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Compressed n-propane extraction of lipids and bioactive compounds from Perilla (*Perilla frutescens*)

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ABSTRACT

An experimental 2^2 design with center point in three replicates was used to investigate the influential factors, such as pressure and temperature, in the extraction of oil from *Perilla frutescens* using compressed n-propane as a solvent, and to compare with the classical Soxhlet method. The experiments were performed in the temperature range of 40–80 °C and pressure of 8–16 MPa at a constant flow rate of 1.0 cm^3/min n-propane. All the conditions applied in the extraction using compressed n-propane provided good results and similar yields. However, the highest yield of extraction was obtained by the Soxhlet classical methodology. Moreover, higher induction times and concentrations of tocopherol and phytosterol were determined in oils resulting from compressed n-propane extraction. The Sovová mathematical model indicated a good fit for all conditions investigated.

1. Introduction

Perilla (*Perilla frutescens* (L.) Britton) is a native plant from Asia that belongs to the Lamiaceae family and is widely used in cooking and traditional medicine [1]. The grains contain about 51% of total lipids and, of this total, approximately 60% corresponds to alpha-linolenic acid [2]. Furthermore, perilla oil has a high content of phytosterols and tocopherols [3,4]. The use of these compounds is associated with reduction of inflammatory, cardiovascular, and cancerous diseases, and the reduction of low-density lipoprotein in the blood [5,6]. Currently, the search for natural sources of these bioactive compounds has attracted a growing interest in the development of products with high aggregate value, which requires high-quality products and low cost [7].

The classical methods of vegetable oil extraction involve the pressing of grains and the use of organic solvents, such as ether or n-hexane. However, the pressing results in low yields and the use of organic solvents has the disadvantages of long extraction times, toxicity of the solvents, and possible thermal degradation of thermolabile compounds [8]. In this context, alternative technologies are required to replace these methodologies, such as supercritical fluid extraction or compressed gas. These processes are advantageous due to not generating toxic residues, facilitating solvent removal, operating in a condition of relatively low temperature, and offering the possibility of controlling the properties of selectivity and solvation by adjusting the temperature and pressure [9].

Carbon dioxide (CO_2) is the most used solvent in this extraction process, however n-propane is relatively low-cost, it is not toxic and it has higher solvating power comparing to CO_2, which results in reduction of the solvent consumption, higher efficiency and shorter extraction time [10,11]. Hegel et al. [12] proposed the application of the mixture of solvents, CO_2 + n-propane, in the extraction of vegetable oil and found that increasing CO_2 concentration in the mixture is less effective than the application of pure n-propane.

This methodology has been widely used in the extraction of various vegetable oils, including chia seeds [13], soybean [9], candeia

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m_F	mass flow rate of the solvent (g/min)
S	solubility of oil in the solvent (g oil/g solvent)
t	extraction time (min)
q_0	initial oil concentration in the solid matrix (g oil/g solid)
m_s	solid mass on an oil-free basis (g)
K_{Fa}	solvent-phase mass transfer coefficient (min)
K_{Sa}	solid-phase mass transfer coefficient (min)
r	easily accessible oil mass
t_{CER}	time of start of the extraction of the oil from the inside of particles (min)
t_{FER}	time of the end of the extraction of easily accessible solute (min)
Z and W	dimensionless parameter of Sovová model
t_0	induction time
$m_{oil,j}^{calc}$	calculated mass of the oil extracted (g)
$m_{oil,j}^{exp}$	mass of oil experimentally obtained (g)
n_i	number of experimental data points for the test i

Greek letters

ρ_{bed}	bed density (g/cm^3)
ρ_F	solvent density (g/cm^3)
ρ_a	apparent density
ρ_t	true density (g/cm^3)
ε	bed porosity

[11], palm [14] and amaranth [15]. Studies on the extraction of perilla oil with supercritical fluid and/or compressed gas are scarce. The mathematical modeling allows the determination of the process parameters and the estimation of its feasibility in large-scale, based on mass balancing equations that describe the mass transport phenomena and provide the kinetic extraction curves [16]. Studies have employed the Sovová model, to describe this process, and excellent results have been obtained [11,14,17].

The aim of this study was to evaluate the influence of temperature and pressure on the extraction of perilla oil using compressed n-propane as solvent in terms of total yield, concentration of tocopherol and phytosterol, and oxidative stability in comparison with the classical Soxhlet methodology. Furthermore, the Sovová model was applied to calculate mass transfers of the process and to describe the experimental kinetic curves.

2. Materials and methods

2.1. Sample preparation

Three batches of 5 kg of perilla grains were purchased in the local market of Maringa (PR, Brazil). The grains were ground in a wiley mill (Marconi, Model MA-580) to obtain a fine flour that was sieved through a 14-mesh Tyler-series sieve (W.S. Tyler, USA). The sample was homogenized and vacuum packed in polyethylene bags and frozen at −18 °C.

2.2. Proximal composition

Moisture, ash, and crude protein contents were determined as described by Cunniff [18]. The factor used to convert total nitrogen to crude protein was 6.25. Crude fiber was determined according to methodology of Instituto Adolfo Lutz [19], and other carbohydrates (C) were calculated by mass difference using Eq. (1):

$$C = 100 - (\%\,\text{ash} + \%\,\text{moisture} + \%\,\text{CP} + \%\,\text{TL} + \%\,\text{CF}) \quad (1)$$

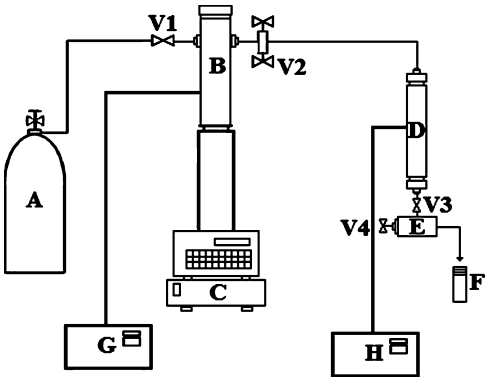

Fig. 1. Experimental apparatus used for compressed n-propane extraction: (A) n-propane reservoir; (B) syringe-type pump; (C) pump control; (D) extraction column; (E) thermoregulator; (F) glass tubes; (G) e (H) thermostatic baths; (V1, V2, V3) needle valve e (V4) expansion micrometric valve.

where C, other carbohydrates; CP, crude protein; TL, total lipids, determined by classical Soxhlet methodology; and CF, crude fiber.

2.3. Classical lipid extraction methodology

A total of 4.0 g of grain-milled and sieved perilla was used to determine the total lipid content in a Soxhlet extractor (Nova Etica, Brazil) using ethyl ether and petroleum ether (1:1 v/v) as solvents, for 16 h at boiling point according to the procedure described by Instituto Adolf Lutz [19].

2.4. Extraction procedure for compressed n-propane

The extractions were performed in a laboratory scale unit, which consists of a solvent reservoir (n-propane, White Martins S.A., 99.5% purity), two thermostatic baths (Quimis, model Q214M2), a syringe pump (Teledyne Isco, model 500D), a stainless steel extractor vessel with an internal volume of 53.4 cm^3 (2 cm diameter and 17 cm high) and a micrometric valve (Autoclave Engineers) attached to a thermoregulator (Tholz, modelo CTM-04E), as shown in Fig. 1.

Approximately 25.0 g of perilla sample were introduced into the extraction column. The n-propane flow rate was constant at 1.0 cm^3/min. An experimental 2^2 factorial design with center point in triplicate was used to evaluate the influence of factors, such as temperature and pressure. These factors were chosen according to reports in the literature that evaluated the extraction of lipids in oilseed using compressed n-propane [20–22]. The experiments were conducted with a temperature variation of 40–80 °C and pressure 8–16 MPa. The extracts were collected in glass tubes and were weighed at four initial cycles of 5 min and six cycles of 10 min. The total time of extraction was 80 min.

2.5. Analysis by scanning electron microscopy

The grains of perilla were evaluated before and after compressed n-propane extraction (CPE) in a scanning electron microscope (SEM; Shimadzu, model ss-550 SuperScan) to an accelerating voltage at 15.0 kV. The samples were pre-metallized to improve the conductivity of the material, consisting of depositing a film of gold in a metallizer (Shimadzu, Model HF-50 ion coater).

2.6. Simultaneous analysis of tocopherols and phytosterols

Phytosterols and tocopherols were simultaneously evaluated by gas chromatography (GC; Thermo-Finnigan, model Thermo Focus GC) coupled to mass spectrometry (MS; Thermo-Finnigan, model DSQ II) according to Du and Ahn [23]. The compounds