Volatile composition of the extracts of Pitanga (*Eugenia uniflora* L.) obtained from the optimization process of supercritical CO_2 extraction.

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SC-CO_2 presents critical *PVT* properties, and under such conditions, the variation of these properties can be intense changing the solubility and selectivity of the CO_2, which can be controlled by the pressure (*P*) and temperature (*T*). Extracts from tropical fruits are interesting for their bioactive property and, in addition, the SFE has been a powerful technique in the separation process to get these extracts.

Pitanga is a fruit with a pleasant aroma, and it is consumed *in natura* or processed as jellies, jams, and juices. The solubility of *Pitanga* extracts in supercritical CO_2 was observed in different conditions of *P* and *T* aiming at producing better yields and composition analyses. The composition of the extracts, from the tests conducted in a complete central composite 2^2 factorial experimental design, was analyzed by GC/MS to identify the compounds that may characterize the fruit aroma or its bioactive principles such as those present in the leaves. The best yields were obtained at 200 bar and 50^{o}C. The relative area percentage for every peak of the majority of volatile compounds was higher at 50%. Among the chemical classes are the sesquiterpenes (α-Cubebene, Germacrene B and Spathulenol) and ketones (β-Damascenone, 2(4H)-Benzofuranone, 5,6,7,7a-tretraidro-4-4-7a-trimethyl, Selina-1,3,7(11)-trien-8-ona, 2-Benzothiazolinone, and 3,7-Ciclodecadien-1-one, 10-(1-methylethenyl)-(E, E)-) compounds, also present in the leaves essential oil. Since the fruit extracts have compounds such as those present in the leaves and the Pitanga juice used in the Brazilian popular medicine as a diuretic, anti-rheumatic and anti-diabetic, they could also present bioactive properties. The extracts also contained caffeine, vitamin E, γ-sitosterol, a phytosterol used to reduce blood cholesterol levels and part of the fruit wax as the long chain hydrocarbons present in the extracts (Eicosane, Heneicosane, n-Docosane, Eicosane, 10-methyl-, n-Tricosane, n-Pentatriacontane, n-Hexatriacontane, and Tritetracontane). Volatile phenolic compounds such as the 2,4-Bis(dimethyl benzyl)phenol, to which antioxidant properties are attributed, are present in the extracts in high proportion.

INTRODUCTION

Clean technology has been used to obtain extracts from a large number of natural products. The high SC-CO2 density allows excellent solubility power, low viscosity, and high diffusivity that promote a considerable penetration in the solute. The extracts from Brazilian natural products have attracted worldwide interest for their biologic properties, and the supercritical fluid extraction (SFE) has been a powerful technique used in separation

processes to obtain a large amount of products in industry and research sectors. Several recent studies, such as this, have suggested new industrial applications for this technology.

The genus *Eugenia* L. from Mytaceae family presents 3,000 species in tropical and subtropical regions. Originally from South America, it is found in Brazil, Uruguay, and Argentina. Its cultivation could also be found in the United States, the Caribbean Islands, India, China, Egypt, Nigeria, and Australia. In Brazil, the first commercial-scale plantings of *Pitangueira* (1,300 to 1,700 ton/year) occurred in the city of Bonito /PE [1]. It is an edible fruit with sweet and agreeable aroma with remarkable amount of pro-vitamin A (11.98 $mg100g^{-1}$) and vitamin C (21.50 $mg100g^{-1}$) [2, 3, 4]. Pitanga fruits are also rich in natural pigments with antioxidant activity [5, 6].

In the Brazilian folk medicine, *Pitanga* is considered digestive, stimulant, antipyretic, antidiarrheic and antirheumatic. The composition of the essential oil and therapeutic properties of the *Pitanga* leaves have been reported in the scientific literature. The aroma of the essential oil from the Pitanga leaves is classified as similar to "mushroom", "timber" and "spice" because of the furanodiene notes, "green bush", and "cucumber" attributed to the presence of selinotrienone (Selina-1, 3,7 (11)-trien-8-one). The properties of essential oils from the leaves of *Pitangueira* have been showed antiinflammatory action [7], contractile activity that facilities the intestinal transit [8], as an inhibitor of increased levels of triglyceride in blood plasma [9], and antimicrobial activities [10]. Because of these biological properties, the interest in the identification, isolation, and synthesis of components of this plant has been growing. Hence, the component Selina-1,3,7 (11)-trien-8-one has been synthesized [3].

Many of the compounds present in the essential oil from the leaves of *Pitanga* are also present in the fruit, even the ketone: Selina-1, 3,7 (11)-trien-8-one [11]. The objective of this work was to study the solubility and the composition of the supercritical extracts from *Pitanga* in order to optimize the operating conditions of pressure (*P*) and temperature (*T*) to obtain the best yield of extracts rich in bioactive compounds.

MATERIALS AND METHODS

The seeds were extracted from the fruit *in natura* (originally from Bonito/PE/Brazil). The fruit was then frozen at –30°C/40min and stored in sealed bags under vacuum at –18°C. Following, it was lyophilized (Liophylizator LI - Terroni Equip. Científicos Ltda., Brazil), wrapped in a vacuum sealed plastic package, and it was finally stored at –15°C. The humidity of the lyophilized fruit (14.48% ± 0.96) was determined by the gravimetric method at 105°C until constant weight.

In the fruit process, the pulp was manually separated from the seeds to remain similar appearance to the fruit *in natura* with spherical shape. The external diameter (13.72 ± 1.26 mm) was determined empirically using metric measures. The real density (350.55 kgm^{-3}) was determined through direct mass measurements of each lyophilized fruit (0.3505 g ± 0.0743) and the volume was measured by the apparent volume of water caused by displacement due to the immersion of the dried fruit. The apparent density (67.46 ± 3.43 kgm^{-3}) was also determined empirically. The mass of lyophilized *Pitanga* in the total volume of the extractor (fixed bed) of 300 cm^3 was weighed. The porosity of the bed (ε) was calculated by the relation between the apparent density (ρ_a) and real density (ρ_r): $\varepsilon = 1 - \frac{\rho_a}{\rho_r}$; resulting in 0.80 or 80%. The high porosity of the bed is expected since we chose to work with loose packing particles which are large and porous.

Liquefied CO_2 from the cylinder passed through a cooling unit to prevent its vaporization and it was driven by a positive-displacement liquid pump (Eldex, AA-100-S, Napa, CA) into a pre-heating tank. Lyophilized fruits in the extractor were maintained in contact with SC-CO_2 for 30 min (static period) up to the desired *P* (from 129 to 271 bar) and thermostatically controlled (from 36 to 64^{o}C). The equilibrium pressure was monitored by calibrated Bourdon gauges (accuracy ± 0.5%) while the desired *T* was controlled by immersion in a water bath with control accuracy of ± 1^{o}C (Sulab, Campinas, Brazil). These conditions of *P*-*T* were applied by using of complete central composite 2^2 factorial experimental designs in order to verify the yield and the flavor intensity of the fruit [12] using response surface analysis and also the composition of the extracts.

The pure extract collected during the three-hour extraction was weighed in an analytical scale (Sartorius, BL 210s). The exit flow of SC-CO_2 was controlled in order to keep *P* constant in the system, and the flow rate (0.0024 kg.min^{-1} ± 0.0003) was measured under ambient *P*-*T* conditions (0.94 bar and 25^{o}C). The yield was calculated by relating the total extract and the mass of the *Pitanga* in the extractor.

Samples of the extracts obtained in the complete central composite 2^2 factorial experimental designs were analyzed by gas chromatography and mass spectrometry (GC/MS) (Shimadzu GC/MS 2010 Plus). The GC injector was equipped with split/splitless at 250^{o}C and a BP1 capillary column (30m × 0.25mm × 0.25 μm). Helium was used as the carrier gas with an internal pressure of 15 psi. The ratio for the split was 1:50 and the volume of the sample injection was 2.0 μL. The GC oven temperature was programmed to operate at 50ºC for 2 min and, from 50 to 180ºC at 4ºC/min. The ion source temperature was 280^{o}C. Data acquisition was performed for the mass range from 50 to 600 *m/z*. The ionisation energy of the electrons was 70 eV (EI). The compounds were identified by comparing the experimental mass spectra with those of the NIST62.LIB mass spectra library. The retention indices (Kovats) were determined by a standard mixture of a homologous series of n-alkanes (C_9 – C_{25}) prepared in ethyl acetate under the same conditions used in chromatographic separation of volatile in the sample.

RESULTS AND DISCUSSION

Using response surface analysis, it was possible to verify that the extract yield was relatively lower in regions in which *P* is higher than 225 bar or lower than 175 bar for the range of *T* studied. With respect to *T*, poorer yields were obtained in the regions under 40^{o}C and higher than 60^{o}C [12]. This statistical analysis restricts the *P*-*T* conditions in which yields are greater (200 bar and 50^{o}C). However, this study could become impracticable if compounds of interest (with bioactive properties) are not present in the extracts.

Consequently, the composition of the extracts was analyzed to identify the components in greater concentration and those that could possibly contribute to the characteristic *Pitanga* flavor or bioactive properties such as those present in the leaves.

The percentage area of the majority of peaks of the volatile compounds in the *Pitanga* extracts accounted for more than 50%. Among the chemical classes present in the extracts, sesquiterpenes (α-Cubebene, Germacrene B and Spathulenol) and ketones (β-Damascenone, 2(4H)-Benzofuranone, 5,6,7,7a-tretraidro-4-4-7a-trimethyl, Selina-1,3,7(11)-trien-8-ona, 2-Benzothiazolinone and 3,7-Ciclodecadien-1-one, 10-(1-methylethenyl)-(E, E)-) were found. These compounds also present in the *Pitanga* leaves essential oil [2, 13]. The fact that the supercritical extracts from the fruit presented volatile components such as those present in the leaves, in addition to the used of the *Pitanga* juice in Brazilian folk medicine as a diuretic,

antirheumatic, antifebrile, antidiabetic, and antidiarrheic, means that these extracts could carry biologic properties.

The extracts were also rich in phenolic volatile compounds which are attributed to antioxidant properties. Lee et al., 2007 [14] evaluated the antiinflammatory and antioxidant activities of Natsugumi (*Elaeagus multiflora*) extract, a typical fruit from China, Korea, and Japan. The authors observed the presence of the 2,4-Bis(dimethylbenzyl) phenol, the same compound in the supercritical extracts of *Pitanga* fruit. When considering the percentage area of the relative peaks chromatogram, this compound is present at considerable quantity. The concentration of volatile phenolic compounds varied within the samples; thus, it was higher in the extracts with higher yield.

Caffeine, Vitamin E, which is also an antioxidant present in fruits, and the γ-sitosterol, which is a phytosterol with chemical structure similar tocholesterol and used to reduce cholesterol levels in the blood in the treatment of hypercholesterolemia, are also present in the *Pitanga* extracts. Phytosterols are components constituents of vegetal wax as well as the long-chain hydrocarbons present in the Pitanga extracts (Eicosane; Heneicosane; n-Docosane; Eicosane, 10-methyl-; n-Tricosane; n-Pentatriacontane; n-Hexatriacontane and Tritetracontane.

CONCLUSIONS

Assessing the identification of the composition of the extracts, the *Pitanga* flavor, and the presence of phenolic compounds in the region in which the highest yield (0.56%) was obtained, it can be concluded that the extracts obtained by using supercritical technology provide a product with aromatic and antioxidant properties that could be utilized as raw material in food, medicaments, and cosmetics. Nevertheless, it is worth mentioning that such raw material is highly purified and free of organic solvents since it is a product obtained by using a Generally Recognized as Safe (GRAS) solvent. The volatile compounds that give the characteristic flavor of *Pitanga* are in greater concentration in the extracts obtained above 50^{o}C for the range of *P* studied. However, it is necessary to run biologic tests to verify the applicability of this extracts. Based on this study, it is possible conclude that the parameters (*P-T*) could be utilized on an industrial-scale production when the objective is to obtain extracts rich in *Pitanga* characteristic flavor or antioxidants.

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