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Short communication

Extraction, chemical characterization and antioxidant activity of andiroba seeds oil obtained from pressurized *n*-butane

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ABSTRACT

In this work, andiroba oil extraction (*Carapa guianensis*) was investigated using pressurized *n*-butane as solvent at 25, 35 and 45 °C and pressures of 7, 10 and 13 bar. Andiroba oil yield as high as 18 wt% was obtained at the lowest temperature and pressure (25 °C and 7 bar) for an extraction period of 45 min. Regarding chemical composition, the major components observed in the extracts were palmitic and oleic acids, while for the phenolic compounds values between 11.2 and 35.7 (mg EAG/100 g) were experimentally observed. For the antioxidant activity, DPPH radical scavenging method employed for the extraction conditions of 25 °C and 13 bar and 45 °C/7 bar provided inhibition percentages of 22.2% and 17.4%, respectively. Extraction with *n*-butane thus showed to be a promising alternative technique to conventional extraction methods, as very mild operating conditions and eco-friendly solvent can be used to provide good results without any residues in the final product.

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1. Introduction

Carapa guianensis is a tree belonging to the Meliaceae family, and is popularly known as andiroba. Its occurrence is preferably in wetland forests and such species usually produces 180–200 kg of seeds per year, containing approximately 60 wt% in oil (Embrapa, 2004). The oil extracted from the seeds has a transparent light yellow color, solidifies at temperatures below 25 °C, has a bitter taste and reaches rancidity rapidly after extraction. Andiroba is widely used in folk medicine as antirheumatic, analgesic, anti-inflammatory and healing agent. It is also used in the manufacture of candles as insect repellent, soaps and cosmetics (Baldissera et al., 2013; Senhorini et al., 2012).

The major unsaturated fatty acids present in andorba oil triglycerides are palmitic acid and oleic acid and glycerin at minor content. The seeds contain lipids, fibers, minerals and fatty acids, and chemical analyses have identified anti-inflammatory, healing and insecticide agents, which are mainly attributed to the presence of limonoids (Qi et al., 2004).

In the conventional oil extraction process, the most commonly employed solvent is a mixture of isomers of n-hexane, which comes from petroleum distillation. The process involves extraction and subsequent purification steps, which can have significant impact on the oil quality (smell, taste and also color), functionality (composition of fatty acids, vitamins and antioxidants) and processing costs (extraction yield, equipment and energy cost) of the final product (Hammond et al., 2005).

Numerous solvents have been proposed to replace n-hexane in the extraction of vegetable oils from plant matrices due to a growing environmental concern as well as searching for safer processes. Based on the traditional extraction techniques there is a great interest in using pressurized n-butane or highly volatile non-polar solvents, as an alternative to conventional solvents for removal of organic bioactive compounds from various herbaceous matrices. When gases at ambient conditions such as propane and n-butane are liquefied, their physical and chemical properties become optimum and they can be considered non-toxic solvents (Yang et al., 2004).

In fact, the extraction with compressed fluids may be attractive due to the possibility of operation at mild temperatures, change in the process selectivity by tuning operating pressure, hence changing solvent power, and also easy solvent recovery from the mixture with essentially no solvent residue (Ribeiro et al., 2006, 2008; Wu

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and Lee, 1999; Ochoa et al., 2001; Zosel, 1982; Benado, 1991; Walters and Dodds, 1999; Franke, 2001). Another advantage is the operation at ambient or below-ambient temperatures, which minimizes thermal degradation of proteins, antioxidants and other nutritionally valuable compounds (Herrero et al., 2013). Moreover, the possibility of solvent recovery with negligible system pressure depressurization, thus minimizing recompression solvent costs, through the use of membranes (Tres et al., 2009, 2014a,b) is also a benefit.

Though carbon dioxide appears to be the elected alternative solvent, n-butane can also be a good choice because its critical pressures are relatively low compared to that of carbon dioxide resulting in a stronger interaction with the non-polar compounds (Mohamed and Mansoori, 2002; Illés et al., 2000; Ndiaye et al., 2006). When compared to carbon dioxide, n-butane presents a similar dielectric constant (solvent polarity), which is around 1.8 and 1.6 for carbon dioxide (Weast et al., 1988). Besides, *n*-butane is plenty available, cheaper and it can be used in much lower pressures compared to carbon dioxide. Besides the mild temperature and pressure operating conditions, the use of short-chain hydrocarbons, like propane and n-butane, allows reduction of extraction time, while improving the quality of the oil obtained. However, literature is scarce on the vegetable matrices extraction using pressurized n-butane, though some tests have been reported and proved profitable and effective (Tres et al., 2014a,b) and some results have been reported with the use of compressed propane (Silva et al., 2015a,b; Santos et al., 2015; Zanqui et al., 2015a,b; Jesus et al., 2013; Corso et al., 2010; Pederssetti et al., 2011; Nimet et al., 2011; Freitas et al., 2008).

The wide availability of raw materials for the production of vegetable oils and the oil seed biodiversity makes the researches in the area attractive, especially with regard to the process optimization, obtaining a product with exclusive quality and with benefits. Obtaining andiroba oil through pressurized *n*-butane as solvent was evaluated as to the chemical composition of the oil obtained, phenolic compounds and antioxidant activity.

2. Experimental

2.1. Materials

Andiroba seeds were purchased from Amazon Oil Industry Company (Ananindeua, Pará, Brazil). Seeds were dried at 40 °C for 24 h and then grinded and classified into the Tyler standard sieve size range of 9–42 mesh. The comminuted particles were all placed into glass flasks under nitrogen atmosphere with external aluminum coating to keep the chemical properties of the seeds prior to extraction. For the extraction experiments, n-butane was the solvent investigated (White Martins Industrial Gases Ltda., 99.5% of purity).

2.2. Pressurized fluid extraction

The experimental unit consisted basically of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 130 cm^3 jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of 0.12 bar, a collector vessel with a glass tube, and a cold trap (Rodrigues et al., 2004; Jacques et al., 2007).

Amounts of around 30 g of dry finely comminuted seeds were charged into the extraction vessel. The solvent was pumped at a constant flow rate of 2 mL/min into the bed, which was supported by two 300 mesh wire disks at both ends. The solvent was kept in contact with the herbaceous matrix for at least 30 min to allow system stabilization. Then, the extract was collected by opening the micrometer valve, and the solvent mass flow was accounted for by the pump recordings. Thereafter, the mass of the extracted oil was weighed, and the glass tube was reconnected to the equipment. This procedure was performed until no significant mass was extracted or, as in some cases, the extraction period exceeded a pre-established limit.

Such experimental conditions were selected after performing a full 2^2 factorial experimental design, considering as the main variables the extraction temperature in the range of 25–45 °C and the extraction pressure from 7 to 13 bar. Solvent density was estimated using the HBT (P–V–T) correlation for compressed liquids (Reid et al., 1987) or taken from experimental literature values (Sage et al., 1942), making possible to estimate the mass of solvent charged into the extraction vessel.

The experiments were accomplished isothermally at constant pressure. A whole experimental run lasted in general 6 h, including all steps involved: sample weighing, temperature stabilization (baths, extractor), extraction, and depressurization. Duplicate runs were performed for all conditions, leading to an overall relative standard deviation of the yields of about 0.2%.

2.3. Chromatographic analysis

The chemical composition of andiroba oil was determined using a GC–MS (Gas Chromatograph QP5050A Shimadzu) equipped with a capillary column (30 m × 0.25 mm–0.25 μm film). The carrier gas was helium (Praxair Industrial Gases, 99.9% purity) with a constant flow rate of 1.1 mL/min. The column was heated to 40 °C for 3 min, heated to 200 °C at 4 °C/min, programmed at 5 °C/min up to 220 °C and maintained at 220 °C for 5 min. The extract was diluted in n-hexane and injected at 250 °C. The identification of major compounds was based on mass spectrum of the substance compared with the database of the GC–MS system (Standard Reference Data Series of the National Institute of Standards and Technology–98 NIST MS library) and the relative amounts of individual components were calculated based on the GC peak area.

2.4. Determination of total phenolic compounds

The total phenolic content was determined by the Folin-Ciocaulteu method based on the method described by Shahidi and Naczk (1995). Triplicate extract solutions protected from light were prepared at concentration of 0.5 mg/mL by adding water and the Folin-Ciocalteu reagent after 3 min, together with 0.2 mL of a saturated solution of sodium carbonate. After one hour, absorbance at 765 nm was read in a spectrophotometer. Quantitation was performed based on a gallic acid standard curve being the total phenolic compounds expressed as mg gallic acid equivalent (GAE) per 100 g of dry extract.

2.5. Determination of total antioxidant activity by DPPH free radical scavenging

The DPPH (2,2-diphenyl-1-picryl hydrazyl) assay was carried out according to the method of Brand-Williams et al. (1995). The DPPH solution was prepared by dissolving 24 mg DPPH in 100 mL chloroform and diluting it until the absorbance reached the value of 1.1 ± 0.02 units at 517 nm. The DPPH solution (1.9 mL) was mixed with 50 mL of chloroform and 50 mL of *C. xanthocarpa* extract, and incubated for 24 h at 25 °C. A control was prepared by adding 1.9 mL of DPPH solution and 100 mL of chloroform. The absorbance was measured at 517 nm against a blank (pure chloroform) in a spectrophotometer (Biochrom Libra S22). All results were expressed as an inhibition percentage, which was calculated as a DPPH radical scavenging. The concentration of antioxidant that causes a 50% decrease in the radical absorbance (IC_{50}) was estimated for the DPPH radical assay by non-linear regression analysis, using

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