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Olive leaves offer more than phenolic compounds – Fatty acids and mineral composition of varieties from Southern Brazil

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ABSTRACT

The chemical composition of olive leaves cultivated in Southern Brazil was evaluated regarding proximate composition, total phenolic compounds, fatty acids profile, and macro- and microelements. For the varieties Ascolano, Arbosana, Negrinha do Freixó, Koroneiki, and Grappolo the concentrations of ash, protein, lipid, and total carbohydrates ranged from 4.37% to 6.00%, 10.50% to 13.10%, 9.13% to 9.80%, and 8.74% to 32.63%, respectively. Arbosana was the variety with the highest concentration of total phenolic compounds (35.71 mg GAE g^{-1}), and the higher concentration of saturated fatty acids (37.26%, mainly palmitic acid). All varieties presented similar concentrations of oleic (from 19.80 to 21.50%), linoleic (from 6.84 to 8.26%), and linolenic (from 34.40 to 41.30%) acids. The high content of linolenic acid found in olive leaves turns this by-product into an interesting source of *n*-3 PUFA (linolenic acid) in addition to phenolic compounds. The mineral elements observed in higher concentrations in the varieties studied were Al, Ca, Fe, K, Mg, P, and S. However, if the recommended daily intake is considered for consumption of 50 g of leaves, the amount of Fe consumed reached the recommended dose while the amount of Cu exceeds the recommended value for all varieties. The results showed the importance of the constitution of these varieties, which could be used as supplements in food or to feed animals and increase the nutritional value of their products.

1. Introduction

The *Olea europaea* is an evergreen tree that has been cultivated for more than 7000 years and is found throughout the world, particularly in Mediterranean countries (Fares et al., 2011; Lalas et al., 2011). The cultivation of olives covers ten million ha of land (Coutinho et al., 2009) and the data for the years 2009 and 2010 indicate a world consumption of olive oil and olives equivalent to 2,902,000 and 2,199,000 tons, respectively (Consejo Oleícola Internacional, 2012). Despite the ideal conditions for olive growing in some regions of Brazil, its production is insufficient and today the country imports the majority of olive oil and olives commercialized in the domestic market (Teramoto et al., 2010). Brazil is among the ten countries with the highest consumption of olive oil and olives around the world (Consejo Oleícola Internacional, 2012). In recent years, the production of olives in Brazil has been promoted mainly in the southern region of the country, and currently the area cultivated with olive trees is close to 500 ha (Embrapa, 2012).

Despite the great interest in the production of olives and olive oil, nowadays several interesting properties of olive leaves have been reported. These byproducts of olive cultivation show antioxidant potential (Benavente-Garcia et al., 2000), useful in the treatment of type 2 diabetes (Boaz et al., 2011) and protection of cells against the oxidative damage caused by hydrogen peroxide without genotoxicity (Anter et al., 2011). Poudyal et al. (2010) showed that rats fed with a diet rich in carbohydrates and lipids and supplemented with olive leaves extract attenuated cardiac, hepatic, and metabolic changes. Botsoglou et al. (2010) and Martins et al. (2009) demonstrated that the use of leaves in animal feed improves the meat quality, thus reducing lipid oxidation. Moreover, olive leaves rich in oil cause a decrease of ruminal protozoa leading to an increase in the efficiency of microbial protein synthesis in the rumen (Molina-Alcaide and Yanez-Ruiz, 2008). For lactating animals, a feed consisting of olive leaves resulted in an improvement

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in milk fat quality compared to diets based on conventional forages (Molina-Alcaide and Yanez-Ruiz, 2008). Therefore, olive leaves could be considered as an important raw-material that have the potential to be used as a natural antioxidant and as an ingredient for the stabilization of vegetable oil (Keceli and Harp, 2014) and animal feed (Molina-Alcaide and Yanez-Ruiz, 2008; Botsoglou et al., 2014; Paiva-Martins et al., 2014), but it is important to mention that they could also be used to improve human health (Anter et al., 2011).

In this way, several studies have been carried out to evaluate the antioxidant properties of olive leaves due to their high content of phenolic compounds (Kiritsakis et al., 2010 Xynos et al., 2012), in special oleuropein and its derivative hydroxytyrosol, which are the substances directly related to the biological effects (Erbay and Icier, 2010). Despite the great interest in phenolic compounds in olive leaves, few studies have been performed to evaluate the presence of other compounds with biological activity in this material, such as fatty acids. The olive oil is composed mainly of palmitic, oleic, and linoleic acids, and in minor amount of palmitoleic, stearic, linolenic, and arachidic acids (Manai-Djebali et al., 2012), and several health benefits associated with olive oil consumption are related to these substances (Miranda et al., 2010). Considering that these compounds are present in olives, Tsiplakou and Zervas (2008) investigated their presence in olive leaves and their use as dietary ingredients in sheep and goat feed, and observed an increase of *cis*-9 *trans*-11 conjugated linoleic acid (CLA) content in the milk of these animals. Therefore, the olive leaves could have other substances with potential health benefits in addition to phenolic compounds, but few studies have been carried out to evaluate them. In this way, an evaluation of phenolic compounds, proximates, and fatty acid composition of olive leaves from the southern region of Brazil is presented as well as the determination of phenolics and minerals and their nutritional significance. Considering the importance of this crop production in Southern Brazil, five varieties of *O. europaea* were studied and a principal component analysis (PCA) was performed in order to evaluate the differences among the compounds produced by each one.

2. Materials and methods

2.1. Chemicals and standards

The following reagents were obtained from VETEC (Duque de Caxias, RJ, Brazil) in analytical grade: citric acid monohydrate, chloroform, methanol, hexane, potassium hydroxide, sodium carbonate, sulfuric acid, and ethanol. Butilhydroxytoluene (BHT) and anhydrous sodium sulfate were obtained from ECIBRA (São Paulo, SP, Brazil). Gallic acid was purchased from Sigma (St. Louis, MO, U.S.A.); Folin Ciocalteau reactive and potassium sulfate were obtained from Proquímius (Rio de Janeiro, RJ, Brazil); copper sulfate from Belga Química (Curitiba, PR, Brazil); sodium hydroxide from Labsynth (Diadema, SP, Brazil); boric acid indicator from CAQ (Diadema, SP, Brazil); and analytical-grade nitric acid was obtained from Merck (Darmstadt, Germany). Distilled and purified water (Milli-Q, 18.2 MΩ cm, Millipore, Billerica, MA, USA) was used to prepare samples and standards. Argon (99.996%, White Martins-Praxair, São Paulo, SP, Brazil) was used in ICP-OES determinations for plasma generation, nebulization, and auxiliary gas. Oxygen (99.9991%, White Martins-Praxair) was used as a reagent in digestions performed under oxygen pressure. For GC analysis, hydrogen (99.9999%, White Martins-Praxair) was used as a carrier gas. The standards used for identification of fatty acids were those available in Mix 37 (SUPELCO, USA). Accuracy of mineral determination was evaluated using a certified reference material (CRM), trace elements in olive leaves (BCR 62), produced by the Community Bureau of Reference (BCR, Brussels, Belgium).

2.2. Samples

Leaves of *O. europaea* varieties Ascolano, Arbosana, Negrinha do Freixó, Koroneiki, and Grappolo were harvested in Chapecó (Santa Catarina State, Brazil; latitude −27°05′47″S and longitude 52°37′06″W, altitude 674 m) in the second week of February (summer) of 2012, from six-year-old trees. Leaves were collected from different parts of several trees in order to minimize the effect of sun exposure and differences related to different maturation stages. After drying in an oven with air circulation (45 ± 5 °C, 48 h), the leaves were ground in a rotor mill (Marconi, MA-340) and the powder was stored at −20 °C and protected from light before analysis.

2.3. Proximate composition determination

Proximate composition was carried out according to methods of the Association of Official Analytical Chemists (AOAC, 1995) in triplicates. Samples were weighed in an analytical balance (model 250 A, max 250 g, 0.1 mg of resolution, BEL, Brazil). Moisture content was measured by loss on drying in an oven with air circulation at 105 °C to constant weight (5 h). Ash content was determined in a muffle furnace at 550 °C to constant weight (7 h). The determination of the protein content of olive leaves was carried out by micro Kjeldahl method. Total carbohydrate content was estimated by difference. The lipid extraction of olive leaves was performed by Bligh-Dyer method (1959) with some modifications. Around 3 g of olive leaf powder were used and 8 mL of chloroform (with 0.02% BHT), 16 ml of methanol, and 6.4 mL of distilled water were added. After, the tubes were shaken on a shaker table (Aaker, Brazil) for 30 min. Then, 8 mL of chloroform and 8 mL of 1.5% (w/v) sodium sulfate solution were added and stirred for 2 min followed by centrifugation for 5 min at 3000 rpm. After, a filtration with qualitative filter paper containing 1 g of anhydrous sodium sulfate was performed and 5 mL were transferred to a beaker previously dried by chloroform evaporation under a laminar flow hood. Finally, the beakers were placed in an oven with air circulation at 105 °C for residual water evaporation and then the lipid residue was weighed.

2.4. Determination of total phenolic content

The extraction of phenolic compounds was based on the procedure described by Mylonaki et al. (2008), with some modifications using 0.5 g of dried olive leaf powder from the different varieties with the addition of 20 mL of 60% (v/v) ethanolic solution (with 1 g L^{-1} citric acid). The extraction was performed for 5 h at 22 ± 2 °C with magnetic stirring (Thelga TMA 10C, MG, Brazil) and under protection from light. An additional extraction step was used in order to perform an exhaustive extraction of phenolics. The extractions were carried out in triplicates. Total phenolic content of leaves was determined according to the Folin–Ciocalteau procedure reported by Singleton and Rossi (1965). Aliquots of 200 μL of extracts were diluted in the ratio 1:40 and were transferred to test tubes with the addition of 1000 μL of Folin–Ciocalteau solution diluted in the ratio 1:10. The tubes were stirred and allowed to stand for 8 min. Then, 800 μL of 7.5% (w/v) sodium carbonate solution was added. After stirring and standing for 2 h, the absorbance was measured at 765 nm using a spectrophotometer (JENWAY UV- 6300 Jenway, UK) calibrated with reference solutions of gallic acid. The total phenolic content was expressed as gallic acid equivalents in milligrams per gram of dried sample (mg GAE g^{-1}).

2.5. Fatty acids profile

For the determination of fatty acids, the lipids extracted by the method of Bligh-Dyer were esterified using the procedure suggested by Hartman and Lago (1973). However, some