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Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: Yield, composition and economical evaluation

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ABSTRACT

Obtaining an extract with high antioxidant activity using environmentally friendly technologies and low-cost raw materials is of great interest. In the present work, a combined extraction process developed by our research group involving ultrasound treatment and agitated solvent extraction was evaluated. This method was compared in terms of yield, composition, and economical feasibility to traditional extraction methods, including ultrasound assisted, agitated bed and soxhlet extraction with ethanol (acidified or not). The proposed method maximizes the extraction of phenolic compounds with acceptable degradation of anthocyanin pigments from an unusual source: Brazilian jabuticaba (*Myrciaria cauliflora*) skins. The use of ultrasonic irradiation continuously supporting a main extraction process has demonstrated increased performance but implies in high consumption of energy and consequently, money. However, the procedure described in this paper appears to be a viable option because it uses shorter ultrasonic irradiation and results in high antioxidant activity extracts, and the anthocyanin profile corroborates literature data (cyanidin-3-glucoside and delphinidin-3-glucoside).

1. Introduction

The desire for a healthier diet allied with the increasing concern of consumers over the use of synthetic additives in food has pushed the food industry to search for new sources of natural pigments (Montes et al., 2005). Anthocyanins are a type of functional pigment responsible for a wide range of colors present in vegetables, flowers, fruits, and derived products. It is known that anthocyanin pigments act as strong antioxidants and are anti-inflammatory, with antimutagenic and cancer chemopreventive activities (Kong et al., 2003). These bioactive properties have already been demonstrated in *in vitro* and *in vivo* studies (Galvano et al., 2004), and an increase of publications in this field has been observed in recent years.

In a recent review paper, Santos and Meireles (2009) compiled the recent studies on the health-promoting properties of anthocyanins. This review demonstrated that consumption of dietary phytochemicals, of which anthocyanins represent a considerable part, may promote several health benefits: reduction in the risk of cardiovascular diseases, diabetes and cancer; a protective effect against hepatic and gastric damage and collagen degradation; an increase of cognitive performance, etc.

Grape peels, grape by-products (constituted mainly by peels) and berries are well known for their antioxidant properties due to the presence of anthocyanins and other phenolic compounds. Many studies have been done to extract and evaluate these compounds on the industrial scale (Santos and Meireles, 2009). In Brazil another source seems promising; jabuticaba (*Myrciaria cauliflora*) is grape-like in appearance and texture, although its skin is thicker and tougher. This fruit has a dark purple to almost black skin color due to a high content of anthocyanins that cover a white gelatinous flesh inside (Terci, 2004).

As the extraction procedure is of great importance for obtaining natural colorants, different research groups have made an effort to develop an efficient extraction procedure. An efficient extraction should maximize anthocyanin recovery with minimal degradation and result in an extract with high antioxidant activity using environmentally friendly technologies and low-cost raw materials. For this purpose this paper aims to demonstrate a potential technique to extract antioxidant compounds (anthocyanins and other phenolic compounds) from jabuticaba skins: utilizing a short ultrasonic irradiation as pre-treatment before conventional solvent extraction. This method is proposed based on the fact that ultrasonic irradiation facilitates the release of extractable compounds and enhances mass transport of the solvent from the continuous phase into plant cells of target compounds (Lee and Row, 2006), mainly during the initial minutes (Zhang et al., 2009). Ethanol was used in all experiments because it is a GRAS (generally-recognized-as-safe) solvent.

It is well known that many flavonoids and related phenolic compounds contribute significantly to the total antioxidant

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activity of many fruits and vegetables (Luo et al., 2002). Given that some recent papers have demonstrated that the biological (antioxidant, antiradical, etc.) activities of jabuticaba skin extracts are due to their compositions (Reynertson et al., 2008) the study of the effect of the extraction method on the extract antioxidant activity was evaluated.

To compare the effect of the extraction method on the process in terms of yield, composition and economic feasibility, different extraction methods were carried out for comparison purposes: ultrasound assisted, agitated bed, and soxhlet extraction using ethanol and acidified ethanol as solvent.

2. Materials and methods

2.1. Plant material

Jabuticaba fruits (*Myrciaria cauliflora*) harvested from a plantation in the State of São Paulo, Brazil, were acquired from a fruit and vegetable market center (CEASA-Campinas, Brazil). Immediately after acquisition, the fruits were stored in the dark in a domestic freezer (−10 °C) (Double Action, Metalfrio, São Paulo, Brazil) until sample preparation. Before extraction, the fruits were manually peeled.

2.2. Extraction procedures

2.2.1. Ultrasound assisted extraction (UAE)

Jabuticaba skins were added to 50-cm^3 Erlenmeyer flasks and then mixed with different volumes of ethanol 99.5% (Ecibra, Santo Amaro, Brazil) to give a feed to solvent ratio of 1:10. Immediately after the addition of the solvent, the flasks were sonicated in an ultrasonicator bath with a 40-kHz frequency (81 W) (model T 1440, Thornton, São Paulo, Brazil) at room temperature for 2 h. After ultrasound extraction, the solvent was separated from the plant residue by simple filtration and evaporated using a rotary evaporator (Laborota, model 4001, Vertrieb, Germany), with vacuum control (Heidolph Instruments Gmbh, Vertrieb, Germany) and a thermostatic bath at 40 °C. The extracts were stored (−10 °C) in the dark until analysis.

2.2.2. Agitated bed extraction (ABE)

The extraction was carried out at 30 °C by placing jabuticaba skins into 125-cm^3 Erlenmeyer flasks containing ethanol (99.5%, Ecibra, Santo Amaro, Brazil) using a feed to solvent ratio of 1:10. Extractions were carried out in a shaker (model MA 420, Piracicaba, Brazil) with agitation (150 rpm) for 2 h. After extraction, the solvent was separated from the plant residue and evaporated, and the extract was stored as described before.

2.2.3. Combined UAE + ABE

Erlenmeyer flasks (125-cm^3) containing jabuticaba skins and ethanol 99.5% (Ecibra, Santo Amaro, Brazil) (feed to solvent ratio of 1:10) were sonicated for 10 min at a frequency of 40 kHz at room temperature; afterwards, the flasks were incubated in a rotary shaker (150 rpm) at 30 °C for 2 h. The solvent–plant residue separation, solvent evaporation and extract storage were done as described before.

2.2.4. Soxhlet extraction

Approximately 25 g of jabuticaba skins and 250 cm^3 of ethanol or acidified ethanol (acidified to pH 3 with HCl) (99.5% Ecibra, Santo Amaro, Brazil) were used (feed to solvent ratio of 1:10). The extraction was done in a soxhlet apparatus for 8 h, and after that the solvent was evaporated, and the extract was stored as described before.

2.3. Extract characterization

2.3.1. Antioxidant activity (AA)

The evaluation of antioxidant activity of the extracts was based on the coupled oxidation of β-carotene and linoleic acid. The technique developed by Marco (1968) consisted of measuring the bleaching of β-carotene resulting from oxidation by the degradation products of linoleic acid. One milligram of β-carotene (97%, Sigma–Aldrich, St. Louis, USA) was dissolved in 10 cm^3 of chloroform (99 %, Ecibra, Santo Amaro, Brazil). The absorbance was tested after adding 0.2 cm^3 of the solution to 5 cm^3 of chloroform, then reading the absorbance of this solution at 470 nm using a UV–Vis. spectrophotometer (Hitachi, model U-3010, Tokyo, Japan). A reading between 0.6 and 0.9 indicated a workable concentration of β-carotene. One milliliter of β-carotene chloroform solution was added to a flask that contained 20 mg of linoleic acid (99%, Sigma–Aldrich, St. Louis, USA) and 200 mg Tween 40 (99%, Sigma–Aldrich, St. Louis, USA). Chloroform was removed using a rotary evaporator (Laborota, model 4001, Vertrieb, Germany), with vacuum control (Heidolph Instruments Gmbh, Vertrieb, Germany) and a thermostatic bath at 40 °C; then 50 cm^3 of oxygenated distilled water (oxygenation for 30 min) was added to the flask with vigorous agitation to form an emulsion. Five milliliters of the emulsion was added to 0.2 cm^3 of the antioxidant solution (7.5 mg of extract/1 cm^3 of distilled water) in assay tubes. To the control solution, 0.2 cm^3 of pure distilled water was added. A blank consisting of 20 mg linoleic acid, 200 mg Tween 40 and 50 cm^3 oxygenated distilled water was used to bring the spectrophotometer to zero. Tubes were manually shaken, and absorbance measurements made at 470 nm immediately after the addition of the emulsion to the antioxidant solution. The tubes were placed in a water bath (model TE 159, Tecnal, Piracicaba, Brazil) at 50 °C. Absorbance measurements were made at 30 min intervals during 3 h. The average deviation of duplicated experiments never exceeded 8%, therefore, no additional statistical analysis was considered necessary. The antioxidant activities (AAs) were calculated by Eq. (1):

$$AA\ (\%) = 100 \times \left(1 - \frac{Abs^{t=0}_{extract} - Abs^{t}_{extract}}{Abs^{t=0}_{control} - Abs^{t}_{control}}\right) \quad (1)$$

2.3.2. Total phenolic compounds

Total phenolic content was estimated using the Folin–Ciocalteau method for total phenolics, based on a colorimetric oxidation/reduction reaction of phenols (Singleton et al., 1965). Briefly, 1 cm^3 of the sample was mixed with 1 cm^3 of Folin and Ciocalteu's phenol reagent. After 3 min, 1 cm^3 of saturated sodium carbonate solution (50% w/w) was added to the mixture, and the volume was adjusted to 10 cm^3 with distilled water. The reaction was kept in the dark for 90 min at room temperature, after which the absorbance was read at 725 nm with a UV–Vis. Spectrophotometer Hitachi, model U-3010 (Tokyo, Japan). For the control sample, 1 cm^3 of distilled water was taken. The results were calculated on the basis of the calibration curve of gallic acid (GA) and expressed as milligrams of gallic acid equivalents (GAEs)/g dry material. The average deviation of duplicated experiments never exceeded 8%; therefore, no additional statistical analysis was considered necessary.

2.3.3. Total monomeric anthocyanins (TMA)

The total monomeric anthocyanin (TMA) content was determined using the pH differential method described by Giusti and Wrolstad (2001), which relies on the structural transformation of the anthocyanin chromophore as a function of pH. A UV–Vis. spectrophotometer (Hitachi, model U-3010, Tokyo, Japan)