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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