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A quantitative study on the phenolic compound, tocopherol and fatty acid contents of monovarietal virgin olive oils produced in the southeast region of Brazil

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ABSTRACT

Consumption of virgin olive oil (VOO) is highly recommended due to its human health benefits. Brazil is now beginning to experimentally produce VOO, and there are no data on its chemical profile. The aim of this work was to determine the phenolic compound, tocopherol and fatty acid contents of 17 monovarietal VOOs produced from olive varieties cultivated in the southeast region of Brazil during two crop years. The chemical composition of Brazilian VOO resembles that found in the literature for well-established VOOs. The analyzed compounds comprised palmitic acid (6–12%), stearic acid (1.6–2.2%), oleic acid (70–84%), linoleic acid (3.2–11.7%), α-linolenic acid (0.6–1.4%), tyrosol (NQ–155 mg kg^{-1}), (+)-pinoresinol (2.9–23 mg kg^{-1}), hydroxytyrosol (ND–38 mg kg^{-1}), luteolin (ND–2.2 mg kg^{-1}), α-tocopherol (29–233 mg kg^{-1}), β-tocopherol (ND–9.6 mg kg^{-1}), and γ-tocopherol (ND—19 mg kg^{-1}). There was a significant difference in the contents of almost all of the analyzed compounds between the two crop years. Principal component analysis demonstrated that some varieties can be differentiated from one another by chemical composition. The results indicated that some Brazilian monovarietal VOOs are promising and that further studies will help to improve the quality of Brazilian VOO.

1. Introduction

Virgin olive oil (VOO) is a product of the extraction of olive fruit and is considered to be one of the best sources of fatty acids and natural antioxidants such as phenolic compounds and tocopherols. Its nutritional properties are highly valued for their positive effects on human health. The chemical composition of VOO consists primarily of monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA), mainly in the form of esters with glycerol (triacylglycerols), which represents more than 98% of its total content. Important minor components in olive oil are sterols, hydrocarbons, phenolic compounds, tocopherols, volatile compounds, terpenols, terpenic acids, free glycerol, free fatty acids, and mono- and diacylglycerols. As a result, olive oil constitutes a complex multi-component matrix and its analysis is not an easy task (Dais & Hatzakis, 2013; Del Coco et al., 2013).

Because the olive tree has been cultivated for thousands of years in the Mediterranean, VOO is one of the main components of the Mediterranean diet. It is highly appreciated all over the world for its taste and aroma, as well as for its nutritional properties (López-Cortés, Salazar-García, Velázquez-Martí, & Salazar, 2013). Different cultivars, pedoclimatic conditions of the orchards, and varying agricultural practices, together with olive ripeness and olive oil extraction techniques, result in a great diversity of olive oil chemical profiles (García-González & Aparicio, 2010).

All of the VOO consumed in Brazil is imported from European (Portugal, Spain, Italy and Greece) and South American (Argentina and Chile) countries. As a result, the price of VOO in Brazil is relatively high, and a considerable part of the population does not have access to this important and healthy vegetable oil. Brazil is beginning to cultivate olives and to produce olive oils to offer a product with lower prices to the consumer in the near future and to create new opportunities for Brazilian agribusiness. However, it is of great importance to determine if the chemical composition of the Brazilian VOO is similar to VOO produced in European, South American and other countries that possess more experience in this field.

Thus, the aim of this work is to provide the first data on the phenolic compound, tocopherol and fatty acid contents of Brazilian VOOs produced in the southeast region of Brazil. In fact, these were the first VOOs extracted in the country, and the results of this work will be

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helpful to agronomists in their search for the best-adapted and optimal VOO-producing olive varieties.

2. Material and methods

2.1. Chemicals and standards

Hexane p.a. and methanol p.a. were purchased from Synth (Brazil). Methanol, acetic acid and isopropanol were all of HPLC grade and were purchased from J. T. Baker (USA), while HPLC grade hexane was from Mallinckrodt (USA). Boric acid (Ecibra, Brazil), sodium chloride p.a. (Nuclear, Brazil), sodium hydroxide p.a. (Nuclear, Brazil), butylated hydroxytoluene, BHT, (Sigma-Aldrich, USA) and boron trifluoride–methanol complex (20% solution in methanol) (Merck, Germany) were also used in these studies. Water was purified in a Milli-Q system (Millipore, USA). Standards of tyrosol, gallic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, caffeic acid, 3,4-dihydroxybenzoic acid, cinnamic acid, vanillic acid, ferulic acid, luteolin and apigenin were acquired from Sigma-Aldrich (USA). The hydroxytyrosol standard was obtained from Cayman Chemical (USA). The oleuropein standard was purchased from Extrasynthèse (France). The syringic acid, sinapinic acid and *o*-coumaric acid standards were acquired from Chem Service (USA). The (+)-pinoresinol standard was purchased from Arbo Nova (Finland). Standards of α-, β-, γ-, and δ-tocopherols, and the standards of C_4 to C_{24} methyl esters (FAME Mix), were acquired from Supelco (USA).

Standard stock solutions of phenolic compounds were prepared by dissolving the appropriate amount of each compound in HPLC grade methanol to a final concentration of 2 g L^{-1} for (+)-pinoresinol, 1 mg L^{-1} for hydroxytyrosol and luteolin, 0.4 g L^{-1} for apigenin, and 5 g L^{-1} for the other 13 compounds. Tocopherol standard stock solutions were prepared by dissolving them in HPLC grade hexane containing 0.01% BHT, at the concentrations of 25 g L^{-1} for the α-, γ-, and δ-tocopherol isomers, and 50 g L^{-1} for the β-tocopherol isomer. The fatty acid methyl ester standard stock solution was prepared in HPLC grade hexane. Standard stock solutions were filtered through a 0.45 μm Millipore PVDF membrane (Millipore, USA), stored at −18 °C and protected from light. Vials containing working solutions were placed under ultrasound for 5 min before injection.

2.2. VOO samples

A total of 17 VOO samples were obtained from Maria da Fé Experimental Farm of the Agricultural and Livestock Research Corporation of the State of Minas Gerais (EPAMIG). Maria da Fé is a city situated in the micro-region of Serra da Mantiqueira in the south of Minas Gerais state (latitude: 22° 18′ 28″ S; longitude: 45° 22′ 30″ W; altitude: 1276 m above sea level). Using the Köppen–Geiger climate classification system, Maria da Fé has a temperate highland tropical climate with dry winters (Cwb). The mean annual temperature is 17 °C and fluctuates between 10.1 °C (minimum) and 23.3 °C (maximum), while the mean annual rainfall is approximately 1738.6 mm.

The samples consisted of VOOs produced from different olive varieties during two different years. From the 2010 crop, samples of MGS Grap 561 (Grappolo 561), Cornicabra, Tafahi 390, Grappolo 575, Arbequina, Alto D'Ouro, Negroa, MGS Neblina and JB1 varieties were available. From the 2011 crop, samples of MGS Mariense (Maria da Fé), Mission, Grappolo 575, Arbequina, Alto D'Ouro, Negroa, MGS Neblina and JB1 varieties were evaluated.

An Abencor® system (Suárez, Aranda, Mendoza, & Rey, 1975) adapted for obtaining sufficient olive oil to perform the chemical analysis was employed for olive oil extraction. Olives from each variety (10 kg) were washed with water to remove impurities and leaves prior being milled in a metallic mill. The olive paste was heated to 28 °C during the homogenizing process, which was performed in a domestic mixer using two types of movements, translation and rotation, during an interval of 50–60 min. Using an analytical balance, 450 g of the olive paste was transferred to a high rotation centrifuge and subjected to centrifugation at 4200 rpm, allowing VOO separation. The VOO phase was separated and placed into plastic packages, where remained for 60 min to allow residual sedimentation. Finally, VOOs were transferred to amber glass bottles.

After arriving at the laboratory, all samples were maintained under refrigeration (4 °C) and protected from light until analysis.

2.3. Sample preparation and extraction procedures

2.3.1. Fatty acids

Approximately 100 mg of each virgin olive oil sample was weighed into test tubes, with the subsequent addition of 4 mL of 0.5 mol L^{-1} NaOH solution in methanol. The tubes were heated in a 100 °C water bath for approximately 8 min until a transparent solution was obtained. After cooling, 3 mL of a 12% BF_3 solution in methanol was added and the tubes were heated again in a 100 °C water bath for 3 min. After cooling, 4 mL of saturated NaCl solution was added with agitation. Next, 4 mL of hexane was added with vigorous agitation. Then, the tubes were left to rest to allow phase separation, and 1 μL from the upper layer was injected into the gas chromatograph. Each sample was prepared in triplicate (n = 3). This procedure was adapted from the work of Joseph and Ackman (1992).

2.3.2. Phenolic compounds

The phenolic compound extraction procedure was based on the work of Pirisi, Cabras, Cao, Migliorini, and Muggelli (2000) and Bonoli, Montanucci, Gallina Toschi, and Lercker (2003). Approximately 2 g of VOO was weighed into a centrifuge tube and 1 mL of hexane and 2 mL of methanol:water (60:40, v/v) were added. This mixture was stirred for 2 min in a vortex apparatus, and the tube was then centrifuged at 5000 rpm for 5 min. The methanol:water layer was separated and the extraction was repeated twice. The extracts were combined and evaporated to dryness at 39 °C under reduced pressure. Samples were resuspended in 1 mL of methanol:water (30:70, v/v) and filtered through a 0.45 μm PVDF membrane (Millipore, USA) before analysis by capillary electrophoresis. All samples were extracted in triplicate (n = 3).

2.3.3. Tocopherols

For the tocopherol analysis, virgin olive oil samples were diluted in hexane (0.1 g in 10 mL of hexane containing 0.01% BHT), filtered through a 0.22 μm PVDF membrane (Millipore, USA), and then directly injected into the column in the HPLC system. This sample preparation was based on the work of Dionisi, Prodoiliet, and Tagliaferri (1995), Guinazi, Milagres, Pinheiro-Sant'ana, and Chaves (2009) and Pinheiro-Sant'ana et al. (2011). Samples were prepared in triplicate (n = 3).

2.4. Separation methods

2.4.1. Fatty acids

A Varian 3800 Gas Chromatograph (Varian Inc., USA) equipped with a flame ionization detector (FID), a split/splitless injector (split ratio 1/80) and a fused-silica capillary column with 90% cyanopropyl-modified polysiloxane (100 m length, 0.25 mm i.d., 0.25 μm film thickness) (NST BIS-6025025, Nano Separation Technologies, Brazil) were used for the determination of fatty acid methyl esters (FAMEs). The optimized parameters were: injector temperature (240 °C); detector temperature (240 °C); carrier gas, hydrogen, flow rate 1.4 mL min^{-1}; detector gas flow rate (H_2/N_2/synthetic air −30/30/300 mL min^{-1}); oven temperature program (197 °C for 23 min, increasing to 225 °C at a rate of 20 °C per min, and kept at the final temperature for 15 min). Peak identification was accomplished by comparing the retention time of the standards with those of the peaks observed in the samples separated under the same conditions. The peak area results are expressed as the percentage of the total FAME peak area. Chromatographic conditions