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Short communication

Pressurized liquid extraction of vitamin E from Brazilian grape seed oil

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Abstract

The goal of this paper is to optimize the pressurized liquid extraction (PLE) of vitamin E from grape seed oil from residues of the wine industry. For this purpose an experimental planning to optimize the extraction of Brazilian grape seed oil by means of PLE with hexane as solvent was applied and the results are compared with conventional methods (Soxhlet and mechanical press extraction). Vitamin E was separated and analyzed using HPLC with UV detection. This study demonstrates the ability of the PLE in extracting grape seed oil rich in vitamin E.

Keywords: Grape seed oil; Pressurized liquid extraction (PLE); ASE; HPLC

1. Introduction

Vitamin E is a fat-soluble vitamin that exists in eight different forms. Each form has its own biological activity, which is the measure of potency or functional use in the body [1,2]. Vegetable oils are the main dietary source of vitamin E (a mixture of tocopherols and tocotrienols) which appears in trace levels but are of great importance due to its medicinal properties; α-tocopherol is the name of the most active form of vitamin E. It is also a powerful biological antioxidant [3]. It decreases the risk of cardiovascular disease, cancer and prevents the sexual impotence [3–5]. In spite of the essential nature of tocopherols in mammalian diets, vitamin E cannot be synthesized by humans. Vitamin E in supplements is usually sold as α-tocopheryl acetate, a form of α-tocopherol that protects its ability to function as an antioxidant [6].

Owing to their lipid soluble antioxidant properties, these compounds inhibit the processes of peroxidation of polyunsaturated fatty acids and other compounds in cell membranes [7]. It is also responsible for being a natural antioxidant that prevents the rancidity of oils during storage [8–11]. Vitamin E is also used in the chemical industry, as additive for food and cosmetic products [12].

Each oil variety has different amounts of vitamin E [13–15]. Grape seed oil is one of the major sources of vitamin E and contains relatively high quantities of tocopherols and tocotrienols in the range of 1–53.06 mg of vitamin E/100 g of oil [11,16–18]. Among the different tocopherol species in foods, α-tocopherol, has the highest vitamin E antioxidant activity [13,19,20].

Tocopherols are sensitive to light and air. Thus, extraction and analytical procedures that require many manipulations can result in the partial degradation of these antioxidants and in considerable quantification errors [8].

High temperatures generally improve the efficiency of extraction due to enhancing the diffusion rate and the solubility of analytes in the solvents [21–23]. Therefore, high-temperature-short-time extraction conditions have been successfully used to retard the degradation of these compounds in fruits, oils and foods [24,25].

Pressurized liquid extraction (PLE) is a process that combines temperature and pressure with liquid solvents to achieve rapid and efficient extraction of analytes from several matrices [26]. A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in the liquid state, even above their normal atmospheric pressure boiling points allowing high-temperature

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extraction. PLE is highly dependent of variables like extraction time, temperature, solvent polarity and ratio solvent/sample, for this reason, it is necessary to optimize the conditions for extracting oils from any matrix.

PLE is used for the extraction of many compounds in vegetal oil as lipids and fatty acids [27–29] due to its short time extraction. It also can be used in the extraction of minor components, such as anthocyanins, carotenoids, phenols, sterols, phospholipids, tocopherols, free fatty acids, and glycerides [22,24,25,27], because these compounds may be degraded during long extractions at higher temperatures.

In this paper it was used PLE to extract grape seed oil from different kinds of Brazilian grape seeds from wine industry waste and HPLC/UV to determine the presence and the amount of vitamin E (in terms of α-tocopherol) in these oils.

2. Experimental

2.1. Grape seed samples

The grape seed used in this work was obtained as a by-product in the wine industry. Six varieties of grapes were used: Herbemont, Seibel, Isabel, Cabernet, Merlot and Muscatel. All of them were cultivated in the state of Rio Grande do Sul—South of Brazil. The residual grape seed was gently assigned by wine manufacture Aurora and Bocato, from Bento Gonçalves, Rio Grande do Sul, Brazil. The grape seed was washed several times with water to remove all the impurities. After drying on an oven at 100 °C, the seeds were crushed in a mill until to produce particles with diameters of 0.014 mm. The milled grape seed were stored in glass vials wrapped with an aluminum foil to prevent degradation by light.

2.2. Reagent and standards

Hexane (extractor solvent) was purchased from Merck (Darmstadt, Germany) and bi-distilled before using. Methanol for liquid chromatography was also Merck but HPLC grade and α-tocopherol standard was obtained from Sigma Aldrich (Steinheim, Germany). All the standard solutions (and work solutions) were prepared in methanol at the desired concentration and filtered through 0.45 μm plastic (PTFE) sterile filters prior to chromatographic analysis. To avoid the oxidation, the solutions were prepared in the same day of the analysis.

2.3. Extraction of grape seed oil

2.3.1. Soxhlet extraction of grape seed

Approximately 10 g of grape seed were extracted in a Soxhlet apparatus for 20 h with hexane, according to the work described by Gómez [30]. The obtained oil was dried in an anhydrous sodium sulfate column and pre-concentrated under reduced pressure to remove all the solvent.

2.3.2. Mechanical press extraction of grape seed

Approximately 50 g of grape seed were put in the mechanical press (Bovenau) for 72 h, under 10 ton, being the oil collected at the bottom in an appropriate vial. The extraction cell was made in the laboratory in stainless steel and totally disassembled, allowing the handling and cleaning of the pieces.

2.3.3. Pressurized liquid extraction of grape seed

About 3 g of homogenized seeds were exactly weighed and placed in a 33 mL stainless steel extraction cell. The cell was closed and mounted in the carousel of extraction of a Dionex ASE 300 (Dionex, Sunnyvale, CA, USA) Accelerated Solvent Extractor equipped with a solvent controller and then extracted. The extract was collected in a 250 mL glass vial and pre-concentrated under reduced pressure to remove all the solvent and finally, dried in an anhydrous sodium sulfate column.

Hexane was the solvent used for the PLE procedure. The extraction program consisted in 30 min static extraction, at 1500 psi, 60 s of purge, 150% flushing, three cycles and 100 °C. After, the raw extract was collected as noted above. The extract was analyzed directly or after concentration by nitrogen.

Many works are described in the literature about PLE and our research group has developed methods for phytotherapic extractions from plants [31,32]. Details of the equipment can be seen in these cited references.

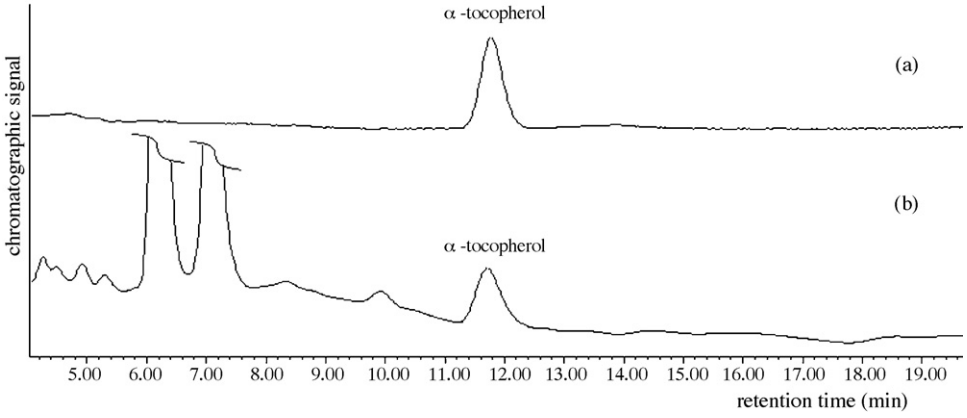

Fig. 1. Chromatogram of α-tocopherol (a) and grape seed oil (b) by HPLC/UV (Conditions described in the text).