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Extraction of bioactive compounds from peach palm pulp (*Bactris gasipaes*) using supercritical CO_2

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ABSTRACT

Natural compounds with biological activity have recently attracted special interest in the agro-industry as sources of additives in nutraceutical food production and pharmaceutical industries. Herein, we evaluated extracts obtained from peach palm fruit (*Bactris gasipaes*) using supercritical carbon dioxide, in terms of yield, total phenolic content, total flavonoids, total carotenoids, and antioxidant activity by β-carotene bleaching method. Extractions were performed at 40, 50, and 60 °C and 100, 200, and 300 bar; additionally, Soxhlet (with petroleum ether) and methanol extraction were conducted. The results showed that supercritical CO_2 allows obtaining extracts rich in carotenoids and, although it presents lower yield than conventional extraction (SOX), supercritical CO_2 represents a technique with greater advantages. The best operation condition for supercritical extraction was 300 bar–40 °C, given that the highest concentration of carotenoids was obtained, without the yield being significantly different from that obtained with 300 bar–60 °C, this extract had antioxidant activity comparable to that of commercial caffeic acid.

1. Introduction

Bioactive compounds are naturally widespread in the plant kingdom because they are synthesized as secondary metabolites with defense functions, besides being responsible for properties of color, astringency, and flavor of fruits and vegetables. They are increasingly important because given their chemical structure, these compounds are suitable for scavenging free radicals found in the human body; said free radicals behave as reactive oxygen species (ROS) enabling development of chronic multifactorial diseases [1].

These compounds are credited for the mass consumption of fruits due to their high content of antioxidants; with beneficial effects in preventing cardiovascular and circulatory diseases, cancer, and neurological diseases, given their anti-inflammatory, anti-allergic, antimicrobial, antithrombotic, and antineoplastic activity [2,3]. Tropical countries like Colombia and Brazil, because of their abundance of exotic fruits, have a huge potential for the exploitation of the resource to obtain bioactive compounds of underutilized fruits like the peach palm fruit (*Bactris gasipaes*), which could be used as active ingredients in pharmaceutical products, in controlling oxidation processes in food processing, in nutraceutical food production with high added value, as well as in the cosmetic industry [4].

Peach palm fruit (*B. gasipaes*) is a palm of the Arecaceae family, cultivated in tropical America from Costa Rica to Brazil and Bolivia in wet and low zones. It is commonly known as "*cachipay*", "*chontaduro*", "*pejibá*", and "*puñuña*" (Amazon). The fruit has a fibrous and fleshy mesocarp of deep yellow or orange color; it may be considered a fruit with high nutritional value due to its high content of fiber, oils, β-carotene, for eight essential amino acids [4–6], and for its energy value. However, the main feature of the recent interest in working with this fruit is the β-carotene content that could be obtained from it as a vitamin A precursor of high antioxidant activity because it can capture free radicals due to its conjugated double-bond system [2].

Extraction with supercritical fluids is a technique that uses the properties of fluids over their critical points to selectively extract soluble components from raw plant materials; additionally, supercritical carbon dioxide is recognized as an ideal solvent to extract bioactive compounds because it is nontoxic, non-explosive, readily available, easy to remove from the final extract, does not cause major disruptions in biocompounds, and its biological properties can be preserved [7–9].

This work sought to evaluate bioactive compounds of extracts obtained from the peach palm fruit using supercritical carbon dioxide (Sc-CO_2) at different pressures and temperatures. For this

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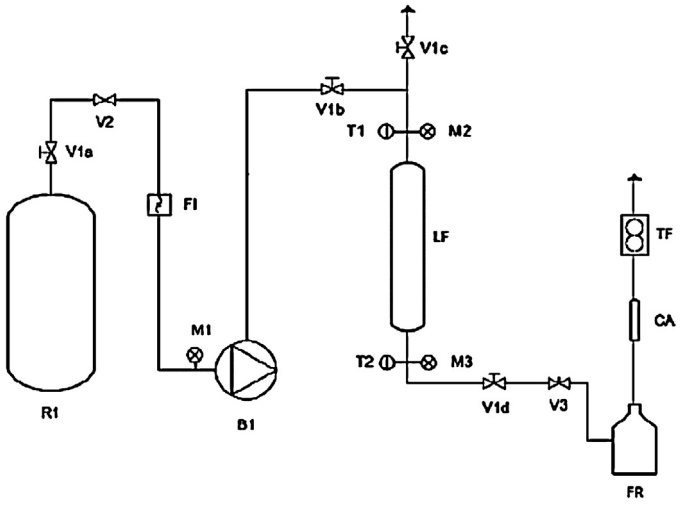

Fig. 1. Extraction flowchart [11]. B1: CO_2 pump, R1: CO_2 reservoir, CA: adsorption column, T: thermocouples, FR: collection bottle, TF: flow totalizer, FI: filter, V1: locking valve, LF: extraction bed, V2: retention valve, M: manometer, V3: valve.

purpose, the following variables were evaluated: extraction yield, total phenolic content (TPC), total flavonoids (TF), total carotenoids and antioxidant activity (AA%) by β-carotene bleaching (BCB) method of each of the extracts obtained.

2. Materials and methods

2.1. Raw material and chemical characterization

Peach palm fruit grown in el Tambo, Cauca (Colombia) were acquired in the municipal market in Cali (Colombia), then the exocarp was removed and the mesocarp was cut, lyophilized, ground, vacuum packed, and refrigerated until its later use.

Chemical characterization of peach palm fruit (proximate analysis) for moisture, ash, crude fat, crude fiber, and protein content was performed by using methods 966.02, 923.03, 920.39, 920.87, and 962.09, respectively, from the Official Analytical Chemists Association (AOAC) [10].

2.2. Supercritical extraction

Supercritical extractions were carried out in a supercritical unit (Fig. 1), which operates up to a maximum pressure of 35 MPa. The unit has a high-pressure pump for the solvent (Thermo Separation Products, model 2000, Florida, USA), two programmable thermostatic baths (Marconi, model MA-159 and Marconi, model MA-184, Piracicaba, SP, Brazil), a flow totalizer (LAO, model G $0.6 \pm 0.001\ m^3$, São Paulo, SP, Brazil), thermocouples, and three control manometers (Record (50.0 ± 0.5) MPa, São Paulo, SP, Brazil). The extraction bed has an internal 3.41 cm diameter and 46 cm height [11].

All extractions were performed using 10.0 ± 0.010 g of raw material, constant CO_2 flow of 3.00 l/min for 91 min and a solvent/raw material S/F ratio (w/w) of 46. The supercritical extractions were performed at different temperature and pressure values; 40, 50, and 60 °C and 100, 200 and 300 bar, respectively (Table 1). After extraction, the collection bottles, kept at 5 ± 1 °C during extraction, were freed of residual CO_2, hermetically re-sealed, isolated from direct light, and stored under refrigeration (Metalfrio Freezer) until its subsequent analysis.

Additionally, Soxhlet extraction (SOX) was conducted with petroleum ether and methanolic extraction (MET) to compare Sc-CO_2 extraction to traditional methods. For MET, 2 g of peach palm fruit were taken and added to a beaker containing 30 mL of methanol (CHEMCO, absolute grade); the solution was stirred for 24 h at 25 °C; then, the solution was vacuum filtered (0.45 μm filter, Vaccuo Tecnalpump TE-0581) and rotor-evaporated (Heidolph 220V). Soxhlet extraction was performed for 6 h using 2 g of raw material and 60 mL of petroleum ether (ECIBRA) as solvent; the ether-extract solution was rotor-evaporated (Heidolph 220V) at 40 °C.

Table 1
Extraction conditions.

Experiment	Temperature (°C)	Pressure (bar)
E1	40	100
E2	40	200
E3	40	300
E4	50	100
E5	50	200
E6	50	300
E7	60	100
E8	60	200
E9	60	300

2.3. Extraction yield

The amount of extract obtained (E) in relation to the amount of raw material (RM) used in each type of extraction is a significant factor in assessing bioactive extracts and on the extraction techniques.

2.4. Chemical characterization: TPC, TF, and total carotenoids

For all Sc-CO_2, MET, and SOX extracts total phenols content (TPC) were quantified, expressed as (mg GAE)/g extract through the Folin–Ciocalteu method [12]. Total flavonoids (TF) were quantified via spectrophotometric method, according to the methodology described by Zhishen et al. [13]. Gallic acid (Sigma Aldrich) was used to construct the calibration curve for TPC at different concentrations and, finally, the following linear equation came about: Abs $B = 0.090 + 0.002$, $R^2 = 0.998$, where Abs is absorbance (nm) and B is phenolic content (mg mL^{-1}).

Catechin was used as pattern for TF (Sigma Aldrich) and the equation obtained was: $C = 0.217$ Abs, $R^2 = 0.999$, where C is the flavonoid content (GAE mg mL^{-1}).

Total carotenoid content was determined according to the methodology described by Szydłowska-Czerniak et al. [14] modified. Extract samples (5.0–8.0 mg) were diluted in 10 mL of *n*-hexane (96% purity, EMSURE Merck); subsequently, the solution was loaded onto the spectrophotometer (FEMTO 800 XI) at 450 nm absorbance, using a 1 cm quartz cell. The calibration curve was prepared by using standard pattern β-carotene (97.0% purity, Fluka Analytical) at different concentrations ranging from 0.02 to 6.1 mg mL^{-1}. The resulting calibration curve was $D = 0.006$ Abs, $R^2 = 0.995$, where D is the total carotenoid content expressed as β-carotene equivalent (mg mL^{-1}).

β-Carotene bleaching is based on a spectrophotometric method monitoring oxidation products due to degradation of linoleic acid. The methodology used was described by Martinez-Correa et al. [15]. Briefly, 5 mL of a dry emulsion of β-carotene and linoleic acid transferred to a test tube and 0.2 mL of extract diluted in ethanol, at a 200-μg/mL concentration was added. Similar standard solutions, quercetin and caffeic acid solutions (200 μg/mL) were used as positive controls (standard solutions). The control solution was prepared the same way, except that the solution was replaced by 0.2 mL of pure ethanolic extract. Both tubes with the extract and the control were subjected to thermal auto-oxidation at 50 °C for 120 min and absorbance was measured at 464 nm (spectrophotometer FEMTO 800 XI) at 30-min intervals, against a