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Study of supercritical extraction from Brazilian cherry seeds (*Eugenia uniflora* L.) with bioactive compounds

Débora Nascimento e Santos, Larissa Lima de Souza, Nilson José Ferreira, Alessandra Lopes de Oliveira *

Food Engineering Department, Faculty of Animal Science, University of São Paulo, Av. Duque de Caxias Norte, 225, Jardim Elite, CEP 13635-900, Pirassununga, SP, Brazil

ABSTRACT

In this study, we obtained extracts from Brazilian cherry (or Pitanga) seeds using supercritical CO_2 (SC-CO_2) and analyzed the volatiles profile. Seeds were placed in contact with the SC-CO_2 under different conditions of pressure (*P*) and temperature (*T*). The yield, the refractive index, the presence of terpenes by thin layer chromatography (TLC) and the volatiles profile by gas chromatography with mass spectrometry (GC–MS) were determined for all the extracts. With respect to the extract yield (0.16–0.48 g/100 g), surface response analysis revealed that the greatest yield was obtained at high *P* and *T* did not affect the process, which indicated that low temperatures can be used to protect the thermosensitive substances. The presence of terpenes with antioxidant activity was detected via TLC analysis, and the GC–MS results indicated γ-elemene and germacrone as the major components and the concentration of the sesquiterpenes in the extract was influenced by the interaction between *P* and *T*.

Keywords: *Eugenia uniflora* L.; GC–MS; Sesquiterpenes; Response surface analysis

1. Introduction

In recent years, interest has been an increasing in the recovery of bioactive compounds from natural sources for the development of functional foods. Supercritical fluid extraction (SFE) has been recognized as an increasingly applied in the food, pharmaceutical and cosmetics areas; it provides higher selectivity and shorter extraction times and does not involve the use of toxic organic solvents. Because of these and other advantages, SFE has been used in various industries (Marsal et al., 2000; Serra et al., 2010). A supercritical fluid is defined as the state in which liquid and gas are indistinguishable. Because of its low viscosity and high diffusion capacity, supercritical fluids exhibit better transport properties than liquids, which allows rapid extraction can be accomplished (Berna et al., 2000; Andreo and Jorge, 2006). Carbon dioxide is an ideal solvent for the extraction of natural products, because it exhibits low toxicity and is non-explosive, readily available and easy to remove from the extracted solutes. The use of CO_2 makes part of this extraction method free of organic solvents and the method is therefore considered environmentally friendly. Additionally, because the critical temperature of CO_2 is 31 °C, thermosensitive natural compounds can be recovered with minimal possibility of damage or decomposition (Salgin, 2007; Danh et al., 2009; Laroze et al., 2010).

The extracts obtained by SFE using CO_2 are generally recognized as safe (GRAS) for use in food production, such as the extraction of oil from a large number of materials whose extracts have shown strong potential for future applications in drugs and food (Zhang et al., 2010). The extraction with supercritical CO_2 is a powerful tool in research on fragrances and essential oils, especially because the

* *Corresponding author*. Tel.: +55 19 3565 4268; fax: +55 19 3565 4284.
E-mail address: alelopes@usp.br (A.L.d. Oliveira).
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isolation of volatile compounds using traditional methods, such as steam distillation or dynamic headspace sampling produces fragrance extracts that may not reflect the real flavor properties of the natural material. With extraction conditions that use mild temperatures, supercritical fluid extraction is the most appropriate method to isolate essential oils from spices, flowers, herbs, leaves, seeds and roots. The extracts are generally regarded as superior to sensory concentrates produced using traditional techniques (Marsili, 2002).

The Pitanga fruit or Brazilian cherry (*Eugenia uniflora* L.) belongs to the botanical family Myrtaceae. It is a native plant to Brazil and the country has been estimated to be the world's largest producer, although it is also found in Northern Argentina and Uruguay (Bezerra et al., 2004; EMBRAPA, 2006). The nutritional value of Pitanga is relevant, because it high concentrations of calcium, phosphorus, pro-vitamin A, vitamin C and phenolic compounds (anthocyanins and carotenoids), the latter of which are known for their antioxidant activity (Mélo et al., 1999; Silva, 2006; Lopes Filho et al., 2008). The leaves and fruits of *Eugenia uniflora* L. in the form of infusions have been widely used in Brazilian folk medicine with several effects, such as exciting, febrifuges, aromatic, antidysenteric, antihypertensive and antirheumatic and its alcoholic extract is used to treat bronchitis, coughs, fevers, anxiety and verminosis (Auricchio et al., 2007). The hydroalcoholic leaves extract decreased levels of the xanthine oxidase enzyme, linked to the onset of gout, and also presented features of vasorelaxant, antioxidant, antidiarrheal, hypotriglyceridemic, hypoglycemic and with antibacterial property (Ogunwande et al., 2005; Amorim et al., 2009).

The work by Oliveira et al. (2006) shows that the Brazilian cherry fruit has monoterpenes similar to those present in the leaves, whose bioactivity has been scientifically proven, explaining the similarity of the effects seen in both extracts. Considering the fruit particularity, the same research group studied the process optimization to obtain cherry extracts by the use of supercritical CO_2 (Malaman et al., 2011). Sensory tests showed the extracts were rich in volatile compounds, leading to the perception of greater intensity of the fruit aroma; and according to the literature, these compounds are also present in the leaves (Pino et al., 2003; Oliveira et al., 2009).

On the seeds, there are few studies reporting its composition. Bagetti et al. (2009) evaluated the nutritional composition and antioxidant activity of these seeds and the results revealed carbohydrates as the most abundant, composed primarily of insoluble fiber. The protein fraction is very small. Among the fatty acids present, 45–47% are polyunsaturated, desirable for the human diet have an effect on reducing the incidence of cardiovascular disease. In another study carried out by Luzia et al. (2010), it was obtained ethanolic extracts from Pitanga seeds showed higher antioxidant potential with a high concentration of phenolic compounds. Among the phenolic compounds found in Pitanga seeds ethanolic extracts obtained by pressurized fluid extraction are present flavonol and conjugate acids with sugars (Oliveira et al., 2014).

In this study, supercritical extract was obtained from the Pitanga seeds and with this objective was to analyze the behavior of pressure and temperature in the yield and the volatile compounds extraction when these conditions was applied.

2. Materials and methods

2.1. Materials

Ripe native fruit were collected on the *Campus* of the Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo (USP), Pirassununga (21°49′46′ S 47°25′33″ W), Brazil. In the Laboratory of High Pressure Technology and Natural Products (LTAPPN), the seeds were selected, washed to remove traces of pulp, and dried in an oven with air circulation at 38 °C for 54 h. The dried seeds were peeled and crushed in hammer mill type (Buhlermiag, Uzwil, Switzerland) and then kept frozen at −20 °C.

Petroleum ether, ethanol (EtOH), chloroform and sodium carbonate were obtained from Synth (Diadema, Brazil). Gallic acid and the homologous series of alkanes C10–C40 were purchased from Fluka (Sant Louis, Unites States). R-(+)-limonene, radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and anisaldehyde were purchased from Sigma Aldrich (Sant Louis, Unites States). Chromatographic plate Silica gel 60 F_{254} and toluene were obtained from Merck GE. We also used ethyl acetate (Mallinckrodt Chemicals, Dublin, Ireland) and methanol (JT Baker, Dublin, Ireland).

The moisture content of the seeds after dryer was determined by gravimetric method which determines the mass loss from the sample by drying to constant weight (AOAC, 1995). The ether extract was obtained by the hot extracting lipids method using Soxhlet with petroleum ether as organic solvent (IAL, 2008). The crushed seeds (200 g) granulometry was determined in a set of six Tyler standard sieve series with stirring for 15 min (ASAE, 2008 – S319.4). For the determination of apparent density, we used the fixed bed of 300 cm^3 (extractor) as standard container volume. For 10 times it was completed this volume with the ground Pitanga seeds and after removal of the extractor, the mass was weighed. The apparent density was obtained by the ratio between the average mass of seeds and volume of fixed bed.

2.2. Supercritical fluid extraction (SFE)

In the extraction procedure it was promoted the contact between the crushed seeds, which were packed in fixed bed extractor 300 cm^3, with supercritical CO_2 to pre-set pressure (P) and temperature (*T*) using a central composite design (CCD). The pressure was performed by a high pressure pump (Eldex, S-100-AA, United States), while the temperature was maintained by operation of a thermostatic bath, in which the extractor has been packaged. The extract, separated from the gas was collected in a flask and CO_2 flow rate (2 g CO_2/min), was measured in a rotameter (LAO, G-1, Osasco, Brazil) under ambient conditions (0.94 bar and 25 °C). At the end of the process, the extracts were weighed and related to the mass of dry seeds packed in the extractor to calculate the overall yield (Malaman et al., 2011).

The operating conditions of the experimental procedure were made according with a CCD with *P* and *T* as independent variables (Table 1) totaling 11 tests (Table 2), which were performed at random. For all tests, it was standardized a time of 16 h of static equilibrium and dynamic extraction period of 6 h. The static extraction time has been applied in order to ensure that the mechanical and thermal equilibrium is guaranteed in the employed operating conditions.