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Comparative analysis of minor bioactive constituents (CoQ_{10}, tocopherols and phenolic compounds) in Arbequina extra virgin olive oils from Brazil and Spain

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ABSTRACT

There is currently an emerging production of olive oil in Brazil but it is still poorly characterized. In this study, we performed a comparative analysis of minor bioactive constituents (CoQ_{10}, tocopherols and phenolic compounds) in extra virgin olive oil from different regions of Brazil and Spain, of Arbequina cultivar. Significant variations ($P < 0.05$) in the concentration of the compounds analyzed were observed among oils from the different growing areas, not only between Spanish and Brazilian samples but also within zones of the same country. All the oils analyzed showed a high content of CoQ_{10}, which ranged from 48 to 85 mg/L. The α − tocopherol was the major isomer quantified and three main groups of phenolic compounds were identified: flavonoids (apigenin, luteolin), phenolic acids (naringenin, p-coumaric acid, vanillic acid) and phenolic alcohols (hydroxytyrosol). Climatic and geographic factors of the production zones greatly influenced the minor fraction composition; positive relationships between altitude and the level of CoQ_{10}, tocopherols and phenolic compounds of the oils were observed, whereas a negative correlation with rainfalls was found. Chemometric analyses demonstrated that oils were differentiated by the chemical composition and origin area and that polyphenols (particularly hydroxytyrosol) held the major weight in the oil classification.

1. Introduction

It is well known that chemical composition of olive oils consists of major (saponifiable fraction) and minor constituents (unsaponifiable fraction). The minor constituents, despite present in lower amounts (up to 2%), are a complex mixture of more than 230 compounds (Lopez et al., 2014; Servili et al., 2014). Among them, phenolic compounds and tocopherols are of great interest, mainly due to their nutritional value, antioxidant potential and health benefits.

The phenolic compounds are secondary plant metabolites that have one phenol ring (phenolic acids/phenolic alcohol) or several aromatic rings with one or more hydroxyl groups (polyphenols) (Ignat et al., 2011; Lopez et al., 2014). Over the last few decades, multiple biological properties, providing antioxidant, anti-inflammatory, chemopreventive and anti-cancer benefits, as well as sensorial proprieties has been attributed to phenol compounds of olive oils (Servili et al., 2014). Recently, their protective effect over blood lipids from oxidative stress has been recognized by the European Food Safety Authority (EFSA, 2011), thus stimulating, even more, the interest for olive oil polyphenols and allowing the use of its health claims (Reboredo-Rodríguez et al., 2016; Martín-Peláez et al., 2013). Tocopherols are known as lipophilic phenols that include 8 occurring forms: 4tocopherols and 4 tocotrienols (α, β, γ and δ). In extra virgin olive oil (EVOO), the most predominant is the α-tocopherol (up to 90% of total), recognized as the most active form of vitamin E in mammals, although different factors such as cultivar and geographic location of the olive trees may influence its concentration (Lopez et al., 2014; Kalogeropoulos and Tsimidou 2014). These natural antioxidants not only provide nutritional value to virgin

Abbreviations: CoQ_{10}, coenzyme Q_{10}; EVOO, extra virgin olive oil; EFSA, European Food Safety Authority; HCA, hierarchical cluster analysis

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Table 1
Geographic coordinates (Latitude and Longitude), altitude (m), annual mean temperatures (°C), annual rainfalls (mm) and minimum and maximum mean temperatures (°C) of the different locations of Arbequina virgin olive oils from Spain (1–9) and Brazil (10, 11).

Oil Sample	Location	Latitude	Longitude	Altitude	Mean Temperatures	Rainfall	Minimum Temperature	Maximum Temperature
1	Granada	37° 03′ N	3° 36′ W	905	17	385	7	26
2	Jaén	38° 03′ N	3° 29′ W	580	17	422	9	26
3	Málaga	37° 06′ N	4° 22′ W	883	20	411	13	27
4	Cádiz	36° 43′ N	6° 01′ W	47	19	636	13	24
5	Sevilla	37° 17′ N	4° 53′ W	416	19	598	11	27
6	Albacete	39° 00′ N	1° 54′ W	677	13	293	6	25
7	Toledo	39° 53′ N	4° 28′ W	459	14	391	7	26
8	Valladolid	41° 53′ N	5° 00′.W	845	13	394	−1	27
9	Lérida	41° 36′ N	0° 35′ W	168	14	677	6	21
10	Rio Grande do Sul	30° 00′ S	52° 52′ W	88	16	1691	3	22
11	Minas Gerais	22° 18′ S	42° 22′W	1310	17	1330	14	21

Geographic coordinates (latitude, longitude and altitude) proximate to olive grove were found using Google Earth program (Google Inc, USA). Climatic data of temperature and rainfall were supplied by the Spanish Meteorology Agency (Aemet, 2015) and the National Meteorology Institute of Brazil (INMET, 2015).

olive oils but also contribute to its stability, protecting from oxidation (Lopez et al., 2014; Kalogeropoulos and Tsimidou, 2014).

Another minor compound with great value is the coenzyme Q10 (CoQ_{10}), an endogenous lipophilic compound that is involved in essential cell regulations and modulations, mainly in the mitochondrial respiratory chain (Jankowski et al., 2016; Thanatuksorn et al., 2009). In the body it exists in either oxidized (ubiquinone) or reduced form (ubiquinol); mainly in its reduced form it is recognized as an effective endogenous antioxidant, although an antioxidant role of the oxidized form cannot be discarded (Pravst et al., 2010; Jankowski et al., 2016). Additionally, it has the ability to recycle α-tocopherol by sparing or regeneration (Pyo, 2010). Due to redox reactions, continuous conversion between ubiquinone and ubiquinol takes place *in vivo* and, moreover, ubiquinone is also reduced during or following the intestinal absorption (Pravst et al., 2010). Therefore, the functions of CoQ_{10} are not affected by the form in which it is consumed (Pravst et al., 2010). Most of the CoQ_{10} in the human body is from endogenous synthesis, but levels decline progressively with increasing age and should be replaced daily by nourishment (Jankowski et al., 2016). In this sense, the EVOO consumption may be a dietary natural source for increasing intake of CoQ_{10} (Venegas et al., 2011; Žmitek et al., 2014). A wide range of possible benefits for human health has been reported for CoQ_{10} (Pravst et al., 2010; Jankowski et al., 2016; Turunen et al., 2004). The levels of these minor bioactive constituents are variable in EVOO. These variations have been attributed to different factors, including agronomic and technological practices, cultivar, ripening stage, climate conditions and geographic origin (Servili et al., 2009; Laincer et al., 2016). Nevertheless, factors influencing the CoQ_{10} content of olive oils have been scarcely investigated (Žmitek et al., 2014).

In recent years, the demand of olive oils is rising over the world, and emerging countries such as Brazil are beginning to produce it. Actually, the Arbequina cultivar is one of the most cultivated in Brazil, and data on physicochemical properties, oxidative stability and fatty acid profile of Arbequina Brazilian oils have been recently published by our research group (Borges et al., 2017). However, little is known about how geographic and climate conditions may affect the minor components of the olive oils in Brazil (Ballus et al., 2014, 2015). Also, there is a lack of information about the similarities and differences between the newly introduced and the autochthonous cultivars. Moreover, to our knowledge, nothing has been published about CoQ_{10} levels of Brazilian olive oil and very little about specific varieties in Spain (Žmitek et al., 2014). Finally, there is also a lack of information of the relationship between CoQ_{10} with other bioactive constituents such as phenolic compounds and tocopherols. With this background, the aims of this work were: i) to characterize the minor constituents CoQ_{10}, tocopherols and individual phenolic compounds of monovarietal Arbequina olive oil produced in Brazil; (ii) to compare it with the olive oils from the same cultivar produced in different regions of Spain and (iii) to classify the oil samples according to their geographic origin, on the basis of the analyzed variables and by applying chemometric analysis.

2. Materials and methods

2.1. Chemicals

All chemical products, standards and solvents for the analysis performed were analytical reagent grade or higher purity (Sigma-Aldrich, St. Louis, MO, USA) and Milli Q water (Millipore, Bedford, MA) was used throughout the assays. CoQ_{10} from Sigma-Aldrich (code: C9538) was used to prepare standard solutions of different concentrations.

2.2. Samples

EVOO from Arbequina cultivar was analyzed. Nine regions of olive oil production in Spain (Granada, Jaén, Málaga, Cádiz, Sevilla, Albacete, Toledo, Valladolid and Lérida, samples 1 to 9) and two regions in Brazil (Rio Grande do Sul and Minas Gerais, samples 10 and 11) were selected to obtain the EVOO samples. The olives were harvested always at the early stage of harvest; the harvest date was: late October to mid-November of 2014 for Spanish samples and March to early April of 2015 for Brazilian samples. The oil was extracted within 24 h, under a two-phase extraction system. The oils (n = 3 from each producing region) were directly donated by the producers, adequately packaged for preserving from light and high temperatures and sent to CSIC laboratories (Granada, Spain) to perform the analysis. As was shown previously (Borges et al., 2017), samples meet quality standards established by European Union regulation n° 2568/91 for extra virgin olive oil. The geographic coordinates (latitude and longitude), altitude (m), annual mean temperatures (°C), annual rainfalls (mm) and minimum and maximum mean temperatures (°C) of the different producing areas of Arbequina virgin olive oils are depicted in Table 1.

2.3. Determination of CoQ_{10}

The samples were analyzed according to Venegas et al. (2011). A quantity of 990 μL of 1-propanol was mixed with 10 μL of the oil, vortex and centrifuged at 11300*g* for 5 min at room temperature. The subsequent supernatant was diluted 1/500 in 1-propanol prior to HPLC injection. CoQ_{10} present in the oil extract were separated by reversed-phase high-performance liquid chromatography (HPLC, Gilson, WI) with a C18 symmetry column (3.5 μm, 4.6 × 150 mm) (Waters Chromatography, Barcelona, Spain) using a mobile phase consisting of methanol, ethanol, 2-propanol, acetic acid glacial (500:500:15:15), and 50 mM sodium acetate at a flow rate of 0.9 mL/min. The electrochemical detector consisted of an ESA Coulochem III with the following setting: guard cell (upstream of the injector) at +900 mV and

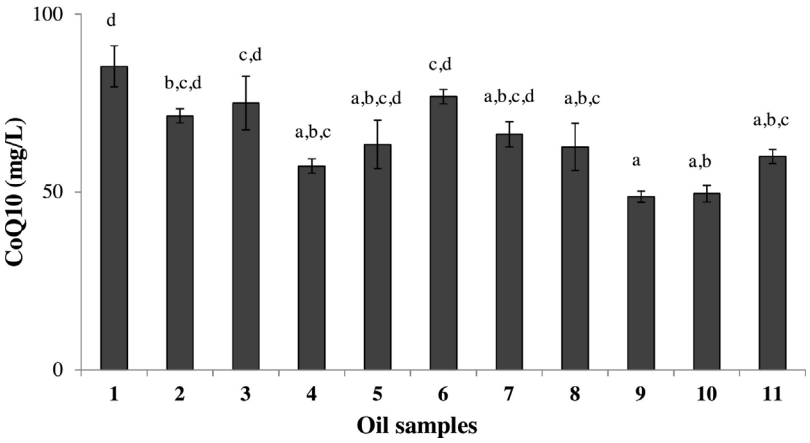

Fig. 1. Coenzyme Q_{10} levels of Arbequina olive oils from Spain (1–9) and Brazil (10–11). (values are means ± SE, n = 3). Different letters indicate significant differences (ANOVA and Tukey test, $P < 0.05$).

conditioning cell at −600 mV (downstream of the column) followed by the analytical cell at +350 mV. While this method is able to detect CoQ_{10} in its reduced (ubiquinol) and oxidized (ubquinone) form, ubiquinol was not detected in our conditions of extraction and analysis. The CoQ_{10} concentrations of the oxidized form were estimated by comparison of the peak areas with those of standard solutions of known concentrations (0, 25, 100, 300, and 600 ng/mL). Values of calibration curve are reported in Supplementary Table S1 in the online version at DOI: http://dx.doi.org/10.1016/j.jfca.2017.07.036. The results were expressed in mg per L of sample.

2.4. Determination of tocopherols

The tocopherols isomers were determined as described by Rueda et al. (2016). Briefly, 1 g of oil was dissolved in 25 mL n-hexane. The samples were analyzed by an HPLC system (Water Alliance 2695 separations module, Milford, MA) equipped with a silica column (4 mm × 250 mm) and eluted with hexane: isopropanol (99.25:0.75 v/v) at a flow rate of 1 mL/min during 25 min. The tocopherols were detected by fluorescence (Water 2475) with excitation wavelength at 290 nm and emission wavelength at 330 nm. A calibration curve using external standards (α-, β-, γ-, and δ-tocopherols) was used for quantification. The results were expressed as mg per kg of sample.

2.5. Individual phenolic compounds

The individual phenolic fraction of samples was performed after an extraction with methanol/water (80:20) according to the International Olive Oil Council (IOOC, 2009). The extracts were analyzed by UPLC-TOF-MS following the method validated by Rivas et al. (2013). The UPLC system consisted of a AcQuity UPLC equipped with a binary pump system (Waters, Milford, MA, USA) using a AcQuity UPLC BEH C18 column (1.7 mm, 2.1 mm x 100 mm inner diameter). The column was kept at 40 °C and the flow rate was 0.4 mL/min. The mobile phase was composed by eluent A, MilliQ water with formic acid (0.1%) and eluent B, methanol with formic acid (0.1%). The elution started at 5% of eluent B for 1 min, then was linearly increased to 100% of eluent B in 11 min and kept isocratic for 5 min; then, it got back to initial conditions in 0.1 min; the equilibration time was 2.9 min. The injection volume was 7 μL, and all samples were filtered through 0.22 mm before chromatography. The UPLC was coupled to a Micromass/Waters LC-TPremier XE benchtop orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer equipped with an ESI interface. Parameters for analysis were set using negative ion mode with spectra acquired over a mass range from *m/z* 100 to 1000. The optimum values of the electrospray ionization MS parameters were as follows: capillary voltage 2.6 kv; drying gas temperature 200 °C, desolvation gas flow 800 L/h. For optimum detection resolution, a solution was prepared of 1 mg/L leucine enkephalin in acetotrinile/H_2O (1:1, v/v) containing 0.1% formic acid continuously infused at a flow rate of 0.05 mL/min by an external rotary pump. This solution was also used for continuous calibration of the equipment, as its perfusion is simultaneous to that of the sample and served as a reference. Mass calibration was performed using a solution of sodium formate (containing 0.05 of formic acid and 5 mM of sodium hydroxide in iso- propanol/H_2O 9:1, v/v). Accurate mass data of molecular ions were processed with MassLynxs (Waters).

Analytical parameters of the methods used are shown as Supplementary file.

2.6. Statistical analysis

Results were analyzed by analysis of variance (one-way ANOVA), with the geographic origin of oils (regions 1–11) as the main factor. Tukey's test was used to compare mean values between oils from the different regions, and differences were established at $P < 0.05$. The relationships of the different variables with the climate characteristics and the altitude of the producing regions were evaluated by Pearson's coefficient. These statistical calculations were carried out using SPSS version 21.0 (IBM Corporation, New York, USA).

In addition, chemometric analysis was performed including all the minor bioactive compounds evaluated in the present study (CoQ_{10}; tocopherols − α, β, γ and phenolic compounds). In a first explorative step a hierarchical clustering analysis (HCA) was carried out to identify eventual similarities between the olive oils samples of different geographic origin, by calculating multidimensional squared Euclidean distances of scores applying the single linkage-clustering method. Posteriorly, to reduce the variables into a small number of factors and explore the contribution of variables to oil differentiation, a factorial analysis (FA) using a varimax rotation was applied. All the chemometric analysis were performed using Stat Graphics Centurion XV software (Stat Point Technologies, Inc., USA, 2006).

3. Results and discussion

3.1. CoQ_{10}

The levels of CoQ_{10} are shown in Fig. 1. According to Pravst et al. (2010), foods containing over than 46 mg CoQ_{10}/L of olive oil are considered a very rich source of CoQ_{10}. Therefore, all the samples evaluated in the present study may be classified as very rich CoQ_{10} sources. As shown in Fig. 1, statistical differences ($P < 0.01$) were found among CoQ_{10} content of the samples ranging between 85.3 ± 5.8 mg/L (region 1, corresponding to Granada, Spain) and 48.7 ± 1.6 mg/L (region 9, Lérida, Spain) (mean ± SE). Brazilian oils showed intermediate values of 49.5 ± 2.3 mg/L and 60.0 ± 1.9 mg/L, for areas 10 and 11, respectively. There is limited information about

CoQ_{10} content in monovarietal EVOO. A previous study comparing different cultivars from several countries (Žmitek et al., 2014) showed that CoQ_{10} is greatly driven by genetic factors; the highest content was observed in the cultivar Hojiblanca (98 mg/L), followed by Picual (63 mg/L) and Arbequina (58 mg/L). Values of 77.5 mg/L of CoQ_{10} content have been found in Spanish EVOO of Picual cultivar (Venegas et al., 2011), similarto the highest levels detected among oils in the present assay (samples from Spanish locations 1, 2, 3 and 6). Higher values, above 90 mg/L, have been observed in commercial Italian EVOO (Cabrini et al., 2001).

These findings support that EVOOs are one of the best natural sources of dietary CoQ_{10} (Žmitek et al., 2014). Since the current intake of CoQ_{10} in developed countries is not sufficient to compensate the age-related decline (Žmitek et al., 2014), promoting the intake of EVOO may be a good alternative to supplementation.

In addition to genetic factors, the CoQ_{10} content of oils may be affected by geographic and climate conditions, as was supported by the results of the present assay, in which significant differences were found between oils from the same cultivar. In this sense, statistical correlations ($P < 0.01$) were found between CoQ_{10} content and geo climate factors of production areas, positive with altitude ($r = 0.461$) and maximum temperature ($r = 0.484$) and negative with rainfalls ($r = -0.494$). Accordingly, the areas of low rainfall and high altitude (Table 1), such as Granada (region 1), produced the oil with the highest CoQ_{10} content. In the same line, high rainfall levels in Brazilian growing areas (regions 10 and 11) could have a negative effect on the CoQ_{10} content of oils, although in the case of oil from region 11 this effect seems to be partly counteracted by the positive influence of the high altitude.

3.2. Tocopherols

Table 2 shows tocopherols content (α, β, γ and total) for Arbequina EVOO from Spain (1–9) and Brazil (10–11). The major isomer quantified was α-tocopherol, representing more than 98% of the total, ranging from 92 to 208 mg/kg of oil, followed by lower amounts of β (0.8–1.9 mg/kg) and γ (0.7–2.15 mg/kg) fractions. No detectable values of δ- tocopherol were observed.

The content of the tocopherol fractions differed between EVOO samples from the different regions ($P < 0.05$). Values of Brazilian oils were within the range found for Spanish samples, with the only exception of the γ −isomer, which was lower among Brazilian oils. Thus, it was shown that the Arbequina oils newly introduced in Brazil have similar tocopherol content that the oils from the autochthonous Spanish cultivar.

In general, α-tocopherol concentration found in the present assay is similar to the values reported for Arbequina EVOO from Spain and the introduced from Argentina, Tunisia and Turkey, which vary from 150 to 300 mg/kg (Beltrán et al., 2010; Dabbou et al., 2010; López-Cortés et al., 2013; Torres et al., 2009; Uluata et al., 2016; Yousfi et al., 2012). The lowest contents of α-tocopherol among samples of the present study were found in Spanish locations 4 (Cádiz) and 9 (Lérida). The low tocopherol level in oil of location 4 could be related tothe low oxidative stability (5.32 h, measured by the Rancimat method) previously described in oils from this region (Borges et al., 2017). Large variations in α-tocopherol content have been observed in Brazilian Arbequina EVOO from Minas Gerais depending on the harvest year (62 and 201 mg/kg for crop years 2010 and 2011, respectively) (Ballus et al., 2014). Thus, values of 147 and 171 mg/kg found in Brazilian samples in the present study (zones of Rio Grande do Sul and Minas Gerais, respectively) were within this wide range.

Some previous data show that tocopherol content of olive oils has a genetic component, i.e. it is highly depending on cultivar, but it may also be affected by climatic conditions, mainly temperature, rainfalls and altitude (Beltrán et al., 2010; Dabbou et al., 2009; Ilyasoglu et al., 2016). Therefore, the growing location area, with different conditions of rainfall and temperature, influence the tocopherol content and composition of the oils (Aguilera et al., 2005), in agreement with present results. In addition, significant correlations were verified relating geo climate conditions with tocopherol content of the samples (Table 4). In concordance with previous research reporting increased tocopherol levels in dry crop seasons (Beltrán et al., 2010; Ilyasoglu et al., 2016), our findings show a negative relationship between rainfalls and γ ($P < 0.01$) and total tocopherol content ($P < 0.05$). On the contrary, some authors do not observe a consistent influence of rainfalls on tocopherol levels in Tunisian oils (Dabbou et al., 2009), since the effects of climatic conditions on tocopherols seem to be cultivar-dependent (Beltrán et al., 2010).

Relationships between the temperature of the growing area and tocopherols have been scarcely studied. In the present study, conflictive correlations among temperature and tocopherols were found, since they were negative with the mean (for α-, γ- and total) and minimum temperatures (for γ-) and positive with maximum temperatures (for α- and total tocopherols) (Table 4). That means that annual mean temperature of the growing zone significantly affects the tocopherol content, but peaks of cold and heat also seem to disturb this variable. Regarding the altitude effects, some authors observe increased tocopherol content in olive oils with increasing the altitude (Dabbou et al., 2009) and, thus, altitude has been proposed as an important factor to be considered in tocopherol level of oils (Kalogeropoulos and Tsimidou, 2014). In this line, positive correlations of altitude with tocopherol concentration of the oils have been found in our study (Table 4). However, oils from areas with the highest altitude were not those with the highest tocopherol content, which suggests that altitude has not a definitive influence and could be counteracted by effects of other geo climatic factors.

As was reported before, the climate conditions have primarily an effect on biochemical reactions during growth and ripening, mainly in some enzymatic reactions that are essential for the tocopherol synthesis (Beltrán et al., 2010; Ilyasoglu et al., 2016). In this sense, increasing the tocopherol content could be an auto-protection mechanism of plants against some stress conditions, such as water stress (Beltrán et al., 2010). However, there is a maximum level depending on the fruit development (Georgiadou et al., 2015). In this line, our results show that climate factors are linked to tocopherols content and may impact them, but the influence of other factors providing synergic or antagonist effects, as the maturation index, cannot be discarded.

Table 2
Tocopherols content (mg/kg) of Arbequina olive oils from Spain (1–9) and Brazil (10–11).

	α-Tocopherol	β- Tocopherol	γ − Tocopherol	Total
Regions				
1	179 e	1.00 a,b,c	1.90 e	182 e
2	202 f	1.03 b,c	0.90 a,b,c	204 f
3	157 c,d	0.85 a,b	1.15 d	159 c,d
4	92 a	0.80 a	0.75 a	93 a
5	164 d	0.90 a,b,c	0.73 a	166 d
6	198 f	1.05 b,c	1.13 c,d	200 f
7	208 f	0.85 a,b	1.00 b,c,d	210 f
8	167 d,e	0.85 a,b	2.15 f	170 d,e
9	128 b	0.75 a	1.05 c,d	130 b
10	147 c	0.85 a,b	0.70 a	148 c
11	171 d,e	0.90 a,b,c	0.80 a,b	173 d,e
SEM	0.87	0.01	0.01	0.86

Means values in each column with different letters are significantly different between regions or countries (n = 3, ANOVA and Tukey test, $P < 0.05$).

3.3. Phenolic compounds

The concentration of phenolic compounds (apigenin, luteolin, naringenin, p-coumaric acid, vanillic acid and hydroxytyrosol) of olive oil samples from different geographical areas of Spain and Brazil is shown in Table 3. Statistical differences between samples ($P < 0.05$) were

Table 3
Phenolic content (μg/kg) of Arbequina olive oils from Spain (1–9) and Brazil (10–11).

	Phenolic content					
	Flavonoids			Phenolic acids		Phenol alcohols
	Apigenin	Luteolin	Naringenin	p-Coumaric acid	Vanillic acid	Hydroxytyrosol
Regions						
1	265 e,f	858 c,d	25.0 a,b	55.0 c	119 c	90.0 a,b
2	309 f,g	1054 d,e	51.5 c,d	59.0 c	59.5 b	287 d,e
3	26.0 a	241 a,b	30.0 b	n.d	n.d	1500 g
4	204 b,c	773 c	16.5 a	24.5 a,b	28.5 a	3.00 a
5	161 b	463 b	63.5 d	13.5 a	n.d	379 e
6	326 g	1257 f,g	50.0 c	97.0 e	124 c	251 c,d,e
7	424 h	1148 e	56.0 c,d	94.0e	121 c	161 b,c,d
8	184 b,c	748 c	16.0 a	91.0 e	n.d	204 b,c,d
9	236 d,e	1236 f,g	58.5 c,d	31.0 b	70.0 b	149 b,c
10	398 h	1482 g	133 e	228 f	n.d	1050 f
11	15.0 a	92.0 a	21.0 a,b	74.0 d	n.d	92.0 a,b
SEM	40.3	133	10.1	18.7	16.2	8.1

Means values in each column with different letters are significantly different between regions or countries (ANOVA and Tukey test, $P < 0.05$).

found in all individual compounds. Strong differences were observed between Brazilian oils, being the content of all phenolic compounds always higher in sample 10 than sample 11. Three main groups of phenolic compounds were identified: flavonoids (apigenin, luteolin), phenolic acids (naringenin, p-coumaric acid, vanillic acid) and phenolic alcohols (hydroxytyrosol). Flavonoids were the major group, ranging from 93 to 36% of the total quantified, followed by phenolic acids (10–30% of the total), with the exception of samples from location 3 (with 14% of flavonoids and only 2% of phenolic acids). The highest levels of hydroxytyrosol were found in oils from Spanish locations 3 (1500 μg/kg, contributing 83% to the total phenols examined) and Brazilian region 10 (1050 μg/kg, 31% of the total). The Brazilian sample 11 showed a different profile of phenolic compounds in comparison with the other samples; with equilibrate quantities of the quantified phenolic groups.

Current data show a large variation of phenolic content for EVOO samples, ranging between 50 and 940 mg/kg (Servili et al., 2014). Nevertheless, available data in the literature are very difficult to compare, since different methods have been used to separate, identify and quantify the phenolic compounds in EVOO samples around the world (Ballus et al., 2015). Besides, commercial standards are not totally available and some of these compounds are identified and quantified on the basis of other ones with similar structures (Ballus et al., 2015; Bakhouche et al., 2013). In this sense, nowadays there is not an official method able to show a complete profile of phenolic compounds and satisfy the new health claims on olive oil phenols, although the scientific community has been working on developing it (Reboredo-Rodríguez et al., 2016).

In accordance with data of the present study, flavonoids (apigenin, luteolin) and phenolic acids (p-coumaric acid, vanillic acid) have been previously found in Arbequina cultivar (Bakhouche et al., 2013; Rivas et al., 2013; Yousfi et al., 2012). However, up to our knowledge, naringenin was not reported yet in Arbequina oil, but was recently detected in oleaster, leaves, olive barks, wastewater and EVOO of Empeltre and Arauco cultivars from Argentina (Bouarroudj et al., 2016; De Fernandez et al., 2014; Leouifoudi et al., 2014; Tóth et al., 2015). On the other hand, hydroxytyrosol is one of the most important phenolic compounds in olive oils (Bakhouche et al., 2013) and it has been widely associated with the high antioxidant capacity in EVOO (Servili et al., 2014). Accordingly, Arbequina oils from region 3 (Málaga, Spain) have a high resistance to oxidation (15.85 h of oxidative stability, by the Rancimat method) (Borges et al., 2017). The health beneficial properties of hydroxytyrosol have been recognized by the EFSA (EFSA, 2011). Concerning geographical area and climate conditions, several authors have studied the relationship of phenolic compounds with them (Bakhouche et al., 2013; Dabbou et al., 2009; Ilyasoglu et al., 2016). In this line, positive correlations between altitude and some of the analyzed phenolic compounds such as naringenin (r = 0.458), p-coumaric acid (r = 0.578) and hydroxytyrosol (r = 0.618) were found in our study (Table 4). In agreement, Dabbou et al. (2009) attribute variations of phenolic profile of Tunisian EVOO to the geographic area characteristics, particularly to altitude. Furthermore, scientific data have shown that water status also influence the phenolic profile of the olive oils, but the effect may differ depending on the compounds, since the enzymes involved in the biosynthetic pathway of phenolic compounds are affected by water stress conditions in a different way (Ilyasoglu et al., 2010; Stefanoudaki et al., 2009). Thus, correlations between climatic conditions and minor olive oil compounds may be explained

Table 4
Pearson's correlation coefficient (r) between climate variables and altitude and analytical parameters (Coenzyme Q_{10}, Tocopherols and Phenolic content).

	Altitude	Temperature	Rainfalls	Minimum temperature	Maximum temperature
Coenzyme Q_{10}	0.461**	0.060	−0.494**	0.073	0.484**
α Tocopherol	0.543**	−0.361*	−0.316	−0.219	0.385*
β Tocopherol	0.376*	−0.074	−0.257	−0.020	0.307
γ Tocopherol	0.437**	−0.396*	−0.446**	−0.588**	0.309
Total	0.547**	−0.365*	−0.321*	−0.226	0.389*
Apigenin	0.314	−0.390*	−0.249	0.097	−0.179
Luteolin	0.315	−0.456**	−0.207	0.075	−0.374*
Naringenin	0.458**	0.000	0.286	0.439*	−0.440*
p-Coumaric acid	0.578**	−0.364*	0.417*	−0.021	−0.534*
Vanillic acid	−0.037	−0.470**	−0.511**	−0.191	0.066
Hydroxytyrosol	0.618**	0.466**	0.064	0.563**	0.066

Symbols indicate significant correlations (* $P < 0.05$, ** $P < 0.01$).

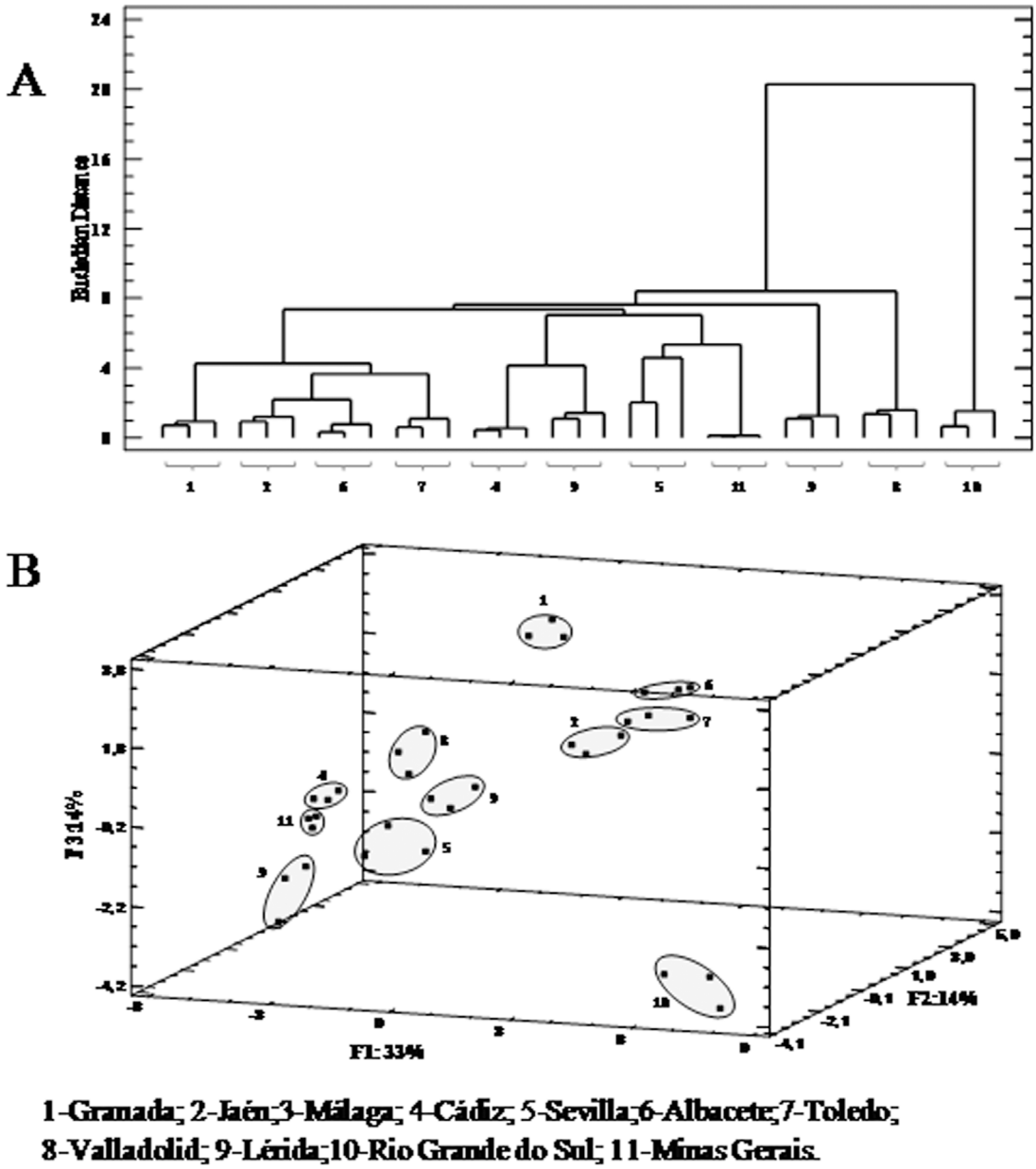

Fig. 2. A- Dendrogram plot showing the conglomeration of olive oil samples from Spain (1–9) and Brazil (10–11) obtained by clustering of CoQ_{10}; tocopherols − α, β, γ and phenolic compounds showing. **B**- PCA 3D plot obtained from CoQ_{10}; tocopherols − α, β, γ and phenolic compounds representing the distribution of olive oil samples from Spain (1–9) and Brazil (10–11).

mainly by the effects in enzymatic activity reactions during the growth and ripening of olive fruits (Ilyasoglu et al., 2016). Accordingly, relationships between climate variables of temperature and rainfalls and phenol composition of oils were found in the present assay (Table 4).

3.4. Chemometric analyses

A hierarchical cluster analysis (HCA) was applied as an initial approach for grouping samples that share common characteristics according to the analyzed variables. HCA was obtained using the Euclidean distance of scores as a similarity criterion and the results are shown in Fig. 2A. The dendogram plot defined five distinctive clusters. Firstly, three separated cluster were observed for samples from Rio Grande do Sul (10), Valladolid (8) and Málaga (3).The fourth cluster was composed by the oils from Cádiz (4), Lérida (9), Sevilla (5) and Minas Gerais (11). The last one was represented by the samples from Granada (1), Jáen (2), Albacete (6) and Toledo (7). It was observed that oil from Rio Grande do Sul showed the biggest Euclidean distance (high significance clustering), i.e the lowest similarities comparing with the other groups.

In the factorial analysis, three factors justifying 76% of total variance were obtained (F1 33%, F2 29%, F3 14%). F1 was explained mainly by phenolic compounds (apigenin 0.92; luteolin 0.92; naringenin 0.81 and p-coumaric acid 0.77), F2 was composed by CoQ_{10} (0.84) and tocopherols (α 0.83; β 0.83 and γ 0.36), while F3 was mainly characterized by hydroxytyrosol (−0.87) and vanillic acid (0.66). According to these factors, a spatial representation of the oils was obtained (Fig. 2B). A clear separation of sample 10 was observed mainly due to F1 and F3, and thus, related with the different content of polyphenols in this oils compared with the other samples, particularly luteolin, p-coumaric acid and hydroxytyrosol. Also according to F1 and F3, samples from locations 1, 2, 6 and 7 showed evident proximity. The remainder oils were relatively grouped but, among them, samples from Málaga (3) were slightly away from the other locations, which may be

associated with its high level of hydroxytyrosol. This entirely means that F2 (governed by CoQ_{10} and tocopherols) was not able to differentiate EVOO samples clearly, according to the content of lipophilic compounds in the samples. However, F2 and particularly F3, with a strong impact of hydroxytyrosol, could classify the samples in three separated blocks, as commented. Thus, phenolic compounds were a useful tool to delimitate EVOO samples from different geographic areas. In general terms, HCA was confirmed by the factorial analysis, which in turn showed the weight of the variables in the classification of the oil samples.

4. Conclusions

Findings of the present study contribute to increasing the knowledge of the EVOO grown in Brazil, a country with an incipient production of olive oil but with great potential for its cultivation. In addition, comparative information with the original cultivar produced in Spain is reported. Significant differences in the minor fraction composition (CoQ_{10}, tocopherols and phenolic compounds) were observed not only between Spanish and Brazilian Arbequina oils but also between oils from the different producing areas within each country. A high level of CoQ_{10} content of olive oils was observed, especially among Spanish oils. Climatic and geographic factors of the production zones seem to greatly affect the content of the parameters analyzed; positive relationships of the altitude with the level of CoQ_{10}, tocopherols and phenolics of the oils were observed, whereas negative correlation with rainfalls werealso shown. Chemometric analyses demonstrated that oils were differentiated according to chemical composition and origin area, and that polyphenols (particularly hydroxytyrosol) held the major weight in the oil classification.

The influence of other factors than those considered in the present study, such as the ripeness index of olives, cannot be discarded, and therefore, the lack of this information may be considered as a limitation of the study.

Conflicts of interest

The authors declare no competing financial interest.

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