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Assessment of subcritical propane, supercritical CO_2 and Soxhlet extraction of oil from sapucaia (*Lecythis pisonis*) nuts

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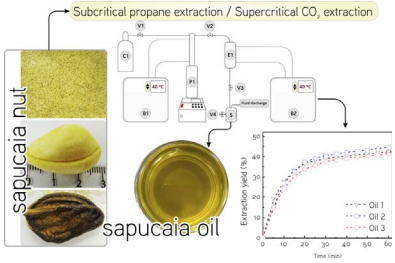
GRAPHICAL ABSTRACT



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ABSTRACT

The extraction of sapucaia (*Lecythis pisonis*) nut oil (SNO) using subcritical propane (SPE) and supercritical CO_2 (with ethanol as co-solvent; $scCO_2$) as solvent was investigated and compared with the conventional (Soxhlet) extraction. Extraction with $scCO_2$ was performed at 333 K and 20 MPa while the SPE extractions were carried out in different conditions to investigate the effects of temperature (293–333 K) and pressure (2–10 MPa) on the oil yield and the chemical compositions of the products. Results show that SPE allowed a fast extraction with a higher yield (46.22%) obtained at 333 K and 10 MPa, representing 93% efficiency compared to Soxhlet. Only temperature had significant ($p < 0.05$) effect on the extraction yield. SPE yielded the oils with highest values of polyunsaturated fatty acids (~36%). Stability to oxidation ranges from 6.53 to 11.17 h. The major triacylglycerols present in SNO are OOO, SOO, POO, PLO, and POS.

1. Introduction

An effective way to valorize tree species and prevent their extinction consists mainly of some reforestation techniques or the reduction of deforestation. Another solution is to find proper use for their principal products (fruits, leaves, seeds), aimed at the maintenance of a cycle that assures protection and survival for the species. Some Amazonian trees that were not adequately studied and products of which are not available in the market have no application in industry, being neglected because of the lack of the required research to stimulate their usage. In this context, *Lecythis pisonis*, a tree which is present in most regions of Brazil [1], is underutilized in terms of the use of its main product, the so-called "sapucaia" nuts. This edible nut presents a high lipid content (51–64%), predominantly linoleic acid [1,2] in a yellowish oil, presenting a characteristic flavor and is also well known for its similarity to Brazil nut (*Bertholletia excelsa*). Although some studies have been conducted with this raw material, its application and usability are still poorly appreciated, because of a lack of information regarding some

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other processes of extraction with minimum changes in its characteristics.

The use of conventional extraction processes that involve organic solvents for extraction is discouraged due to solvent residues and damage to some components, which are important in the final product, mainly due to the use of high temperatures [3]. On the other hand, new techniques which are as effective as the traditional ones and result in high quality products with no solvent remaining have been promoted and extensively stimulated, such as the use of pressurized fluids under sub- or supercritical condition [3–8]. A widely used technique, which is a so-called "green technology", is the use of carbon dioxide at supercritical condition ($scCO_2$; T = 304.25 K, P = 7.39 MPa) [6]. However, $scCO_2$ is less effective in extracting lipid portions, because of the low solubility of triacylglycerides [4,9], and the use of high-pressure or co-solvents (such as ethanol) being necessary to improve the extraction yields. On the other hand, compressed or subcritical propane (critical conditions: T = 369.82 K, P = 4.25 MPa) has been investigated and used as a solvent in which triglycerides and fatty acids show high solubility [8,9], providing higher extraction yields, the major benefit being the use of lower pressure and temperature which leads to a high quality product with minimum damage, no degradation of bioactive compounds, shorter extraction times and completely free of residue [4,5,7,10,11], thus presenting some advantages over $scCO_2$ [5].

Propane in compressed conditions has been successfully applied in the extraction of oils from a wide range of vegetable sources such as macaúba pulp [4], passion fruit seed [7], kiwi seed [8], palm [12], sesame seed [11], pequi [13], canola seed [14], red pepper seed [15], crambe seed [16], flaxseed [17], perilla [18], *Moringa oleifera* [19], Sacha inchi [20], *Araucaria angustifolia* [21], grape seed [22], and sunflower [23].

Publications in the literature report oil extraction from *L. pisonis* nuts by cold extraction [2], and also using organic solvent by Soxhlet [1,24,25] and Bligh & Dyer methods [25]. However, there are no data about oil extraction from sapucaia nuts using compressed propane and supercritical CO_2 processes. Thus, the aim of the study was to obtain sapucaia nut oil using subcritical propane and supercritical carbon dioxide (with ethanol as co-solvent) techniques and investigate the effects of temperature and pressure in the oil extractions. The Soxhlet technique using *n*-hexane as solvent was applied to obtain an oil that was used for comparison purposes. In addition, the oil samples obtained (from different process and techniques) were analyzed for their fatty acid composition, oxidative stability, crystallization and melting behavior, and triacylglycerol composition.

2. Material and methods

2.1. Sapucaia (Lecythis pisonis) nut samples

Sapucaia nut samples were harvested in a small crop area located in Araguanã, State of Maranhão (Brazil). The nuts were dried in an air circulating oven at 313.15 K for 24 h to remove moisture. The shells were then removed using a stainless-steel nut cracker. Peeled nuts were packed in plastic bags, crushed with a small hammer, and passed through a Tyler sieve system composed of sieves with mash numbers 8, 12 and 24. The nut pieces that passed through sieve #8 and were held in the sieve # 12 were used in the extractions, as shown in the schematic Fig. 1.

2.2. Sapucaia nut oil extraction

The oil samples utilized in this study were obtained by three different methods: subcritical propane extraction (LPP1 to LPP5), supercritical CO_2 using ethanol (1:1, w/w) as co-solvent (LPC), and Soxhlet using *n*-hexane (LPS). The oil extracted by Soxhlet was mainly used as control. All the extraction conditions are summarized in Table 1.

2.2.1. Classical extraction

The oil from sapucaia nut samples was extracted using a Soxhlet apparatus (Vidrolabor®, Labor Quimi, Brazil) for 6 h, using *n*-hexane as solvent [26]. In order to obtain enough oil samples for all the analysis, ~25 g of raw material was used in the extraction. Solvent was removed at 316.15 K under reduced pressure using a rotary evaporator (Model 801, Fisatom Ltda., Brazil). The sample was flushed with gaseous N_2 before storage. The oil was kept in an amber glass vessel and stored at 277.15 K until further analysis.

2.2.2. Sub- and supercritical fluid extraction procedures

The propane and CO_2 used in this work were purchased from White Martins S.A. (99.5% purity in liquid phase). The extractions were performed in a bench-scale unit (represented by Fig. 2), described in detail in previous works by our research group [5,8]. Briefly, the experimental setup consists of jacketed-vessel (0.08 m^3 inner volume, L = 0.16 mm and $\Phi = 2.52 \times 10^{-2}$ m) coupled to a thermostatized bath, a micrometering needle valve to control the flow inside the extractor, a syringe-type pump (ISCO, model 500D, Lincoln, NE 68504, USA), and pressure and temperature sensors and transducers. Supercritical CO_2 extraction condition was stablished according to previous tests performed in our laboratory by using 1:1 (w/w) ethanol as co-solvent by directly immersing the sample into the alcohol just before confinement in the extractor.

Extraction using subcritical propane was performed based on a simple 2^2 factorial experimental design with a center point (Table 1), aiming to evaluate the effects of pressure and temperature on the extraction yield. The solvent was pumped at a constant flow rate of 2.0 ± 0.2 $cm^3\ min^{-1}$ for both fluids used.

The oil was collected in an amber glass vessel and its weight was determined at time intervals of 5 min of extraction. The yields were calculated as the ratio of the extracted oil mass to the initial sapucaia nut weight. The analysis of the experimental data, at 95% confidence level, was carried out using the Statistica 10.0 software program (Statsoft™, Inc.).

2.3. Fatty acid composition

The fatty acid composition of the different sapucaia oils was determined using GC. Oil samples were directly methylated using methanolic sodium methoxide as described by Christie (1982) [27] and then injected (1-μL aliquot) into a capillary BPX70 column, 60 m × 0.22 mm internal diameter with 0.25 μm film thickness (SGE Inc., Austin, TX, USA). An Agilent 6890-series Gas Chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) with 7683-series auto-sampler was used to house the column. The oven temperature was programmed to increase from 110 to 230 °C at a rate of 4 °C/min and maintained at 230 °C for 18 min. The injector and detector temperatures were 250 and 255 °C, respectively. Helium was used as the carrier gas at an average velocity of 25 cm/s. Fatty acid composition was expressed as the percentage of the total peak area of all the fatty acids in the oil sample.

2.4. Triacylglycerol composition of sapucaia oils

The triacylglycerol (TAG) composition of the sapucaia nut oils was evaluated by using a high performance liquid chromatography (HPLC) system. About 30 mg of oil sample was placed in a 2 mL HPLC vial. The sample was dissolved by adding 600 μL chloroform and 1 mL 60:40 HPLC-grade acetone:acetonitrile solution. TAG composition of sapucaia oil was obtained by performing the chromatographic analyses with Waters Alliance model 2690 HPLC with a refractive index detector Waters model 2410 (Waters, Milford, MA, USA). A Waters xbridge C18 column with 4.6 mm × 250 mm internal diameter with 5 μm particle size was used to achieve the chromatographic separation of the compounds in the sapucaia oil. Instrument settings were as follows: