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Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (*Tamarindus indica*), sweet variety

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ABSTRACT

The objective of this study was to evaluate the antioxidant activity of tamarind seed extracts, sweet variety. The extracts were obtained by sub and supercritical CO_2 different concentrations of ethanol. The extractions with pure CO_2 were performed in the range of 20–50 °C and 100–250 bar and with ethanol under the conditions of 50 °C and 250 bar as well at 40 °C and ambient pressure. The yields of extractions with pure CO_2 of better quality and quantity of antioxidant were obtained under the conditions of 50 °C and 250 bar. However, the phenolic content of the tamarind seeds extract, sweet variety, obtained with pure ethanol at 50 °C and 250 bar was superior to all the others, including the synthetic antioxidant butyl hydroxytoluene (BHT). Therefore, the ethanolic extracts of tamarind seed, sweet variety, are potential sources of natural antioxidants for the food industry.

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1. Introduction

Tamarind (*Tamarindus indica*) is a fruit plant that belongs to the legume family, native to equatorial Africa, India and Southeast Asia and grows in tropical and subtropical regions, with ideal average temperature of 25° C. It is considered an ideal tree for semi-arid regions, tolerating 5–6 months in dry conditions but does not survive at low temperatures (Pereira et al., 2011).

There are different varieties of *Tamarindus indica* and they can be divided into acidic and sweet. Acidic varieties are commonly found in most countries, therefore easily develops into warm, sunny locations. The varieties of sweet type are not readily available. In Thailand, two types of Tamarind are found in abundance, so-called sweet and sour varieties (Sudjaroen et al., 2005).

The sweet variety is rarely found in Brazil, but in the interior of Bahia, in the city of Sento Sé, the Bebedouro farm has a planted area of “sweet” Tamarind, which is the name that was given to the fruit by not presenting the characteristic acidity of the common

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tamarind. There are few studies in the literature with sweet variety of *Tamarindus indica*.

Fruits, leaves and seeds are natural sources of antioxidants and several studies have bet on this alternative to replacing synthetic antioxidants (Ramalho and Jorge, 2006).

The extraction with supercritical or subcritical fluid has as one of its advantages the obtaining of solvent-free extract with high purity and, as a disadvantage, the high cost due to the use of materials and structure resistant to high pressures (Brunner, 1994).

Tsuda et al. (1994) identified four antioxidants in the seed coat of Indian tamarind: 2-hydroxy-3′,4′ dihydroxyacetophenome; methyl 3, 4-hidydroxybenzoate; 3,4-dihydrophenyl acetate and (−)-epicatechin.

Sudjaroen et al. (2005) identified the profile of polyphenolics in Tamarind pericarp, the extracts obtained by Soxhlet with methanol, was dominated by proanthocyanidins (73.4%) in various forms (+)-catechin (2.0%), procyanidin B2 (8.2%), ( )-epicatechin (9.4%), procyanidin trimer (11.3%), procyanidin tetramer (22.2%), procyanidin pentamer (11.6%), procyanidin hexamer (12.8%) along with taxifolin (7.4%), apigenin (2.0%), eriodictyol (6.9%), luteolin (5.0%) and naringenin (1.4%) of total phenols. The content of tamarind seeds comprised only procyanidins, represented (%) mainly by oligomeric procyanidin tetramer (30.2), procyanidin hexamer (23.8), procyanidin trimer (18.1), procyanidin pentamer (17.6) with lower amounts of procyanidin B2 (5.5) and (−) epicatechin (4.8).

Luengthanaphol et al. (2004) found only work on sweet tamarind variety supercritical fluid extractions performed on the range of 35–80 °C and 10–30 MPa and met -(−) epicatequinana (≈22.mu.g of (−)- epicatechin, per 100 g of the tamarind seed coat sweet Thai. The use of a co-solvent of 10% ethanol resulted in a much higher yield of (−)- epicatechin, (≈13 mg/100 g), under the best conditions found to be 40 °C and 10 MPa.

Mathematical modeling is crucial for predicting the behavior of the extraction process on an industrial scale, providing the data needed to build equipment and projection of industrial plants, contributing to the evaluation of the economic viability of this process (Mezzomo et al., 2009).

In Brazil, the extraction and characterization of the compounds present in the seed of tamarind are unprecedented, requiring further studies on the and the economic viability of this residue.

The objectives of this work were to obtain the *Tamarindus indica* seed extracts, sweet variety, with pure CO_2 and with ethanol in different concentrations in sub and supercritical conditions (temperatures of 20, 35 and 50 °C and pressures of 100, 175 and 250 bar), and evaluation of its antioxidant activity and fatty acid profile.

2. Materials and methods

2.1. Raw material

The raw material was collected in the city of Sento Sé – Bahia, Brazil, in the months of December 2011 and January 2012. The tamarind seeds, sweet variety, were manually removed from the pulp and stored in the refrigerator at 4 °C.

The seeds were dried at 40 °C for 24 h and stored in a transparent bottle coated with foil protecting it from light and heat. Seeds were ground in a knife mill and separated in a sieve shaker 24–48 mesh. The moisture content was determined by the method of direct drying at 105 °C, described by Institute Adolfo Lutz (Brazil, 2005).

Average particle diameter (ds) was determined by sifting 100.00 g of powdered seeds for 30 min and the mass retained in each sieve was used in the calculations, as described by Gomide (1983) in semi analytical balance (Quimis, model Q520-5200), accurate to two decimals, were weighed. The sieves were shaken for 30 min, and then weighed masses retained on each sieve for the calculation of average particle diameter as described by Gomide (1983); *Determination of aApparent specific mass (ρa)* was determined from the ratio of the sample mass used in extractor and the volume occupied by the fixed bed, including the pores of the bed and not the inner pores of the particles. *Real specific mass (ρr)*, was estimated using a gas He pycnometer (AccuPyc II 1340, Micrometrics, 2009). *Bed porosity (ε)* was determined from the ratio by the real and apparent specific mass of tamarind seeds sample, sweet variety, including the pores of the bed and the interior of the particles.

2.2. Supercritical extraction equipment

The supercritical extraction equipment consists of a CO_2 cylinder with 99.9% purity (Praxair, Brazil), a heating tape (Teledyne Isco, model 500 D, syringe pump) a liquid pump Alliance HPLC Lab Series III, stainless steel extractor and two thermostatic baths. The first bath (Julabo, model F32), used to cool the solvent prior to entering the syringe pump and the second bath (Quimis, scientific devices Ltda., Model Q214-M2) to maintain the desired temperatures in the heated extractor.

2.3. Preliminary supercritical extraction

The preliminary extractions with wet and dry samples were held in intermediate conditions of temperature and pressure, 35 °C and 175 bar, and subsequently used in the yield tests for 4 h.

The yields of extracts obtained from the use of wet and dry samples determined the choice of which sample would be used for subsequent tests.

2.4. Kinetic experiment – preliminary extraction curve and kinetic parameters

Kinetic curve was constructed by collecting the solute at time intervals of 15–30 min at 250 bar and 50 °C, maximum conditions subsequently used in the experimental design, for approximately 7 h with a flow rate of 2 mL/min, to reach the diffusion period (DCP) of the extraction curve. From the analysis of the kinetic curve, the extraction time for global yield extraction was determined.

The extraction curves were plotted using the software Microsoft Excel Home and Business 2010, version 14.0.4760.1000, for yield evaluation, being the mass of accumulated extract in the function of extraction time.

2.5. Determination of apparent solubility

Solubility is a required parameter for mathematical modeling in Sovová (1994) and Sovová modified by Martínez and Martínez, (2008) models. This was called preliminary because it is not the main objective of the work. This apparent solubility was obtained by the dynamic method of extraction, where solubility is represented by the slope of the extraction curves, in the stage of constant rate (CER).

According to Danielski (2002), the time of contact between the phases needed to achieve balance is between 0.9 g/min to 1.4 g/min for the oleoresins solubility in supercritical CO_2. Thus, the flow rate determined for this experiment was 1.1 ± 0.2 g/mL, promoting the saturation of the solvent with the oil at the outlet of the extractor.

The extraction kinetic curve was used for determination of the apparent solubility in this experiment.

2.6. Obtaining extracts

The extracts were obtained by Soxhlet, as described by the

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