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Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties

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ABSTRACT

To increase the potential and better exploring of grape seeds that are an important wine-industrial waste, oils of ten traditional Portuguese grape varieties were evaluated in relation to their vitamin E content (tocopherols and tocotrienols), fatty acid profile, as well as, antioxidant properties. Our results showed that the grape-seed oils were a good source of γ-tocotrienol (499–1575 mg/kg), α-tocopherol (85.5–244 mg/kg) and α-tocotrienol (69–319 mg/kg). Concerning fatty acid profile, linoleic (C18:2cc), oleic (C18:1), palmitic (C16:0) and stearic (C18:0) acids were the predominant. Grape-seed oils demonstrated to be a good source of polyunsaturated fatty acids (PUFAs) (63.64–73.53%), whereas monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) ranged between 14.19–21.29% and 11.64–14.94%, respectively. Interesting values of DPPH and ABTS radical scavenging activities were also obtained. This study demonstrated that these seeds may be reused and their oils incorporated in other food products, taking into account the compounds with positive effects on human health that are present in their composition.

1. Introduction

Vitis vinifera L. production is widespread throughout the world, exceeding 68 million tons (FAOSTAT, 2010). In 2010, Portugal produced more than 945 kton of grapes, being one of the most relevant crops in terms of employment and wealth. As grape-seeds comprise about 5% of the fruit weight (Choi & Lee, 2009), more than 3 million tons of grape seeds are discarded annually worldwide and around 47 kton in the particular case of Portugal. Grape seeds are an important part of the pomace, corresponding to 38–52% of dry matter (Maier, Schieber, Kammerer, & Carle, 2009). So, grape seeds are often referred as a significant agricultural and industrial waste (Freitas, Jacques, Richter, Silva, & Caramão, 2008; Kim, Kim, Choi, Jeong, & Lee, 2008; Luque-Rodríguez, Castro, & Pérez-Juan, 2005; Lutterodt, Slavin, Whent, Turner, & Yu, 2011). Thus, finding feasible solutions for treating this residue, including attempts to the development of new products, would constitute an excellent opportunity. Grape-seed oils may be a good option, as numerous health benefits associated with their composition may exist, mainly in what concerns vitamin E and essential fatty acids, particularly the linoleic acid. This fatty acid is referred in the literature as a protector of cardiovascular diseases (Wijendran & Hayes, 2004), while vitamin E has neuroprotective and antitumoral properties; it is able to lower the cholesterol levels and has antioxidant activity (Choi & Lee, 2009). Recently, some studies indicate that aqueous extracts prepared from grape seeds can have antibacterial and antioxidant activities (Adámez, Samino, Sánchez, & González-Gómez, 2012). Furthermore, grape-seed oils have emerged as a product with potential to be used in pharmaceutical and food applications (Bail, Stuebiger, Krist, Unterweger, & Buchbauer, 2008; Furiga, Lonvaud-Funel & Badet, 2009), being already extracted in Italy, Spain and France (Crews et al., 2006). Thus, the possibility of reuse of grape seeds is promising. On the other hand, the fact of living in a society that faces environmental (waste disposal problems) and economic (the need to invest in most vulnerable areas) issues, the recovery and reuse of grape seeds are of great importance (González-Centeno et al., 2010), contributing to lower the production costs, to increase producer income and to create new products for human consumption (Baydar & Ozkan, 2006). Of our knowledge, no study has been performed until now that focused on the potential use of seed oils of Portuguese grape varieties. So, this study aims to characterize seed oils of ten national grape varieties in relation to fatty acid and vitamin E contents, as well as antioxidant activity, in order to valorize this genetic patrimony.

2. Material and methods

2.1. Grape seed samples

Grapes were harvested at their ripening time in Valpaços, northeast of Portugal. Ten red varieties were selected, including: Aragonês, Cornifesto, Marufo, Periquita, Tinta Barroca, Tinta Carvalha, Tinto Cão,

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Touriga Francesa, Touriga Nacional and Trincadeira Preta. After harvest, the grapes were transported under refrigeration and on their arrival at the laboratory the fruits were washed with ultra-pure water (Milli-Q system). After drying with soft paper, the grapes were weighted and the seeds and skins separated from the pulp. The number of seeds and their weights were also determined, as well as the weights of the skins. Afterwards, the seeds were dried at 40 °C.

2.2. Grape-seed oil extraction

For each variety, 5 g of seeds were crushed in a mortar with a pestle. Anhydrous sodium sulfate was added to remove moisture remains. The lipid fraction was obtained by Soxhlet extraction using petroleum ether for 24 h. Each sample was extracted in duplicate.

2.3. Fatty acid composition

Methyl esters of fatty acids were obtained by hydrolysis with a methanolic solution of potassium hydroxide (11 g/L), BF_3/MeOH esterification and extraction with *n*-heptane. Fatty acids were analyzed in a gas chromatograph (GC) (Chrompack, model CP-9001), equipped with a split/splitless injector system and an autosampler (Chrompack CP-9050 model). Fatty acid separation was carried out on a CP Sil-88 column (Varian) with the dimensions 50 m × 0.25 mm × 0.19 μm. Helium was used as carrier gas at a pressure of 120 kPa. Injector and detector temperatures were 250 °C and 270 °C, respectively. Methyl ester separation was carried out with a temperature gradient between 140 and 220 °C and detection was performed with a flame ionization detector (FID). Collection and processing of the data were performed by the CP Maitre Chromatography Data System Program, Version 2.5 (Chrompack International BV). The identification of the chromatographic peaks was made by comparing the retention time of the sample peaks with a standard mixture of 37 fatty acid methyl esters (FAME Mix-37 Supelco).

2.4. Vitamin E

An accurate lipid portion was diluted in hexane, followed by the addition of the internal standard, tocol (Matreya, USA). Tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) were determined on an HPLC Jasco chromatograph, equipped with a pump (PU-980 Model), a mixing chamber HG-980-30 and an autosampler Plus AS2057 model. Detection was performed by the fluorescence detector FP2020 Plus model at 290 nm (excitation) and 330 nm (emission) wavelengths. A normal phase silica Supelcosil LC-SI (Supelco) column was used with a mobile phase of hexane:dioxan (97:3 v/v) at ambient temperature. Standard solutions of tocopherols and tocotrienols were graded according to their molar absorptivity. The linear working range was always checked for each of the compounds.

2.5. Antioxidant activity of the grape-seed oils

2.5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The free radical scavenging activities of the grape-seed oils were determined by using 2,2-diphenyl-1-picrylhydrazyl free radicals. Briefly, the oil samples were diluted in ethyl acetate (1:10) and 1 ml of this solution was added to 4 ml of DPPH solution (1 mM in ethyl acetate). After 30 min at room temperature in the dark, the absorbance was measured at 515 nm. Ethyl acetate was used instead of seed oils as a control. The ability to scavenge the DPPH radicals was determined using Eq. (1).

$$DPPH\ scavenging\ effect(\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100, \quad (1)$$

where A_{DPPH} was the absorbance of the control reaction and A_{Sample} was the absorbance in the presence of the sample.

2.5.2. ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity

The ABTS radical scavenging capacities of the grape-seed oils were determined as described by Zuleata, Esteve, and Frígola (2009), based on the capacity of a sample to inhibit this radical ($ABTS^{\cdot+}$) compared with a reference antioxidant standard. Briefly, the $ABTS^{\cdot+}$ radical was prepared by the reaction of 25 ml of ABTS solution (7 mM) with 440 μl of potassium persulphate (140 mM). This mixture was kept at room temperature in the dark for 12–16 h, the time required for forming the radical. The $ABTS^{\cdot+}$ solution was then diluted with ethanol to obtain an absorbance of 0.700 (±0.20) at 734 nm. 100 μl of each grape-seed oil was mixed with 2 ml of $ABTS^{\cdot+}$ solution and the absorbance was measured 6 min after mixing. The antioxidant capacities of the grape-seed oils were calculated by a standard curve obtained by measuring the absorbances of Trolox solutions (0.025–0.5 mM). The results were expressed in μmol of Trolox equivalents per ml of oil.

2.6. Statistical analysis

Statistical analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, IL), version 18.0. The normality and variance homogeneity were checked by Shapiro–Wilk and Levene tests. As both failed the non-parametric Kruskall–Wallis test was applied. When significant differences were detected, a One-Way ANOVA to rank means and the LSD Post-hoc test were applied. In order to join grape varieties in homogeneous groups a cluster analysis was performed taking into account the fatty acid composition (SFA, MUFA and PUFA) and vitamin E composition (tocopherols (α-, γ-, δ-) and tocotrienols (α-, β-, γ-, δ-)). The subjects were grouped after performing a Hierarchical Cluster Analysis, using the nearest neighbor method and the squared Euclidean distance. The number of clusters to retain was established by the R-squared criteria, as described in Maroco (2003).

3. Results and discussion

3.1. Grape characterization

Grapes of the ten studied varieties were characterized in terms of the weights of the fruits, skins and seeds (Table 1). The grape weights varied within and between varieties, the means ranging between 1.82 and 3.63 g for Tinto Cão and Aragonês, respectively. When considering the ranges, it was observed that Tinto Cão grapes were the lightest (1.26 to 2.60 g) whereas Aragonês were the heaviest (2.82 to 4.38 g). When considering the percentages of skins and seeds, higher values were obtained for the formers. The differences in the number of seeds between varieties were also stated, the means ranging from 1.53 for Trincadeira Preta and 3.30 for Tinto Cão. Generally, it was observed that the grapes presented 1 to 3–4 seeds; however, the varieties that had a higher number of seeds (ex. Tinto Cão and Touriga Nacional) did not show necessarily higher seed weights.

3.2. Grape seed oil contents

Oil contents, expressed in percentage of oil in dry basis, are reported in Table 2. The highest fat content (12.40%) was obtained for Touriga Francesa, followed by Tinto Cão (12.06%), values three times higher than that determined for Marufo (3.95%). These values were smaller than those referred by Tangolar, Ozogul, Tangolar, and Torun (2009), of 10.45% to 16.73% for grape varieties grown in the experimental vineyard of the Cukurova University (Turkey). On the other hand, our values were similar to those obtained by Beveridge, Girard, Kopp, and Drover (2005). These authors referred a maximum oil content, extracted with petroleum ether, of 11.17% for Cabernet Sauvignon and a minimum of 6.64% for