EXTRACTION OF THE 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 a source of ingredients for nutraceutical food and pharmaceutical industries. In this work we evaluated extracts obtained from peach palm fruit (*Bactris gasipaes*), in terms of: total phenolic content (TPC), total flavonoids (TF), total carotenoids and antioxidant activity (AA%), using supercritical carbon dioxide as method of extraction. Extractions were performed at 40°C, 50°C and 60°C temperature and 100 bar, 200 bar and 300 bar pressure, and additionally Soxhlet and methanolic extraction were conducted. The results showed that SC-CO_2, allows obtaining extracts rich in carotenoids and although it presents a lower performance than the conventional extraction (SOX), the SC-CO_2 represents a technique with major advantages. The best operation condition for the supercritical extraction was 300 bar-40°C because of the highest concentration of carotenoids was obtained, without the yield being significantly different from that obtained with 300 bar-60°C, this extract obtained AA% comparable to that of commercial caffeic acid.

Key words: *Bactris gasipaes*, supercritical fluid, bio-compounds, carotenoids, antioxidant activity.

1. Introduction

Bioactive compounds are widely distributed in nature, because they are synthesized as secondary metabolites with defense functions, besides being responsible for the properties of color, astringency and flavor of fruits and vegetables. Its increasing importance in the modern world is based on the fact that these compounds are suitable for capture free radicals found in the human body and they behave as oxidant agents (ROS) enabling the development of chronic multifactorial diseases [1].

Consumption of fruits with high content of antioxidants produces beneficial effects in the prevention of cardiovascular diseases, circulatory, cancer and neurological diseases, because of its anti-inflammatory, anti-allergic, antimicrobial, antithrombotic and antineoplastic activity [2]. Tropical countries as Colombia and Brazil, due to their richness in exotic fruits constitute a huge potential for exploitation of this resources, in order to obtain bioactive compounds of underutilized fruits as peach palm fruit (*Bactris gasipaes*).

Peach palm fruit (*Bactris gasipaes*) is a palm of the *Arecáceae* family, cultivated in tropical America from Costa Rica to Bolivia in wet and low zones, is commonly known as cachipay, chontaduro (Colombia), pejibá y pupunha (Brasil). In Colombia is grown in the Pacific between Urabá and Nariño, in addition to the Amazon region. It has a fibrous and fleshy mesocarp of deep yellow or orange color, it may be considered as a fruit with high nutritional value due to its high fiber content, oils, β-carotene, and cotains 8 of the 20 essential amino acids [3,4]. The main feature of recent interest with this fruit is the content of β-carotene that can be obtained from it as a precursor of Vitamin A and high antioxidant activity because it has ability to capture free radicals due to its conjugated system of double bonds [2].

Extraction with supercritical fluids is a technique that uses the properties of the fluids above their critical point for extract soluble compunds from natural raw materials, additionally carbon dioxide is constituted as ideal solvent for the bioactive extraction because it no toxic, non-explosive, availability [5,6,7].

The objective of this work was to evaluate bioactive compounds of extracts from the peach palm pulp (*Bactris gasipaes*) using carbon dioxide in supercritical state (scCO_2) at different pressure and temperature conditions. For this purpose were evaluated these variables; extraction yield, total phenolic content (TPC), total flavonoids (TF), total carotenoids and antioxidant activity by beta-carotene bleaching method (DBC). Additionally, a comparison was made with traditional extraction methods.

2. Materials and methods

2.1 Raw material and chemical characterization

Peach palm Fruits (*Bactris gasipaes*), grown in the Tambo (Cauca, Colombia) were purchased in municipal market in Cali (Colombia), then exocarp was removed, and the mesocarp (pulp) was lyophilized, ground , packed and stored under refrigeration (Figure 1 and 2).

Figure 1. *Bactris gasipaes* in natura

Figure 2. Peach palm fruit conditioned for extraction

Chemical characterization of peach palm fruit (Weende analysis) for moisture, ash, crude fat, crude fiber and protein content, was performed using the methods 966.02, 923.03, 920.39, 920.87 and 962.09 of the Official Analytical Chemists Association (AOAC) [8].

2.2 Supercritical Extraction

Supercritical extractions were carried out in supercritical unit which operates up to a maximum pressure of 35 MPa (Figure 3). The unit consists of 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), a flow totalizer (LAO, model G 0.6 ± 0.001 m3, São Paulo, SP), thermocouples and three control manometers (Record, (50.0 ± 0.5) MPa, São Paulo, SP), the extraction bed has an internal diameter of 3.41 cm and 46 cm in height.

All extractions were performed using 10.0 ± 0,010 grams of material, a constant flow of CO_2 of 3.00 l/min and a relation solvent / raw material [S/F (w/w)] of 46. Supercritical extractions were performed at different temperature and pressure value: 40, 50 and 60°C and 100, 200 and 300 bar respectively (Table 1). After the extraction the collection bottles and stored under refrigeration (Freezer Metalfrio).

Additionally Soxhlet extraction was conducted (SOX) with petroleum ether and methanol (MET) with the purpose of comparing scCO_2 extraction. For MET, 2g 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 hours at constant 25°C temperature, then the solution was filtered under vacuum (0.45 µm filter, Vaccuo Tecnal TE-0581) and rotor-evaporated (Heidolph 220V). SOX was performed for 6 hours using 2g of raw material and 60ml of petroleum ether (ECIBRA) as solvent, the ether-extract solution was rotor-evaporated (Heidolph 220V) at 40°C.

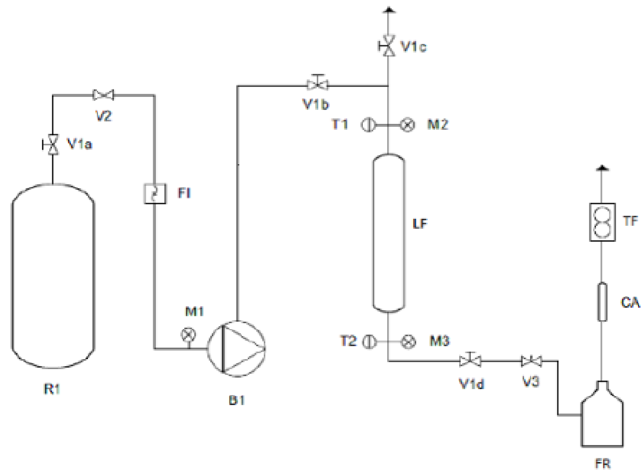

Figure 3. Extraction unit [9]

B1: Pump CO2
CA: Adsorption column
FR: Collection bottle
FI: Filter
LF: Extraction bed
M: Manometer
R1: CO2 Reservoir
T: Thermocouples
TF: Flow Totalizer
V1: Locking Valve
V2: Retention Valve
V3: Valve

Table 1. Experimetal 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

Extraction yield was determined form the amount of extract obtained in relation to the amount of raw material (d.b) used in each extraction procedure (Equation 1).

$$\boldsymbol{Y}(\%) = \frac{E}{RM} \text{x } 100 \tag{1}$$

where:
Y: Extraction yield (%)
E: Extract obtained (g)
RM: Amount of raw material (g, d.b)

2.3 Chemical characterization: TPC, TF and total carotenoids

For all extracts total phenols were quantified (TPC) and expressed as (mg GAE)/g extract, by the Folin-Ciocalteu method [10], gallic acid (Sigma Aldrich) was used to construct the calibration curve for TPC in different concentrations and finally the following linear equation came about: $B = 0.090\ Abs + 0.002$ (R^2 = 0.998), where *Abs* is the absorbance (nm) and B is phenolic content (mg ml^{-1}). Total flavonoids (TF) by spectrophotometric method according to the methodology described by Zhishen *et al*. [11], catechin was used as pattern for TF (Sigma Aldrich) and the equation obtained was: $C = 0.217Abs$ ($R^2 = 0.999$), where C is the content of flavonoids (GAE mg ml^{-1}).

The total carotenoid content was determined according to the methodology described by Szydłowska *et al*. [12]. The extract samples (5.0-8.0mg) were diluted in 10ml of n-hexane (96% purity, EMSURE Merck), then the absorbance was read in spectrophotometer (FEMTO 800 XI) at 450 nm, using a 1cm quartz cell. The calibration curve was prepared using standard β-carotene (97.0% purity, Fluka Analytical) at different concentrations (0.02-6.1 mg/ml). The resulting calibration curve was $D = 0.006Abs$ ($R^2 = 0.995$), where D is the total carotenoid content expressed as β-carotene equivalent (mg ml^{-1}).

Antioxidant activity was measured with β-carotene bleaching is based on a spectrophotometric method of follow up to oxidation products due to degradation of linoleic acid. The methodology used was described by Martinez-Correa *et al*. [13]. Briefly, 5 ml of a dried emulsion of β-carotene - linoleic acid and transferred to a test tube adding 0.2ml of extract diluted in ethanol, at a concentration of 200 µg/ml, similarly to the standard solutions (solutions of quercetin and caffeic acid ,200µg/ml) are used as positive controls. Control solution is prepared the same way except that the solution was replaced by 0.2ml ethanol. Both tubes were submitted to thermal auto-oxidation at temperature of 50°C for 120 min and the absorbance was measured at 464 nm (spectrophotometer FEMTO 800 XI) at intervals of 30 minutes, control solution that was prepared with 5ml of the emulsion without the β-carotene adding 0.2 ml of ethanol. The antioxidant activity (% AA) is calculated as percentage of inhibition relative to the control that represents 100% oxidation (Equation 2):

$$A.A(\%) = \left[\frac{(A_0^c - A_t^C) - (A_0^s - A_t^s)}{(A_0^c - A_t^C)}\right] x\,100 \qquad (2)$$

where "C" represents control solution, and "s" sample, and A_0 and A_t are absorbance values at 0 and t minutes.

2.4 Statistical Analysis

All experiments were performed by duplicate, results were analyzed in the SAS statistical software (v. 9.1.3), was also performed Tukey test with a confidence range ≥ 95%.

3. Results and discussion

3.1 Chemical characterization

The table 2 shows the composition of peach palm pulp (mesocarp), dry matter content was significantly high in comparison with other tropical Colombian fruits and is similar to reported by Leterme *et al*. [14], however a significant variation in the mineral content was presented for the same study. In amazonian fruit reported dry matter content lower than other colombian tropical fruits [5], but with high protein content and ether extract.

Table 2. Composition percentage of peach palm pulp (*Bactris gasipaes*)

Component	Content (%)
Dry matter	30,84
Ash	2,64
Protein	8,17
Oil	18,73
Neutral Fiber	8,18
Carbohydrates	62,28

Table 2 also shows that the fruit has a high lipid content (17.73%), which together reddish color in the mesocarp may also be indicative of high carotenoids content, and consequently a high extraction yield since the lipid phase of these foods is rich in carotenoids [15,16]. The contents of neutral fiber, carbohydrates and proteins have similarity to those reported by Rios et al. [17] for different tropical colombian varieties of peach palm.

3.2 Extractions

The Figure 4 shows the extraction yield extractions performed, all $scCO_2$ extractions showed lower values compared to SOX (up to 394%) and had no significant differences with MET (except those at 100 bar). Supercritical extraction yields generally reported by other authors are [6,7,18,19], this due to the selectivity and affinity of solutes in $scCO_2$ in relation to conventional extraction methods which obtain high yields

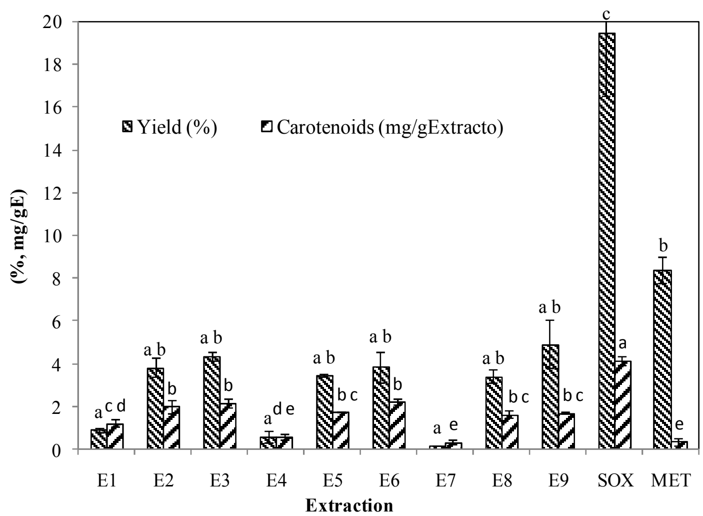

Figure 4. Extraction yield and carotenoid concentration

Disadvantage of conventional extractions (MET, SOX), even they had yields higher than $scCO_2$ is the limitation in the subsequent recovery, separation and purification of the extracts, the extract has some limitations for food applications [20]. Supercritical extraction yield takes into account a complex balance between the reduction of the density of CO_2 and the increasing vapor pressure of the compounds [21], $scCO_2$ yields increases with the increasing pressure (Figure 4). However it is observed that in the treatment E9 the behavior pattern changes, introducing the highest performance with a value of 4.9%. The statistical analysis performed for $scCO_2$ extractions showed significant differences only with the variation of pressure but not for temperature, as reported in other studies [7,22].

3.3 Chemical Characterization of extracts

Total Carotenoids: Carotenoids are the most interesting bioactive compounds for peach palm fruits, which have characteristic reddish color and are rich in oil, these pigments are responsible for color of the fruit, are also the precursors of Vitamin A, responsible for immune system regulation and oxidation inhibitors and peroxidation of fats [23]. Figure 4 shows carotenoid concentration in $scCO_2$ extracts increases with extraction yield and pressure, different behavior was observed for 300 bar -60 °C (E9) which has higher yield than obtained at 300 bar- 40°C-50°C (E3, E6), with low carotenoids concentration. Carotenoid content in extracts obtained E1 to E9, showed maximum at high pressures and at low temperatures, at high temperatures thermal degradation of carotenoids can be important [16, 22]. Regard to conventional extractions, SOX showed the highest carotenoids concentration (4.157 mg/g), with significant differences compared with MET and $scCO_2$, since for its polarity allows higher yields, as reported by Vagi *et al*. [24] and Cadoni *et al*. [23], opposite behavior happens with MET.

The analysis of variance for $scCO_2$ extracts obtained with showed that both temperature and pressure produced significant differences in the final carotenoids concentration, however, the experiments conducted at 300bar and 40°C and 50°C showed no significant difference from those obtained at 200bar - 40°C and 50°C, so that for the extractions with purpose of recovery of carotenoids, it is enough to work at 200bar.

Total phenols (TPC) and total flavonoids (TF): For none of the extracts obtained with $scCO_2$, SOX and MET, were detected phenolic and flavonoids compounds by spectrophotometry (TPC, TF <0.0102 mg/ml). Contreras *et al*. [25] reports for peach palm fruit TPC lower for Colombian exotic fruits, additionally nonpolarity of $scCO_2$ solubilizes and extracts nonpolar compounds; which corresponds to lipid fraction, therefore is not an effective method for obtaining extracts rich in phenolic compounds with high polarity [26]. Martinez-Correa *et al*. [13] reports that primary extraction of the nonpolar compounds (e.g. $scCO_2$) promotes subsequent removal of the polar compounds (e.g. phenolics) using conventional solvents such as ethanol and water, thereby obtaining more concentrated polyphenol extracts, than those obtained with use the same solvents but without previous extraction.

3.4 Antioxidant Activity (AA)

Figure 5 shows the antioxidant activity (%) reported for MET, SOX and $scCO_2$ extracts at 60 minutes, as can be seen the higher AA for $scCO_2$ extracts was obtained for E9 (10.3%) and its activity was statistically similar to presented for commercial caffeic acid. MET also presented value statistically equal to caffeic acid and E9 extract, however MET extract presented the lowest carotenoids concentration.
The highest antioxidant capacity was obtained for SOX extract reaching 25% inhibition; it also presented the highest overall yield and carotenoids concentration, what could be related to the high antioxidant activity. Nonpolar nature of compounds extracted with SOX allows these compounds can be located in water/lipid interface thus protecting the emulsion and inhibiting the oxidation of β-carotene [13], this explains AA value of quercetin obtained (AA= 26%.)

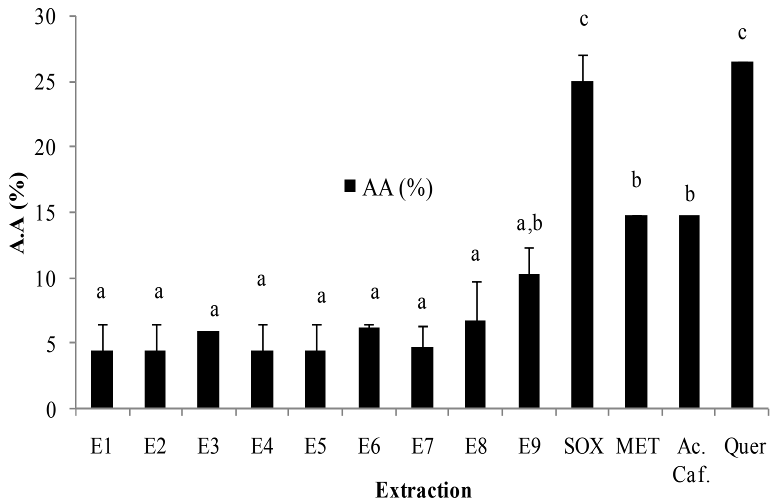

Figure 5. Antioxidant activity of the different types extracts compared with commercial antioxidants for 60 minutes

Extracts obtained from sc CO_2 had lower ability to inhibit oxidation reactions that MET, even with a high carotenoid concentration; this behavior suggests that antioxidant activity determined by the DBC method would not be directly related to the carotenoid content in the extracts, or this is basically a capacity of phenolic compounds and not for carotenoids nature, as reported by Rodriguez-Amaya [27].

4. Conclusions

This study showed that use of supercritical CO_2, produces carotenoid-rich extracts from peach palm mesocarp that. The recommended operating condition for supercritical extraction is 300 bar-40 °C since obtained the highest concentration of carotenoids, with yield to obtained for 300 bar-60°C. $scCO_2$ extracts obtained an AA (%) comparable to commercial caffeic acid, which promotes potential use as source of carotenoids for pharmaceutical, natural colorant in food or in cosmetic industries.

Acknowledgements

The authors thank to the staff of the Laboratory LASEFI, Unicamp, for their cooperation and guiance in this project.

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