenary crop and the main production area is around the Mediterranean
84 basin, which accounts for more than 95% of the total olive oil produced worldwide. Thanks to
85 the similar climate characteristics to the southern European countries, the crop has expanded
86 to other regions, such as North Africa, North America and South America, as well as Asia. In
87 the context of South America, Argentina, Chile, and Uruguay are already considered producer
88 countries, while Brazil is at the begin (Borges et al., 2017; International Olive Council,
89 2017).

90 Brazil has been increasingly believing and investing in the olive tree cultivation,
91 presenting ever more strongly the culture to society and contributing to the development of
92 agriculture and agribusiness in the country. The olive tree has adapted well in the Mantiqueira
93 mountain range, between Minas Gerais and São Paulo, and in regions of Rio Grande do Sul.
94 Although olive growing in Brazil is a little over ten years old, the current scenario is already
95 very promising and signaling potential for growth. According to the Brazilian Institute of
96 Olive Growing the Brazilian production of olive oil in 2018 was estimated at 150,000 liters,
97 representing an increase of a 42.8% in relation to the production of the last year and, taking

into account that Brazilian olive crops are still very young (only 40% of them are ready to produce), the projection for 2025 is reach 20,000 hectares planted that represents an increment of 300% in relation to the area produced at this time. Brazil currently cultivates Arbequina, Arbosana, Manzanilla, Koroneiki, Coratina, Picual, Ascolana, and Grappolo cultivars, being Arbequina the main one, for having adapted very well to the climate and soil of the producing regions (Instituto Brasileiro de Olivicultura, 2018).

Regarding Brazilian regulation, the norms that standardize the classification and commercialization of virgin olive oil are described in Normative Instruction No. 1, of January 30, 2012, of the Ministry of Agriculture, Livestock and Food Supply of Brazil (Brasil. Ministério da Agricultura Pecuária e Abastecimento, 2012) and are based on IOC and EC regulations.

For almost ten years Brazil have produced olive oils whose chemical characteristics has been reported in literature (da Silva, de Oliveira, Zambon, Pio, & Gonçalves, 2014; Ballus et al., 2014; Ballus et al., 2015). Since there is no published scientific information regarding the sensory characteristics of Brazilian virgin olive oils evaluated by trained tasters, according to the IOC official protocol, no data available on the volatile compounds of virgin olive oil produced in Brazil, and neither any data with respect to correlations between chemical and sensory parameters, this research contributes to the scientific knowledge about virgin olive oil produced by an emerging country in the olive tree cultivation. In this context, the aim of this work was to evaluate chemical and sensory features of commercial Brazilian virgin olive oils.

Materials and Methods

Sampling

Brazilian virgin olive oils from Arbequina, Grapollo, Koroneiki and Coratina cultivars, representative of those cultivars produced in Brazil (Instituto Brasileiro de Olivicultura, 2018;

123 Teramoto, Sachs, Garcia, Oliveira, & Duarte, 2017) were studied. Twelve commercial virgin
124 olive oils of the crop harvest 2016/17, each representative of large batch, were considered
125 (Table 1). All of them derived from olive trees cultivated in Brazil in conditions of altitude
126 between 900 and 1200 meters, rainfall above 1500 mm per year and a temperature range from
127 10 to 24 °C. Virgin olive oils were obtained under a two-phase centrifuge extraction system.
128 Samples in triplicate were sent to the University of Bari Aldo Moro, Department of Soil, Plant
129 and Food Sciences, Food Science and Technology Unit, where they were analyzed.

130 **Routine analyses**

131 Free fatty acids (FFA), expressed as percentage of oleic acid, peroxide value (PV),
132 expressed as meq O_2 kg^{-1} of oil, and specific extinction coefficients at 232 and 270 nm (K_{232}
133 and K_{270}) were determined according to EU standard methods (Official Journal of the
134 European Communities, 1991).

135 **Fatty acid composition**

136 Fatty acid composition were determined according to EU standard methods (Official
137 Journal of the European Communities, 1991).

138 **Fatty acid alkyl esters determination**

139 Fatty acids methyl esters (FAME) and ethyl esters (FAEE) were determined according to
140 EU Commission Regulation 61/2011 (Official Journal of the European Communities, 2011)
141 as described in Squeo, Grassi, Paradiso, Alamprese, & Caponio (2019).

142 **Pigments determination**

143 Chlorophylls and carotenoids were determined according to Makhlouf, Squeo, Barkat,
144 Trani, & Caponio (2018) with some modifications. The chlorophyll content was evaluated by
145 the absorption spectrum according to the American Oil Chemists' Society (2017), and
146 expressed as mg of *pheophytin a* per kg of oil. The concentration of total carotenoids was

147 calculated measuring the absorption of 0.25 g of oil dissolved in 10 mL UV-hexane at 449
148 nm.

149 **Tocopherols determination**

150 Total tocopherols (α, β, γ, and δ) were determined by HPLC according to Gliszczynska-
151 Swigło & Sikorska (2004). Samples of oils were weighted (0.02-0.03 g) and dissolved in 1
152 mL of 2-propanol. Vortex-mixed samples were directly injected to HPLC column (C18, 150 ×
153 4.6 mm i.d. with a particle size of 3 μm, Thermo Scientific, Waltham, MA, USA). The mobile
154 phase consists in a mixture of acetonitrile and methanol (1:1 v/v) at a constant flow rate of 1
155 mL min^{-1} in isocratic elution. The injection volume was 20 μL. The quantification of
156 tocopherols was reached by means of FLD detector (Dionex 3400RS, Waltham, MA, USA),
157 set at excitation wavelength of 295 nm and an emission at 325 nm, using the external
158 calibration method on the basis of three calibration curves (α- γ- δ-tocopherols, R^2 99.99,
159 99.95 and 99.96, respectively). Results were reported as ppm (mg kg^{-1}) of oil for α-tocopherol
160 and for the sum of γ- and β-tocopherols.

161 **Phenolic compounds determination**

162 VOO phenolic compounds were extracted and determined according to Squeo, Caponio,
163 et al. (2019) e Difonzo et al. (2017) with some modifications. For the extraction, about 1 g of
164 the oil was added with 1 mL of hexane and 5 mL of methanol/water (70:30 v/v). After 10 min
165 vortexing, and centrifugation at 3941 × *g* for 10 min at 4 °C (SL 16R Centrifuge, Thermo
166 Fisher Scientific Inc., Waltham, MA, USA), the methanol/water phase was recovered and
167 submitted a subsequent centrifugation at 8867 × *g* for 5 min at 4 °C. The methanol/water
168 phase was then filtered through nylon filter (pore size 0.45 μm, Sigma, Ireland) to an amber
169 glass vial. Total phenols were determined spectrophotometrically by Folin-Ciocalteu assay.
170 To 100 μL of appropriately diluted extract, was added 100 μL of Folin-Ciocalteu reagent.
171 After 4 min, 800 μL of 5% Na_2CO_3 were added and then incubated at 40 °C for 20 min. The

absorbance was read after cooling down at room temperature at 750 nm. The results were expressed as mg of gallic acid equivalents per kg of oil. For phenolic compounds analysis by HPLC, the extraction was performed using 5 g of oil added with 250 μL of 100 ppm gallic acid solution (internal standard), 1 mL of hexane and 2 mL of methanol/water mixture and following the same procedure reported before. Then, an aliquot of the methanol/water solution of phenolic compounds (250 μL) was transferred to a HPLC vial with 250 μL of water/acetic acid (99:1, v/v). UHPLC binary system (Dionex Ultimate 3000 RSLC, Waltham, MA, USA) equipped with a quaternary pump (HPG 3200RS), auto-sampler (WPS 3000), stationary phase compartment (TCC 3000), diode array detector (3000 RS) and the Chromeleon software for data acquisition and processing was used. The stationary phase was an Acclaim C18 analytical column (150 × 4.6 mm i.d.) with a particle size of 3 μm (Thermo Scientific, Waltham, MA, USA). The mobile phases were (A) water/acetic acid (99:1, v/v) and (B) methanol/acetonitrile/acetic acid (50:49:1 v/v/v) at a constant flow rate of 1 mL min^{-1}. The column temperature was set at 30 °C. The gradient program was as follows: 95% A for 1 min; 80% A in 10 min; 56% A in 12 min; 41% A in 10 min; 10% A in 14 min, for a total run duration of 46 min. Diode array detection was monitored at 280 nm, and spectra were recorded at wavelength range 200-380 nm. The identification of phenolic compounds was performed by comparing the peak retention times with those obtained by the injection of pure standards and, in absence of these, with data in literature (International Olive Council, 2009). The quantification was achieved using a gallic acid internal standard on the 280 nm spectrum and the results expressed as mg of gallic acid equivalents (GAE) per kg of oil (Tamborrino et al., 2017).

Volatile compounds

Volatile compounds were determined adding 1 ± 0.010 g of oil into 20 mL vials, sealed with a screw top aluminum cap and silicon/PTFE septum, and 100 μL of a 60 mg kg^{-1}

197 solution of 1-octanol in purified olive oil as internal standard for quantification and submitted
198 to the (SPME/GC-MS) in the conditions reported in Tamborrino et al. (2017). The extraction
199 was performed by exposing a 50/30 μm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA,
200 USA) in the headspace of the sample at 40 °C for 20 min. When the extraction process was
201 completed, the fiber was inserted into the injector port of the gas chromatograph for thermal
202 desorption of volatiles in splitless mode. The GC/MS instrumentation included an Agilent
203 model 6850 gas chromatograph coupled to a mass spectrometer Agilent 5975. The volatile
204 compounds were separated on a HP-Innowax (60 m × 0.25 mm, 0.25 μm film thickness) polar
205 capillary column (Agilent) under the following conditions: flow 1.5 mL min^{-1}; injector
206 temperature, 250 °C; pressure of the carrier (helium), 30 kPa. The oven temperature was held
207 for 5 min at 40 °C, then increased by 4 °C min^{-1} to 220 °C and held constant for 10 min. The
208 mass spectrometer was operated in the electron impact mode (electron energy = 70 eV), and
209 the ion source temperature was 230 °C. A continuous scan mode was employed with a scan
210 time of 7.7 scans s^{-1} over a mass range of 33-270 amu. The volatile compounds were
211 identified by comparison of their mass spectra with those present in the NIST and Wiley
212 libraries. Results have been expressed as mg kg^{-1} of 1-octanol equivalents (OE).

213 **Antioxidant activity**

214 Antioxidant activity was carried out as reported in Difonzo et al., (2018). Extracts were
215 analyzed for their capacity to scavenge the stable DPPH radical. A solution of DPPH 0.08
216 mM in ethanol was prepared. In cuvettes for spectrophotometry, 50 μL of each sample were
217 added to 950 μL of DPPH solution. After 30 min in the dark, the decrease of absorbance was
218 read at 517 nm. The results were expressed in μmol Trolox equivalents (TE). For all the
219 spectrophotometric determinations a Cary 60 Agilent spectrophotometer (Cernusco, Milan,
220 Italy) was used.

221 All determinations were carried out in duplicate.

Panel test

The panel test was carried out according to EU Commission Regulation EEC/2568/91 and its subsequent modifications (Annex XII) (Official Journal of the European Communities, 1991). A panel from Department of Soil, Plant and Food Sciences, Food Science and Technology Unit, of the University of Bari Aldo Moro, formed by eight trained tasters, evaluated the samples. After performing the olfactory tests, the gustatory sensations were evaluated considering positive attributes, i.e., fruity and bitter, and negative attributes, as the presence of defects, i.e., fusty/muddy sediment, musty-humid-earthy, winey-vinegary, acid-sour and rancid. The tactile sensation of pungency was either analyzed. The notes given by each taster were compiled by the leader of the panel, the statistical evaluation was carried out by the median of the fruity and median of the defects, and the results indicated the classification of the olive oils in extra virgin, virgin, and *lampante*.

Data analysis

The associations between variables were evaluated by Pearson's coefficient. Analysis was performed using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA. Descriptive statistics were calculated by means of Minitab 17 (Minitab Inc., State College, PA, USA). Principal Component Analysis (PCA) is a powerful and useful tool to explore multivariate dataset, study variable's correlations and highlight samples clusters. PCA was carried out on the correlation matrix by means of Minitab 17 (Minitab Inc., State College, PA, USA) and the results have been reported as score plot and loading plot.

Results and Discussion

Chemical parameters

The results of the chemical quality parameters are reported in Table 2. Values obtained for the routine analyses (FFA, PV, K_{232}, and K_{270}) were in accordance with EU regulations for

247 extra-virgin olive oil (EVOO) classification (Official Journal of the European Communities,
248 1991) and comparable to those reported in literature for Brazilian olive oils (Borges, Pereira,
249 et al., 2017). The characteristic olive oil fatty acid profile was observed in all samples, being 7
250 saturated (miristic, palmitic, margaric, stearic, arachidic, behenic, and lignoceric acids), 3
251 monounsaturated (oleic, palmitoleic, and gadoleic acids), and 2 polyunsaturated (linoleic and
252 linolenic acids) (Ballus et al., 2014; Borges, Pereira, et al., 2017; de Oliveira, Ramos, Pio, &
253 Cardoso, 2012; Official Journal of the European Communities, 2016). Palmitic, linoleic and
254 oleic acid showed the highest range among samples (Table 2). Similar ranges of these fatty
255 acids in Brazilian olive oils were reported by Ballus et al. (2014).

256 Regarding antioxidants and pigments, the average values of chlorophylls and carotenoids
257 were higher than those reported by literature for Brazilian olive oils – which reported values
258 ranged from 1.3 to 1.7 mg kg^{-1} for chlorophylls and from 2.7 to 3.9 mg kg^{-1} for carotenoids
259 (Borges et al., 2017) – whereas tocopherol content was in accordance with literature (Borges,
260 López, Pereira, Cabrera-Vique, & Seiquer, 2017). Our findings are worthy considering the
261 important role of carotenoids in the protection of lipids from oxidation (Ambra, Natella,
262 Lucchetti, Forte, & Pastore, 2017; Choe & Min, 2006). The total phenolic content (TPC), not
263 determined by other authors in Brazilian virgin olive oils (Ballus et al., 2014; Borges, Pereira,
264 et al., 2017; Borges, López, et al., 2017), showed high variability among samples ranging
265 from 40 to 280 mg kg^{-1}, with the lowest values found for Arbequina oils and the highest for
266 Coratina oil (sample 12, Table 1). The antioxidant activity of the VOOs, similarly to TPC,
267 showed high variability (range 976-1790 μmol TE g^{-1}). It is well known that phenols, which
268 constitute a complex matrix of hydrophilic substances, are mainly responsible for the VOOs
269 antioxidant activity (Ballus, Meinhart, de Souza Campos, & Godoy, 2015; Fuentes et al.,
270 2018; Ramos-Escudero, Morales, & Asuero, 2015). A significant Pearson's correlation
271 ($r=0.8508$, $p<0.0001$, Table S1), between TPC and antioxidant activity measured by DPPH

assay was observed, similarly to the positive correlation already reported between TPC and induction time found by different authors on virgin olive oils collected in Europe (Caponio, Alloggio, & Gomes, 1999; Franco et al., 2014; Fuentes et al., 2018; Owen et al., 2000). VOOs phenols are mainly represented by secoiridoids (3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA, oleuropein), phenolic acids (vanilic acid and *p*-coumaric acid), phenolic alcohols (tyrosol and hydroxityrosol), flavonoids (apigenin and luteolin), and lignans (pinoresinol). Among them, 3,4-DHPEA and its derivatives (3,4-DHPEA-EDA, 3,4-DHPEA-EA, and *p*-HPEA) are reported to have the higher antioxidant activity when compared with other biophenols of VOOs (Ragusa et al., 2017).

In the VOOs under study, twelve phenolic compounds were identified (Figure 1), being diphenols conjugated to the elenolic acid, especially the 3,4-DHPEA-EDA and 3,4-DHPEA-EA, the most abundant compounds followed by pinoresinol, hydroxytyrosol, *p*-HPEA-EDA, cinnamic acid, luteolin, and tyrosol. The other phenolic compounds were detected in amount lower than 1 mg kg^{-1}. 3,4-DHPEA-EDA and hydroxytyrosol presented the highest variability among samples, with an overall range of 34.12 and 13.98 mg kg^{-1}, respectively (Table 2). On the whole, the obtained values are comparable to those found by Borges et al (2017) and lower than those determined by Ballus et al. (2015) on Brazilian oils confirming that phenols are strongly affected by cultivar as well as climatic conditions, agronomical and technological factors, olives ripeness degree, and oil extraction system, as well known (Ballus et al., 2014; Borges et al., 2017; Dabbou et al., 2009; Caponio et al., 2018). Moreover, the concentration of hydroxytyrosol and its derivatives were strongly correlated ($r=0.9601$, $p<0.0001$) with antioxidant activity suggesting that this class of phenols plays an important role on the antioxidant properties of the Brazilian VOOs studied. Similar findings were reported by Franco et al. (2014) studying olive oil samples from several Spanish cultivars. Several studies highlighted the antioxidant and anti-inflammatory properties of the VOOs phenols profile

297 establishing a relationship between health protective effects of the VOOs consume and
298 prevention of the several diseases such as obesity, cardiovascular disease cancer and
299 Alzheimer's disease (Muto et al., 2015; Nocella et al., 2018; Parkinson & Cicerale, 2016;
300 Rossi et al., 2017). According to the European Regulation (Official Journal of the European
301 Communities, 2012) VOO phenols contribute to blood lipids protection against oxidative
302 injury when the oil contains at least 5 mg of hydroxytyrosol and its derivatives (e.g.
303 oleuropein complex and tyrosol) per 20 g of olive oil. Among analyzed VOOs, Coratina
304 sample (n. 12 of Table 1) showed the highest amounts of hydroxytyrosol.

305 Another important quality parameter for EVOOs, proposed by the International Olive
306 Council (International Olive Council, 2010), are the fatty-acid alkyl esters (FAAEs) defined
307 by the sum of fatty-acid methyl esters (FAMEs) and fatty-acid ethyl esters (FAEEs) produced
308 by esterification of fatty acids with low-molecular-weight alcohols, such as methanol and
309 ethanol, respectively (Di Serio et al., 2017; Mariani & Bellan, 2008). In particular, methanol
310 is associated with overripe olives that lead to cellular damage and subsequent its release from
311 the breakdowns of pectins through the enzyme pectin methylesterase action while ethanol is
312 associated with fermentative processes during olives storage in inadequate conditions (Valli et
313 al., 2013). In turn, such conditions also lead to sensory defects such as fusty and winey-
314 vinegary flavor (Morales, Luna, & Aparicio, 2005; Reiners & Grosch, 1998). For these
315 reasons, the European Union focused their attention only on the FAEEs setting the maximum
316 value for EVOO at 35 mg kg^{-1} of oil (Official Journal of the European Communities, 2016).
317 The detected values led to classify the oils as extra virgin; in particular FAEEs ranged from
318 2.56 to 19.22 mg kg^{-1} while FAMEs ranged from 1.50 to 13.88 mg kg^{-1}. To the best of our
319 knowledge, no previous data are present in literature on FAAEs content of Brazilian extra
320 virgin olive oils.
321

Sensory characteristics and Pearson's correlation with chemical parameters

Sensory notes of VOO are considered essential to consumers' approval. Its unique and delicate fragrance and flavor (Campestre et al., 2017; Fiorini et al., 2018; Kalua et al., 2007) is attributable to the volatile profile, that stimulate the human sensory receptors, mainly produced endogenously by oxidation of the polyunsaturated fatty acids (linoleic and linolenic acids) through the lipoxygenase (LOX) pathway (Kalua et al., 2007). Moreover, most of the volatile compounds associated to green and fruity notes are formed during the climacteric stage of the olive ripening. The climacteric stage, in which the production of ethylene is the highest inducing biochemical changes and increasing the enzyme activities, is the period in which the extracted VOO has the large amounts of aromatic volatile compounds (Rahmani & Csallany, 1998). Milling and malaxation are crucial since the aromatic substances are formed through the action of enzymes released in these steps of extraction processes (Olías, Pérez, Ríos, & Sanz, 1993; Ranalli, Tombesi, Ferrante, & De Mattia, 1998). *Trans*-2-hexenal, hexanal, and *trans*-2-hexan-1-ol – associated with green and fruity aroma perceptions – are the major volatile compounds responsible of the positive aroma of VOOs (Morales et al., 2005; Reiners & Grosch, 1998). However, the presence of volatiles from lipid auto-oxidation and aerobic or anaerobic fermentation, due to non-optimal both olive management and oil storage conditions, characterizes the off-flavor of the oil being associated with sensory defects (Caponio et al., 2015; Fiorini et al., 2018; Kalua et al., 2007).

The relationship between volatile profile and VOO quality has been largely investigated in the countries traditionally producer of virgin olive oil (Angerosa, 2002; Caponio et al., 2015; García-Vico et al., 2017; Hbaieb, Kotti, Gargouri, Msallem, & Vichi, 2016). Data about volatile compounds of virgin olive oils produced in other regions of the world are scarce or nonexistent. There is no data available in literature on the volatile profile of VOO produced in Brazil. Since 2008, when Brazil extracted the first virgin olive oil, producers have been

improved their agricultural and processing conditions in order to produce an olive oil with high quality in both chemical and sensory aspects.

Table 3 shows the volatile compounds composition of the investigated samples. Overall, seventeen volatile compounds were identified (Figure 2): seven aldehydes (butanal, 2 methyl, butanal, 3 methyl, *cis*-3-hexenal, *trans*-2-hexenal, *trans*-2-pentenal, hexanal, nonanal), two ketones (3-pentanone, 1-penten-3-one), three alcohols (*trans*-2-hexen-1-ol, 2-penten-1-ol, ethyl alcohol), three carboxylic acid (propanoic acid, butanoic acid, acetic acid), and two other compounds (3-ethyl-1,5-octadiene, octane). As expected, aldehydes were the class of volatiles more abundant evidencing a general good quality of the investigated oils. The major aldehydes identified were *trans*-2-hexenal, whose values ranged from 74.42 to 252.75 mg kg^{-1}, and hexanal with values that ranged from 4.79 to 17.45 mg kg^{-1}. The low amount of nonanal, detected only in two oils and associated with fatty/waxy/pungent notes and ethyl alcohol, detected only in four oils and associated with fermentative process (Morales et al., 2005), as well as the high amount of *trans*-2-hexen-1-ol, more abundant alcohol whose values ranged from 5.89 to 71.51 mg kg^{-1}, confirm this assumption. Moreover, among volatiles associated with off-flavors the most abundant was acetic acid, associated to sour/vinegar notes (Morales et al., 2005; Reiners & Grosch, 1998), with values ranging from 5.38 to 122.05 mg kg^{-1}. Its presence could be associated to non-optimal olive management pending processing (Angerosa et al., 2004).

In addition to the chemical quality parameters, the organoleptic parameters are also required by European Regulation 2568/91 and subsequent modification, so that the presence of fruity and absence of defects are mandatory conditions to be an extra virgin olive oil. Then, the investigated samples were analyzed for positive sensory attributes and the presence of defects. Table 4 shows median values of fruity, bitter, pungent, and the kind of defect of the virgin olive oils studied. Overall, data showed a quite low fruity and pungent intensity and,

372 with the exception of sample 12, the absence of bitter notes. This could be attributable to the
373 general low amount of phenolic compounds (Table 2), such as oleuropein related compounds,
374 which are significantly and positively correlated to the bitter taste, as reported by other
375 authors (Siliani, Mattei, Innocenti, & Zanoni, 2006; Beltrán, Ruano, Jiménez, Uceda, &
376 Aguilera, 2007; Favati, Condelli, Galgano, & Caruso, 2013). According to the median of the
377 defects and the median of the fruity, ten of the samples were classified as VOO, one as EVOO
378 (sample 7), and one was classified as lampante (sample 5) having fruity equal to zero. With
379 the exception of sample 5, all the others olive oil samples presented notes of fruity, which
380 median value ranged from 0.4 to 3.0. Pungency was also identified with a median ranged
381 from 0.3 to 1.7. The main defects identified were fusty/muddy and winey-vinegary. Fusty is a
382 typical defect of oil deriving from olives stored for long periods before extraction, whereas
383 vinegary defect is typical in oil from overripe olives, both defects arising from fermentation
384 processes (Angerosa et al., 2004).

385 A significant and positive Pearson's correlation was observed between *trans*-2-hexenal
386 content and fruity median ($r=0.6526$, $p<0.05$, Table S1), acetic acid content and vinegary
387 defect ($r=0.7854$, $p<0.0001$, Table S1), total FAAEs content and vinegary defect ($r=0.7505$,
388 $p<0.05$, Table S1) and FAEEs content and vinegary defect ($r=0.8418$, $p<0.0001$, Table S1).
389 Also Morales & Luna, (2000) and Morales et al. (2005) reported strong correlation between
390 the presence of acetic acid and winey-vinegary defect in virgin olive oil while Gómez-Coca,
391 Moreda, & Pérez-Camino (2012) demonstrated the association between high amounts of
392 FAEEs and defects that arise from fermentation. Corroborating these data, Di Serio et al.
393 (2017) reported that FAEEs are correlated to the fermentation processes, which are also
394 responsible for organoleptic defects in olive oil, such as winery/vinegary.

395 Overall, the obtained data suggested that raw material managing of Brazilian olive oil
396 mills should be improved. Indeed, together with the volatile compounds responsible for the

basic positive attribute of VOO, typically found in oils from healthy and fresh fruits, those compounds indicating anaerobic and aerobic fermentations were the negative ones generally found.

Principal component analysis (PCA)

Figure 3 reports the multivariate explorative analysis (PCA) carried out on the dataset. Thirty-nine variables were used to explore the data, namely the fatty acid composition, pigments, tocopherols, TPC, antioxidant activity, single phenolic compounds (3,4 DHPEA-EDA, 3,4 DHPEA-EA, *p*-HPEA-EDA, *p*-HPEA-EA, Hydroxytyrosol, Tyrosol, Apigenin, Luteolin and Pinoresinol), single volatile compounds (*trans*-2-Hexenal, Hexanal, *trans*-2-Hexen-1-ol, 2-Penten-1-ol and Acetic acid), sensory descriptors (fruity and pungent) and methyl end ethyl esters of fatty acids. The first two components explained around 60% of the total variability and allowed a good separation of the samples according to the varieties. Indeed, the Arbequina oils lie on the negative quadrant of the PCs and were characterized by the highest amount of $C_{16:1}$, $C_{17:0}$, $C_{18:2}$, total saturated and total polyunsaturated fatty acids. Further, according to the loadings (data not shown), these oils were richer in *p*-HPEA-EA and Hexanal. Interestingly, the two blends in which Arbequina was one of the constituent lie on the boundary of the region circumscribed by the pure Arbequina oils, highlighting the good ability of the variables considered and of the PCA in grabbing and describing the oil features. Accordingly, the blend Arbequina/Grappolo moved toward the neighboring quadrant in which the pure Grappolo oils are located. These samples had higher content of $C_{20:1}$, FAMEs, FAEEs, 2-Penten-1-ol and Acetic Acid. The loading plot also highlighted the good correlation between the amount of Acetic acid and the alkyl esters content of the oils. Only one sample of Arbequina was far from the others (number 7), due to the higher content in total tocopherols, in *trans*-2-Hexenal and, accordingly, in the fruity note. The geographical origin influenced to

a less extent the oils features since the Arbequina oil from the southern area was confused to the others from the southeastern region. Coratina and Koroneiki oils lie far from all the other samples having, the former, the higher amount of phenolic compounds, antioxidant activity and pungent note and *trans*-2-Hexen-1-ol. Koroneiki oil seemed characterized by intermediate features between Arbequina and Coratina samples.

Conclusions

The obtained data give a comprehensive overlook about the quality of commercial Brazilian virgin olive oils. Significant correlations between the panel test results and the minor components of VOO, such as volatile compounds, were observed. Our findings are relevant not only for contributing to the production of scientific knowledge about the chemical and sensory quality of virgin olive oil, but also to indicate the importance of conducting research on Brazilian olive oil in order to help finding the proper agricultural and technological practices adapted to subtropical climate conditions and resulting in a high quality virgin olive oil, in both sensory and chemical approaches.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We are grateful to the olive oil producers that kindly provide us the samples. Authors contribution: LZ conceived the study, conducted the analysis, analyzed data, performed statistical analysis and wrote the manuscript. GS and GD participated in the conception of the study, conducted the analysis, analyzed data, performed statistical analysis and revised the manuscript. EIB participated in the conception of the study and interpretation of data and

revised the manuscript. FC conceived and designed the study, analyzed data and revised the manuscript critically.

Figures captions

Figure 1. Chromatogram of the phenolic compounds of Brazilian virgin olive oil. Peaks are indicated as follows: (I.S.) Gallic acid (internal standard), (1) Hydroxytyrosol, (2) Tyrosol, (3) Vanillic acid, (4) Siringic acid, (5) *p*-Coumaric acid, (6) Cinnamic acid, (7) 3,4-DHPEA-EDA, (8) *p*-HPEA-EDA, (9) Pinoresinol, (10) 3,4-DHPEA-EA, (11) Luteolin, and (12) Apigenin.

Figure 2. Chromatogram of the volatile compounds of Brazilian virgin olive oil. Peaks are indicated as follows: (1) Octane, (2) Butanal, 2 methyl, (3) Butanal, 3 methyl, (4) 3-Pentanone, (5) Ethyl alcohol, (6) 1-Penten-3-one, (IS) 1-propanol (internal standard), (7) 3-Ethyl-1,5-octadiene, (8) hexanal, (9) *trans*-2-Pentenal, (10) *cis*-3-Hexenal, (11) *trans*-2-Hexenal, (12) Nonanal, (13) 2-Penten-1-ol, (14) Acetic acid, (15) *trans*-2-Hexen-1-ol, (16) Propanoic acid, and (17) Butanoic acid.

Figure 3. Results of the PCA. (A) Score plot and (B) loading plot.

References

Ambra, R., Natella, F., Lucchetti, S., Forte, V., & Pastore, G. (2017). α-Tocopherol, β-carotene, lutein, squalene and secoiridoids in seven monocultivar Italian extra-virgin olive oils. *International Journal of Food Sciences and Nutrition*, *68*, 538–545.

American Oil Chemists' Society. (2017). AOCS Official Method Cc 13i-96. Determination of Chlorophyll Pigments in Crude Vegetable Oils. In *Official methods and recommended practices of the AOCS* (7th ed.). Washington, DC: AOCS Press.

Angerosa, F. (2002). Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *European Journal of Lipid Science and*

Technology, *104*, 639–660.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G. (2004). Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *Journal of Chromatography A*, *1054*, 17–31.

Bajoub, A., Bendini, A., Fernández-gutiérrez, A., & Carrasco-Pancorbo, A. (2018). Olive oil authentication : A comparative analysis of regulatory frameworks with especial emphasis on quality and authenticity indices , and recent analytical techniques developed for their assessment . A review. *Critical Reviews in Food Science and Nutrition*, *58*, 832–857.

Ballus, C. A., Meinhart, A. D., Campos Jr, F. A. de S., da Silva, L. F. de O., de Oliveira, A. F., & Godoy, H. T. (2014). A quantitative study on the phenolic compound , tocopherol and fatty acid contents of monovarietal virgin olive oils produced in the southeast region of Brazil. *Food Research International*, *62*, 74–83.

Ballus, C. A., Meinhart, A. D., de Souza Campos, F. A., & Godoy, H. T. (2015). Total Phenolics of Virgin Olive Oils Highly Correlate with the Hydrogen Atom Transfer Mechanism of Antioxidant Capacity. *Journal of the American Oil Chemists' Society*, *92*, 843–851.

Beltrán, G., Ruano, M. T., Jiménez, A., Uceda, M., & Aguilera, M. P. (2007). Evaluation of virgin olive oil bitterness by total phenol content analysis. *European Journal of Lipid Science and Technology*, *109*, 193–197.

Bernardini, E., & Visioli, F. (2017). High quality , good health : The case for olive oil. *European Journal of Lipid Science and Technology*, *1500505*, 1–9.

Bertoncini, E., & Testa, U. (2014). Análise sensorial de azeite de oliva. *Informe Agropecuário Da Empresa de Pesquisa Agropecuária de Minas Gerais*, *35*, 58–65.

Borges, T. H., López, L. C., Pereira, J. A., Cabrera-Vique, C., & Seiquer, I. (2017). Comparative analysis of minor bioactive constituents (CoQ10, tocopherols and phenolic

compounds) in Arbequina extra virgin olive oils from Brazil and Spain. *Journal of Food Composition and Analysis*, *63*, 47–54.

Borges, T. H., Pereira, J. A., Cabrera-Vique, C., Lara, L., Oliveira, A. F., & Seiquer, I. (2017). Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. *Food Chemistry*, *215*, 454–462.

Brasil. Ministério da Agricultura Pecuária e Abastecimento. (2012). Instrução Normativa No 1 de janeiro de 2012. Brasília: Diário Oficial da União.

Campestre, C., Angelini, G., Gasbarri, C., & Angerosa, F. (2017). The compounds responsible for the sensory profile in monovarietal virgin olive oils. *Molecules*, *22*, 1–28.

Caponio, F., Alloggio, V., & Gomes, T. (1999). Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chemistry*, *64*.

Caponio, F., Squeo, G., Brunetti, L., Pasqualone, A., Summo, C., Paradiso, V. M., … Bianchi, B. (2018). Influence of the feed pipe position of an industrial scale two-phase decanter on extraction efficiency and chemical-sensory characteristics of virgin olive oil. *Journal of the Science of Food and Agriculture*, *98*, 4279–4286.

Caponio, F., Squeo, G., Monteleone, J. I., Paradiso, V. M., Pasqualone, A., & Summo, C. (2015). First and second centrifugation of olive paste: Influence of talc addition on yield, chemical composition and volatile compounds of the oils. *LWT - Food Science and Technology*, *64*, 439–445.

Cerretani, L., Salvador, M. D., Bendini, A., & Fregapane, G. (2008). Relationship Between Sensory Evaluation Performed by Italian and Spanish Official Panels and Volatile and Phenolic Profiles of Virgin Olive Oils. *Chemosensory Perception*, *1*, 258–267.

Choe, E., & Min, D. B. (2006). Mechanisms and Factors for Edible Oil Oxidation. *Comprehensive Reviews in Food Science and Food Safety*, *5*, 169–186.

525 da Silva, L. F. O., de Oliveira, A. F., ZAmbon, C. R., Pio, R., & Gonçalves, E. D. (2014).
526 Índice de qualidade em amostras de azeite de oliva extraídas de cultivares de oliveiras do
527 Sul de Minas Gerais. *Informe Agropecuário Da Empresa de Pesquisa Agropecuária de*
528 *Minas Gerais*, *35*, 34–49.
529 Dabbou, S., Issaoui, M., Esposto, S., Sifi, S., Taticchi, A., Servili, M., … Hammamia, M.
530 (2009). Cultivar and growing area effects on minor compounds of olive oil from
531 autochthonous and European introduced cultivars in Tunisia. *Journal of the Science of*
532 *Food and Agriculture*, *89*, 1314–1325.
533 de Oliveira, M. C., Ramos, J. D., Pio, R., & Cardoso, M. das G. (2012). Características
534 fenológicas e físicas e perfil de ácidos graxos em oliveiras no sul de Minas Gerais.
535 *Pesquisa Agropecuaria Brasileira*, *47*, 30–35.
536 Di Serio, M. G., Giansante, L., Di Loreto, G., Faberi, A., Ricchetti, L., & Di Giacinto, L.
537 (2017). Ethyl esters versus fermentative organoleptic defects in virgin olive oil. *Food*
538 *Chemistry*, *219*, 33–39.
539 Dierkes, G., Krieger, S., Duck, R., Bongartz, A., Schmitz, O. J., & Hayen, H. (2012). High-
540 Performance Liquid Chromatography − Mass Spectrometry Pro fi ling of Phenolic
541 Compounds for Evaluation of Olive Oil Bitterness and Pungency. *Journal of*
542 *Agricultural and Food Chemistry*, *60*, 7597–7606.
543 Difonzo, G., Pasqualone, A., Silletti, R., Cosmai, L., Summo, C., Paradiso, V. M., & Caponio,
544 F. (2018). Use of olive leaf extract to reduce lipid oxidation of baked snacks. *Food*
545 *Research International*, *108*, 48–56.
546 Difonzo, G., Russo, A., Trani, A., Paradiso, V. M., Ranieri, M., Pasqualone, A., … Caponio,
547 F. (2017). Green extracts from Coratina olive cultivar leaves : Antioxidant
548 characterization and biological activity. *Journal of Functional Foods*, *31*, 63–70.
549 Favati, F., Condelli, N., Galgano, F., & Caruso, M. C. (2013). Extra virgin olive oil bitterness

550 evaluation by sensory and chemical analyses. *Food Chemistry*, *139*, 949–954.
551 Fiorini, D., Boarelli, M. C., Conti, P., Alfei, B., Caprioli, G., Ricciutelli, M., … Pacetti, D.
552 (2018). Chemical and sensory differences between high price and low price extra virgin
553 olive oils. *Food Research International*, *105*, 65–75.
554 Franco, M. N., Galeano-Díaz, T., López, Ó., Fernández-Bolaños, J. G., Sánchez, J., De
555 Miguel, C., … Martín-Vertedor, D. (2014). Phenolic compounds and antioxidant
556 capacity of virgin olive oil. *Food Chemistry*, *163*, 289–298.
557 Fuentes, E., Paucar, F., Tapia, F., Ortiz, J., Jimenez, P., & Romero, N. (2018). Effect of the
558 composition of extra virgin olive oils on the differentiation and antioxidant capacities of
559 twelve monovarietals. *Food Chemistry*, *243*, 285–294.
560 García-Vico, L., Belaj, A., Sánchez-Ortiz, A., Martínez-Rivas, J. M., Pérez, A. G., & Sanz, C.
561 (2017). Volatile Compound Profiling by HS-SPME/GC-MS-FID of a Core Olive
562 Cultivar Collection as a Tool for Aroma Improvement of Virgin Olive Oil. *Molecules*,
563 *22*, 1–12.
564 Gliszczynska-Swigło, A., & Sikorska, E. (2004). Simple reversed-phase liquid
565 chromatography method for determination of tocopherols in edible plant oils. *Journal of*
566 *Chromatography A*, *1048*, 195–198.
567 Gómez-Coca, R., Moreda, W., & Pérez-Camino, M. (2012). Fatty acid alkyl esters presence
568 in olive oil vs . organoleptic assessment. *Food Chemistry*, *135*, 1205–1209.
569 Hbaieb, R. H., Kotti, F., Gargouri, M., Msallem, M., & Vichi, S. (2016). Ripening and storage
570 conditions of Chétoui and Arbequina olives: Part I. Effect on olive oils volatiles profile.
571 *Food Chemistry*, *203*, 548–558.
572 Instituto Brasileiro de Olivicultura. (2018). Projeção do mercado oleícola para os próximos
573 anos. Retrieved October 2, 2018, from https://www.ibraoliva.com.br
574 International Olive Council. (2009). Determination of Biophenols in olive oils by HPLC.

575 COI/T.20/Doc No 29.
576 International Olive Council. (2010). Determination of the content of waxes, fatty acid methyl
577 esters and fatty acid ethyl esters by capillary gas chromatography COI/T.20/Doc. No
578 28/Rev. 1.
579 International Olive Council. (2015a). Sensory Analysis of Olive Oil Method for the
580 Organoleptic Assessment of Virgin Olive Oil COI/T.20/Doc. No 15/Rev. 8. Madrid.
581 International Olive Council. (2015b). Trade Standard Applying to Olive Oils and Olive-
582 pomace Oils COI/T.15/NC No 3/Rev. 8. Madrid.
583 International Olive Council. (2017). World olive oil figures. Retrieved September 14, 2017,
584 from http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures
585 Kalua, C. M., Allen, M. S., Bedgood, D. R., Bishop, A. G., Prenzler, P. D., & Robards, K.
586 (2007). Olive oil volatile compounds, flavour development and quality: A critical
587 review. *Food Chemistry*, *100*, 273–286.
588 Makhlouf, F. Z., Squeo, G., Barkat, M., Trani, A., & Caponio, F. (2018). Antioxidant activity
589 , tocopherols and polyphenols of acornoil obtained from Quercus species grown in
590 Algeria. *Food Research International*, *114*, 208–213.
591 Mariani, C., & Bellan, G. (2008). Individuazione di oli di qualità inferiore negli oli di oliva
592 extravergini. *Rivista Italiana Sostanze Grasse*, *82*, 3–20.
593 Martín-Peláez, S., Covas, M. I., Fitó, M., Kusar, A., & Pravst, I. (2013). Health effects of
594 olive oil polyphenols : Recent advances. *Molecular Nutrition & Food Research*, *57*,
595 760–771.
596 Morales, M. T., & Luna, G. (2000). Sensory and chemical evaluation of winey-vinegary
597 defect in virgin olive oils. *European Food REsearch and Technology*, *211*, 222–228.
598 Morales, M. T., Luna, G., & Aparicio, R. (2005). Food Chemistry Comparative study of
599 virgin olive oil sensory defects. *Food and Chemical Toxicology*, *91*, 293–301.

Muto, E., Dell'Agli, M., Sangiovanni, E., Mitro, N., Fumagalli, M., Crestani, M., … Caruso, D. (2015). Olive oil phenolic extract regulates interleukin-8 expression by transcriptional and posttranscriptional mechanisms in Caco-2 cells. *Molecular Nutrition and Food Research*, *59*, 1217–1221.

Nocella, C., Cammisotto, V., Fianchini, L., D'Amico, A., Novo, M., Castellani, V., … Carnevale, R. (2018). Extra Virgin Olive Oil and Cardiovascular Diseases: Benefits for Human Health. *Endocrine, Metabolic & Immune Disorders - Drug Targets*, *18*, 4–13.

Official Journal of the European Communities. (1991). Commission Regulation (EEC) No 2568/91 of 11 July 1991. Brussels: Publications Office of the European Union.

Official Journal of the European Communities. (2001). Council Regulation (EC) No 1513/2001 of 23 july 2001. Brussels: Publications Office of the European Union.

Official Journal of the European Communities. (2011). COMMISSION REGULATION (EU) No 61/2011 of 24 January 2011 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Publications Office of the European Union.

Official Journal of the European Communities. (2012). REGULAMENTO (UE) N. o 432/2012 DA COMISSÃO de 16 de maio de 2012.

Official Journal of the European Communities. (2016). COMMISSION DELEGATED REGULATION (EU) 2016/2095 of 26 September 2016 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.

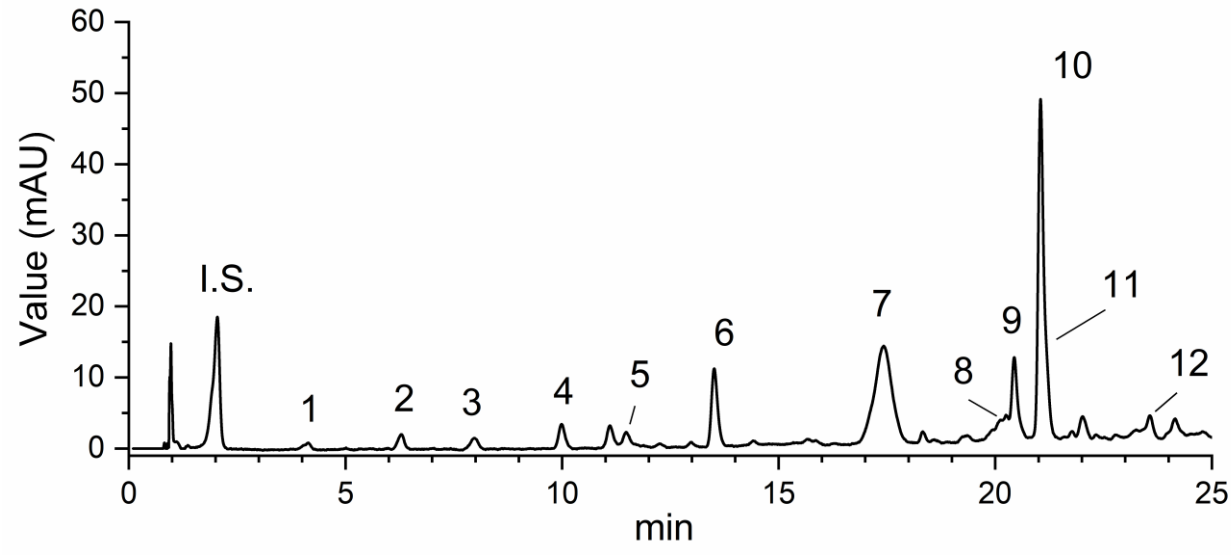
Olías, J. M., Pérez, A. G., Ríos, J. J., & Sanz, L. C. (1993). Aroma of Virgin Olive Oil: Biogenesis of the "Green" Odor Notes. *Journal of Agricultural and Food Chemistry*, *41*, 2368–2373.

Owen, R. W., Mier, W., Giacosa, A., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2000).

625 Phenolic compounds and squalene in olive oils : the concentration and antioxidant
626 potential of total phenols , simple phenols , secoiridoids , lignans and squalene. *Food and*
627 *Chemical Toxicilogy*, *38*, 647–659.
628 Parkinson, L., & Cicerale, S. (2016). The Health Benefiting Mechanisms of Virgin Olive.
629 *Molecules*, *21*, 1–12.
630 Procida, G., Cichelli, A., Lagazio, C., & Conte, L. S. (2015). Relationships between volatile
631 compounds and sensory characteristics in virgin olive oil by analytical and chemometric
632 approaches Giuseppe Procida , a * Angelo Cichelli , b Corrado Lagazio c and. *Journal of*
633 *the Science of Food and Agriculture*, *96*, 311–318.
634 Ragusa, A., Centonze, C., Grasso, M., Latronico, M., Mastrangelo, P., Fanizzi, F., & Maffia,
635 M. (2017). Composition and Statistical Analysis of Biophenols in Apulian Italian
636 EVOOs. *Foods*, *6*, 1–15.
637 Rahmani, M., & Csallany, A. S. (1998). Role of Minor Constituents in the Photooxidation of
638 Virgin Olive Oil. *Journal of the American Oil Chemists' Society*, *75*, 837–843.
639 Ramos-Escudero, F., Morales, M. T., & Asuero, A. G. (2015). Characterization of bioactive
640 compounds from monovarietal virgin olive oils: Relationship between phenolic
641 compounds-antioxidant capacities. *International Journal of Food Properties*, *18*, 348–
642 358.
643 Ranalli, A., Tombesi, A., Ferrante, M. L., & De Mattia, G. (1998). Respiratory rate of olive
644 drupes during their ripening cycle and quality of oil extracted. *Journal of the Science of*
645 *Food and Agriculture*, *77*, 359–367.
646 Reiners, J., & Grosch, W. (1998). Odorants of Virgin Olive Oils with Different Flavor
647 Profiles. *Journal of Agricultural and Food Chemistry*, *46*, 2754–2763.
648 Rossi, M., Caruso, F., Kwok, L., Lee, G., Caruso, A., Gionfra, F., … Incerpi, S. (2017).
649 Protection by extra virgin olive oil against oxidative stress in vitro and in vivo. Chemical

650 and biological studies on the health benefits due to a major component of the
651 Mediterranean diet. *PLoS ONE*, *12*, 1–21.
652 Siliani, S., Mattei, A., Innocenti, L. B., & Zanoni, B. (2006). Bitter taste and phenolic
653 compounds in extra virgin olive oil: an empirical relationship. *Journal of Food Quality*,
654 *29*, 431–441.
655 Squeo, G., Caponio, F., Paradiso, V. M., Summo, C., Pasqualone, A., Khmelinskii, I., &
656 Sikorska, E. (2019). Evaluation of total phenolic content in virgin olive oil using
657 fluorescence excitation – emission spectroscopy coupled with chemometrics. *Journal of*
658 *the Science of Food and Agriculture*, *99*, 2513–2520.
659 Squeo, G., Grassi, S., Caponio, F., Paradiso, V. M., Alamprese, C., & Caponio, F. (2019). FT-
660 IR extra virgin olive oil classification based on ethyl ester content. *Food Control*, *102*,
661 149–156.
662 Tamborrino, A., Squeo, G., Leone, A., Michele, V., Romaniello, R., Summo, C., … Caponio,
663 F. (2017). Industrial trials on coadjuvants in olive oil extraction process : Effect on
664 rheological properties , energy consumption , oil yield and olive oil characteristics.
665 *Journal of Food Engineering*, *205*, 34–46.
666 Teramoto, J. R. S., Sachs, R. C. C., Garcia, V. L., Oliveira, A. da S. S. de, & Duarte, M. C.
667 (2017). Atividade antimicrobiana das folhas de duas variedades de oliveira e a
668 contextualização deste coproduto da produção paulista e mundial de azeite de oliva.
669 *Revista Intellectus*, *1*, 63–83.
670 Valli, E., Bendini, A., Maggio, R. M., Cerretani, L., Toschi, T. G., Casiraghi, E., & Lercker,
671 G. (2013). Detection of low-quality extra virgin olive oils by fatty acid alkyl esters
672 evaluation: A preliminary and fast mid-infrared spectroscopy discrimination by a
673 chemometric approach. *International Journal of Food Science and Technology*, *48*, 548–
674 555.
675

676



677

678 **Figure 1.** Chromatogram of the phenolic compounds of Brazilian virgin olive oil. Peaks
679 are indicated as follows: (I.S.) Gallic acid (internal standard), (1) Hydroxytyrosol, (2)
680 Tyrosol, (3) Vanillic acid, (4) Siringic acid, (5) *p*-Coumaric acid, (6) Cinnamic acid, (7) 3,4-
681 DHPEA-EDA, (8) *p*-HPEA-EDA, (9) Pinoresinol, (10) 3,4-DHPEA-EA, (11) Luteolin, and
682 (12) Apigenin.

683

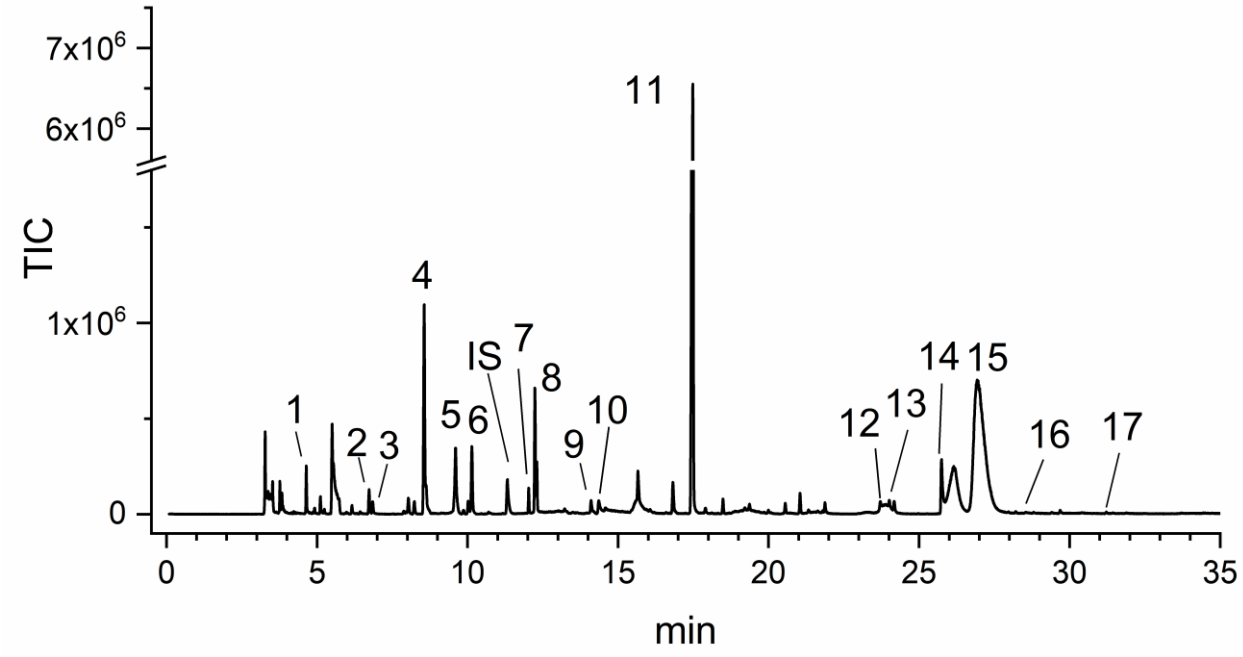


Figure 2. Chromatogram of the volatile compounds of Brazilian virgin olive oil. Peaks are indicated as follows: (1) Octane, (2) Butanal, 2 methyl, (3) Butanal, 3 methyl, (4) 3-Pentanone, (5) Ethyl alcohol, (6) 1-Penten-3-one, (IS) 1-propanol (internal standard), (7) 3-Ethyl-1,5-octadiene, (8) hexanal, (9) *trans*-2-Pentenal, (10) *cis*-3-Hexenal, (11) *trans*-2-Hexenal, (12) Nonanal, (13) 2-Penten-1-ol, (14) Acetic acid, (15) *trans*-2-Hexen-1-ol, (16) Propanoic acid, and (17) Butanoic acid.

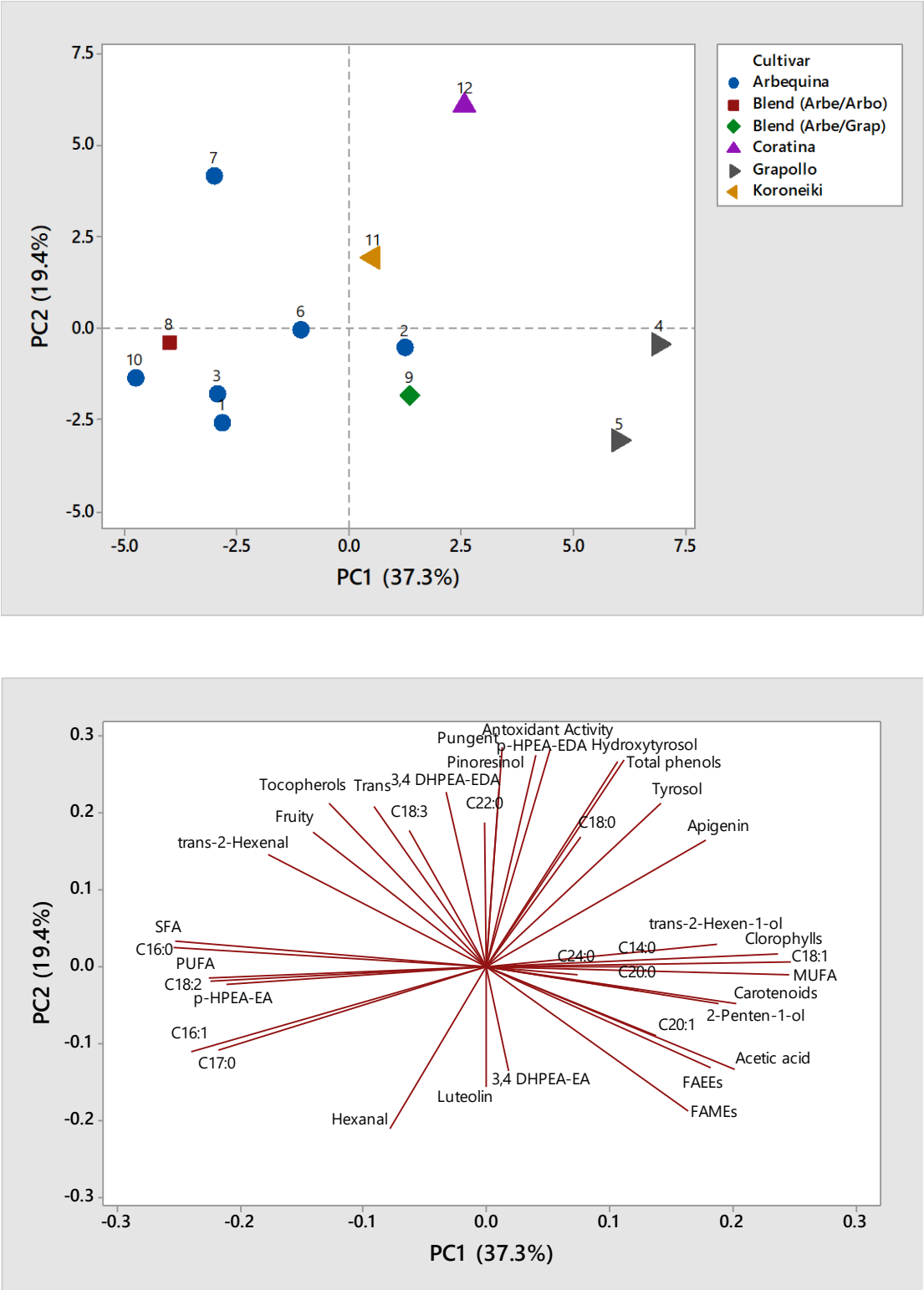


Figure 3. Results of the PCA. (A) Score plot and (B) loading plot.

Table 1. Cultivar and producing regions of the Brazilian virgin olive oil samples.

Olive oil sample	Cultivar	Region/State
1	Arbequina	Southeast/Minas Gerais
2	Arbequina	Southeast/Minas Gerais
3	Arbequina	Southeast/Minas Gerais
4	Grappolo	Southeast/Minas Gerais
5	Grappolo	Southeast/Minas Gerais
6	Arbequina	Southeast/Minas Gerais
7	Arbequina	Southeast/São Paulo
8	Arbequina/Arbosana	Southeast/Minas Gerais
9	Arbequina/Grappolo	Southeast/Minas Gerais
10	Arbequina	Southern/Parana
11	Koroneiki	Southern/Parana
12	Coratina	Southern/Parana

728

729

Table 2. Descriptive statistics of the dataset under study (*n*=12).

Chemical parameter	Mean value	Min value	Max value	Range	Median	IQR
Routine quality parameters						
FFA (g 100 g^{-1})	0.53	0.34	0.80	0.46	0.55	0.20
PV (meq O_2 kg^{-1})	9.1	8.2	10.5	2.2	8.9	1.4
K_{232}	1.79	1.49	2.22	0.73	1.78	0.33
K_{270}	0.17	0.14	0.21	0.07	0.17	0.03
Fatty acid composition (g 100 g^{-1})						
$C_{14:0}$	0.02	0.01	0.02	0.01	0.02	0.01
$C_{16:0}$	12.91	8.60	16.72	8.12	13.32	3.19
$C_{16:1}$	1.46	0.54	2.51	1.97	1.35	1.44
$C_{17:0}$	0.25	0.13	0.37	0.24	0.28	0.18
$C_{18:0}$	1.66	1.49	2.19	0.70	1.61	0.16
$C_{18:1}$	74.80	64.40	83.56	19.16	74.58	7.71
$C_{18:2}$	7.82	4.15	13.19	9.04	8.18	3.86
$C_{18:3}$	0.37	0.30	0.47	0.17	0.37	0.03
$C_{20:0}$	0.51	0.42	0.61	0.19	0.50	0.10
$C_{20:1}$	0.17	0.12	0.22	0.10	0.16	0.06
$C_{22:0}$	0.02	0.01	0.03	0.02	0.02	0.01
$C_{24:0}$	0.02	0.01	0.03	0.02	0.02	0.01
TFA	0.01	0.01	0.02	0.01	0.01	0.01
SFA	15.38	10.93	19.27	8.34	15.93	3.10
MUFA	76.42	67.13	84.28	17.15	75.81	6.64
PUFA	8.20	4.50	13.59	9.09	8.57	3.80
Antioxidants and pigments (g kg^{-1})						
Chlorophylls	33.12	14.06	59.93	45.87	35.04	31.37
Carotenoids	18.02	10.69	26.18	15.49	17.99	10.52
Tocopherols	177	123	222	99	181	50
TPC	119	40	280	240	99	117
3,4 DHPEA-EDA	7.55	0.60	34.72	34.12	4.57	8.50
3,4 DHPEA-EA	8.41	5.05	10.25	5.20	8.55	1.98
p-HPEA-EDA	2.39	0.81	7.28	6.47	1.98	2.09
p-HPEA-EA	0.57	0.37	0.81	0.44	0.56	0.28
Syringic acid	0.84	0.55	1.37	0.82	0.79	0.29
Cinnamic acid	1.27	0.00	5.08	5.08	0.80	2.29
Vanillic acid	0.62	0.21	1.15	0.94	0.62	0.23
p-Coumaric acid	0.33	0.00	1.08	1.08	0.30	0.21
Hydroxytyrosol	2.34	0.00	13.98	13.98	1.37	2.31
Tyrosol	1.44	0.33	5.42	5.09	1.00	1.17
Apigenin	0.78	0.35	1.73	1.38	0.62	0.82
Luteolin	1.69	0.78	2.26	1.48	1.75	0.53
Pinoresinol	3.09	1.80	4.85	3.05	2.93	0.95
Antioxidant activity (μmol TE g^{-1})	1255	976	1790	814	1175	428
Fatty acid alkyl esters (g kg^{-1})						
FAMEs	6.27	1.50	13.88	12.38	5.80	3.92
FAEEs	7.28	2.56	19.22	16.66	6.14	3.91
Total FAAEs	13.55	5.20	28.43	23.23	11.68	5.62

IQR, inter quartile range; FFA, free fatty acids; PV, peroxide value; TFA, total trans fatty acids; SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids; TPC, total phenolic compounds; FAMEs, fatty acids methyl esters; FAEEs, fatty acids ethyl esters; FAAEs, total fatty acids alkyl esters.

730

Table 3. Volatile compounds (mg OE kg^{-1} oil) identified in the Brazilian virgin olive oils samples (mean value ± standard deviation).

Compounds (Sensory descriptor/aroma*)	Olive oil sample**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aldehydes												
Butanal, 2 methyl (Malty)	2.10 ±0.37	1.93 ±0.57	2.41 ±0.43	0.83 ±0.12	-	1.94 ±0.30	1.00 ±0.26	1.32 ±0.09	1.36 ±0.05	2.09 ±0.23	0.78 ±0.06	-
Butanal, 3 methyl (Malty)	1.10 ±0.13	1.43 ±0.30	1.61 ±0.16	-	0.49 ±0.41	1.82 ±0.20	1.10 ±0.76	1.09 ±0.03	0.70 ±0.03	2.00 ±0.10	0.74 ±0.02	-
cis-3-Hexenal (Green/leaf-like)	3.76 ±0.63	1.53 ±0.67	3.32 ±0.53	1.23 ±0.13	-	2.4 7±0.52	2.16 ±2.49	3.43 ±1.52	1.41 ±0.84	1.23 ±0.42	1.21 ±1.38	2.24 ±2.54
trans-2-Hexenal (Green/apple-like/bitter almonds)	184.17 ±25.21	78.80 ±10.02	185.82 ±15.13	74.42 ±15.42	96.39 ±23.59	120.77 ±35.78	225.07 ±11.58	252.75 ±68.17	90.08 ±5.94	184.20 ±1.94	104.31 ±7.15	222.86 ±26.30
trans-2-Pentenal (Green/apple/bitter almond)	2.65 ±0.30	-	2.33 ±0.48	0.90 ±0.01	-	1.63 ±0.81	2.79 ±0.18	2.02 ±0.34	1.11 ±0.11	-	1.40 ±0.07	-
Hexanal (Green/green apple)	17.45 ±1.99	8.03 ±1.25	16.89 ±0.62	8.47 ±2.17	12.35 ±0.38	5.68 ±1.58	8.53 ±1.98	10.73 ±2.69	6.91 ±1.22	10.19 ±0.89	4.79 ±0.40	6.03 ±0.35
Nonanal (Fatty/waxy/pungent)	2.10 ±1.21	-	-	-	-	-	-	-	-	0.95 ±0.14	-	-
Total aldehydes	*213.33 ±28.56*	*91.70 ±12.81*	*212.38 ±12.92*	*85.85 ±17.83*	*109.23 ±22.80*	*134.31 ±39.18*	*240.66 ±12.25*	*271.35 ±72.85*	*101.57 ±8.18*	*200.66 ±1.10*	*113.23 ±6.18*	*231.12± 29.20*
Ketones												
3-Pentanone (Sweet)	9.23 ±0.21	10.19 ±2.40	7.99 ±1.59	13.97 ±1.96	12.25 ±2.97	6.46 ±2.04	5.34 ±1.13	3.85 ±0.22	-	3.47 ±0.10	-	9.79± 2.81
1-Penten-3-one (Green)	9.14 ±0.37	2.07 ±0.19	7.72 ±0.57	-	-	3.86 ±0.59	13.22 ±1.40	7.86 ±1.57	-	8.20 ±0.23	-	-
Total ketones	*18.37 ±0.58*	*12.26 ±2.59*	*15.71 ±2.16*	*13.97 ±1.96*	*12.25 ±2.97*	*10.33 ±2.64*	*18.56 ±2.54*	*11.71 ±1.79*	-	*11.67 ±0.13*	-	*9.79 ±2.81*
Alcohols												
trans-2-Hexen-1-ol (Green/leaves)	17.36 ±1.38	30.51 ±3.80	48.12 ±9.84	52.41 ±29.79	56.08 ±25.24	37.52 ±13.06	7.54 ±0.79	16.75 ±3.22	40.77 ±44.20	5.89 ±0.27	13.52 ± 7.41	71.51 ±1.52
2-Penten-1-ol (Banana-like)	0.49 ±0.33	0.77 ±0.51	0.69 ±0.28	4.82 ±0.31	4.24 ±4.64	3.06 ±4.10	1.33 ±1.38	0.93 ±0.56	0.39 ±0.02	0.48 ±0.01	0.59 ± 0.53	1.19 ±1.14
Ethyl alcohol (Alcohol)	1.07 ±0.78	0.50 ±0.19	-	0.53 ±0.26	0.56 ±0.36	-	-	-	-	-	-	-
Total alcohols	*18.93 ±2.48*	*31.78 ±4.50*	*48.80 ±10.12*	*57.76 ±29.22*	*60.87 ±29.51*	*40.58 ±17.15*	*8.87 ±0.59*	*17.67 ±3.78*	*41.16 ±44.23*	*6.36 ±0.26*	*14.12 ± 7.94*	*72.71 ±0.37*
Carboxylic acids												
Propanoic acid (Pungent/sour)	1.33 ±1.32	2.04 ± 0.04	-	3.18 ±0.73	3.04 ±0.21	-	-	-	-	-	-	-
Butanoic acid (Rancid/cheese)	-	-	-	4.65 ±1.10	18.90 ±1.13	-	-	-	-	-	-	-
Acetic acid (Sour/vinegary)	40.55 ±42.04	29.97 ± 7.64	5.88 ±0.27	122.05 ±40.85	121.07 ±28.20	24.95 ±6.32	10.99 ±5.60	10.10 ±7.28	6.20 ±1.37	5.38 ±1.87	7.80 ±3.43	7.74 ±2.36
Total carboxylic acids	*41.88 ±43.35*	*32.01 ±7.67*	*5.88 ±0.27*	*129.89 ±42.68*	*143.01 ±27.28*	*24.95 ±6.32*	*10.99 ±5.60*	*10.10 ±7.28*	*6.20 ±1.37*	*5.38 ±1.87*	*7.80 ±3.43*	*8.10 ±2.35*
Other compounds												
3-Ethyl-1,5-octadiene	1.24 ±0.20	8.52 ±4.47	4.38 ±0.49	2.10 ±1.98	4.16 ±0.52	1.69 ±1.16	8.80 ±0.51	3.07 ±2.53	6.94 ±1.10	5.76 ±0.78	5.77 ±0.64	6.36 ±0.65
Octane Sweet/alcane	1.39 ±0.35	1.32 ±0.31	0.78 ±0.04	1.42 ±0.35	2.13 ±0.81	0.50 ±0.07	0.45 ±0.08	0.77 ±0.13	2.04 ±0.23	1.44 ±0.05	1.38 ±0.12	0.48 ±0.04

*(Kalua et al., 2007; Angerosa et al., 2004);
** See Table 1 for cultivar specification.

732

733

Table 4. Results of the panel test on the Brazilian virgin olive oil samples expressed as median value.

Olive oil sample*	Fruity	Bitter	Pungent	Defect	Principal defect
1	1.0	0.0	0.4	1.1	fusty/muddy
2	0.6	0.0	0.5	3.3	fusty/muddy
3	0.6	0.0	0.4	2.0	fusty/muddy
4	0.6	0.0	0.6	1.5	winey-vinegary
5	0.0	0.0	0.5	3.3	winey-vinegary
6	0.8	0.0	0.6	1.4	fusty/muddy
7	3.0	0.0	1.7	0.0	-
8	3.0	0.0	0.6	0.7	fusty/muddy
9	0.3	0.0	0.3	2.3	fusty/muddy
10	1.0	0.0	0.0	1.9	fusty/muddy
11	0.4	0.0	0.6	1.1	fusty/muddy
12	1.4	0.3	1.1	1.0	fusty/muddy

* See Table 1 for cultivar specification.

734

735