The Journal of Supercritical Fluids 130 (2017) 176–182

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Quality of perilla oil *(Perilla frutescens)* extracted with compressed CO_2 and LPG

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ARTICLE INFO

Keywords:
Perilla
Carbon dioxide
Liquefied petroleum gas
α-linolenic acid
Phytosterols

ABSTRACT

Perilla seed oil was extracted using compressed fluids, with varied temperature and pressure, in order to assess how they affect extraction yield, antioxidant activity, fatty acids, and phytosterols. Highest yields occurred for 25 MPa/20 °C with extraction using compressed CO_2 (31.80%) and for 0.5 MPa/20 °C with extraction using compressed LPG (42.29%). In this conditions, oil extracted with LPG presented antioxidant activity in DPPH higher than that extracted with CO_2 (50.20 AA% and 46.44 AA%, respectively). Fatty acid profile was similar for both fluids used, with predominant polyunsaturated fatty acids, mainly α-linolenic acid. Furthermore, bioactive compounds, such as squalene, α-tocopherol, and β-sitosterol, were quantified.

1. Introduction

Perilla *(Perilla frutescens),* is a native plant of Asian countries, used in cooking and in traditional medicine. It is, however, still unknown to Western populations [1]. Its seeds contain approximately 35–45% of oil, and are a good source of polyunsaturated fatty acids, especially α-linolenic acid [2]. In addition, its seeds have phytosterols and tocopherols. These components present in the perilla seed offer several health benefits, such as reduction of plasma lipid levels and prevention of cardiovascular diseases [3].

Conventional oil extraction processes use (cold or hot) organic solvents that can have a significant impact on oil characteristics, and on the composition of fatty acid, vitamins, and antioxidants [4]. Therefore, the method of using compressed fluids seems very attractive, especially due to the milder temperature conditions that cause less degradation of relevant compounds [5].

Although not having all the good qualities found for carbon dioxide (CO_2), fluids such as propane and *n*-butane, also evaporate after decompression at atmospheric pressure, eliminating the solvent purification step. It is possible to add that propane and *n*-butane also have greater solvation power when compared to CO_2, extracting higher diversity of substances present [6].

Liquefied petroleum gas (LPG), which contains *n*-butane and propane as its major components, as an interesting solvent. Recently, Scapin et al. [7] reported the use of compressed LPG in the extraction of chia seed oil, showing good results, as regards the extraction yield and the quality of the extract. It is also added that no papers were found in the literature reporting its use for the extraction of perilla seed oil. Thus, the aim of this research was to extract perilla seed oil using compressed CO_2 and LPG under different conditions of pressure and temperature, and avaluate the quality of the extracted oil through its antioxidant activity, physicochemical characterization, fatty acid profile, and phytosterols.

2. Materials and methods

2.1. Samples

Perilla seeds were purchased in the market in the city of Santa Maria, located in the state of Rio Grande do Sul, in Brazil. The seeds were dehydrated in an oven with forced air at 55 °C for 24 h, reaching 4.03 g/100 g of moisture content. In sequence, seeds were ground using an analytical mill cooled at 4 °C (Quimis, Q 298ª21, Brasil) whit an ultrathermostatized bath (Solab, SL-152/10) for 1 min, and placed in dark plastic packaging at −4 °C until later use, following the methodology of Scapin et al. [7].

2.2. Chemical composition of perilla seed

Moisture, ash, protein, and lipid contents were determined according to the AOAC methodology [8]. Fiber was determined by the enzymatic method (Method 985.28) and carbohydrates were found by

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http://dx.doi.org/10.1016/j.supflu.2017.08.007
Received 26 June 2017; Received in revised form 9 August 2017; Accepted 11 August 2017
Available online 18 August 2017

subtracting from the other fractions.

2.3. Perilla oil extraction

2.3.1. Soxhlet extraction

Perilla oil was extracted with *n*-hexane in Soxhlet at 80 °C for 8 h [9] to compare the yields of extraction using compressed fluids with conventional extraction.

2.3.2. Extraction using compressed fluids (CO_2 and LPG)

The experimental procedures were conducted in a system consisting of a solvent cylinder (CO_2 with 99.5% purity, of the brand *Air Liquide*, and LPG (a mixture of propane (50.3%), *n*-butane (28.4%), isobutane (13.7%), ethane (4.8%) and other minor constituents (methane, pentane, isopentane) of the brand *Ultragás*), a stainless steel extraction cell with an internal volume of 100 mL, a high pressure syringe pump (ISCO, 500D) and two thermostatic baths (Quimis, Q214M2).

The extractions were performed using 5 g of dried and milled samples and the solvent (CO_2 or LPG) was pumped into the bed and kept in contact with the sample for 1 h to allow the system to stabilize. The extract was then collected by opening the micrometric valve. The experiments were carried out at constant temperature and pressure. The flow of CO_2 and LPG was maintained at 4 g/min. For the experiment with CO_2, extraction lasted 150 min, and for LPG, 5 min. An experimental design with 2 levels and 2 variables was used (2^2) with triplicate at the central point to carry out the extractions, as shown in Table 1. We also determined the extraction curves from the accumulated extracted weight values as a function of time.

After the extractions, overall yield (X_0) of oil extracted was determined, resulting from the ratio of the total weight of extracted oil (m_e) and the initial weight (m_i) of perilla seed used for each extraction (Eq. (1)) and the oil recovery (R_0) was determined by the ratio of the total weight of extracted oil (m_e) and the weight of oil extracted through Soxhlet ($m_{soxhlet}$) (Eq. (2)).

$$X_0 = \left(\frac{me}{mi}\right)x100 \tag{1}$$

$$R_0 = \left(\frac{m_e}{m_{soxhlet}}\right)x100 \tag{2}$$

For determination of solvent density (ρ_F) in specifics condition of temperature and pressure was used for CO_2 the software Termopro, and for compressed LPG was made by using data for propane, *n*-butane, isobutene and ethane (major components) in acquired in NIST [10]. From these data, was calculate the specific volume for each condition using the fraction of each component in the mixture and the obtained density. Then it was just done the conversion of specific volume of the mixture for density.

2.4. Determining the antioxidant activity of perilla oils

Antioxidant activity was determined by the free radical sequestration method (DPPH· − 2,2-diphenyl-1-picrylhydrazyl) using methodology described by Dal Prá et al. [11], wherein the perilla seed oil samples were diluted in ethanol at a concentration of 20 $mg_{oil}/mL_{ethanol,}$ defined from previous tests. Then, 1500 µL of the diluted sample was added to 1480 µL of the 0.1 mM DPPH solution and 20 µL ethanol. In parallel, a blank was drawn for each sample containing 1500 µL of the diluted sample and 1500 µL of ethanol, and a blank for DPPH containing 1480 µL of DPPH and 1520 µL of ethanol. After 30 min of reaction under no light, the absorbances were measured in UV–vis spectrophotometer (UV-2600, Shimadzu) at 522 nm. The percentage of antioxidant activity against DPPH radical (Eq. (3)) was calculated, where A_{DPPH} is the absorbance of DPPH solution, A and A_B are the absorbances of sample and blank, respectively.

$$AA_{DPPH} = \left(\frac{A_{DPPH} - (A - A_B)}{A_{DPPH}}\right) \times 100 \tag{3}$$

Table 1
2^2 experimental planning with triplicate at the central point.

	CO_2		LPG	
Experiment	P (MPa)	T(°C)	P (MPa)	T(°C)
1	10 (−1)	20 (−1)	0.5 (−1)	20 (−1)
2	25 (+1)	20 (−1)	2.5 (+1)	20 (−1)
3	10 (−1)	60 (+1)	0.5 (−1)	40 (+1)
4	25 (+1)	60 (+1)	2.5 (+1)	40 (+1)
5	17.5 (0)	40 (0)	1.5 (0)	30 (0)
6	17.5 (0)	40 (0)	1.5 (0)	30 (0)
7	17.5 (0)	40 (0)	1.5 (0)	30 (0)

2.5. Perilla oil physical and chemical characterization

At points where higher yield occurred in extractions with compressed CO_2 and LPG, the following was determined: Acidity Index (Cd 3d-63); Wijs iodine index (Cd 1-25); Peroxide index (method Cd 8-53); Abbé refractometer index (Cc 7-25), in accordance with the AOCS methodology [12].

2.6. Fatty acids profile of perilla oils

As samples obtained with CO_2 and pressurized LPG (about 100 mg) were derivatized in fatty acids methyl esters (FAME) as described by Hartman and Lago [13]. FAME were analyzed according to Scapin et al. [7] method, using a gas chromatograph equipped with flame ionization detector (GC-FID; Varian, model Star 3400 CX, CA, USA) and an automatic sampler (Varian, model 8200, CA, USA). The results were expressed as a percentage of fatty acids by using an internal standard methyl tricosanoate (Sigma-Aldrich, St. Louis, USA) of the known mass and the correction factors of FID [14].

2.7. Determination of phytosterols in the perilla oils

The same analytical procedure for FAME preparation was used for phytosterols. The compounds α-tocopherol, β-sitosterol and squalene were analyzed according to Scapin et al. [7] method. The determination of their concentration were performed by external calibration using a gas chromatograph equipped with flame ionization detector (GC-FID – Varian – model Star 3400 CA, USA) and automatic sampler (Varian – model 8100 – CA, USA). The qualitative analyses were performed in a gas chromatograph coupled to a mass spectrometer (GC/MS; Shimadzu QP-2010-Plus, Tokyo, Japan).

2.8. Statistical analysis

The statistical analysis of the results was performed using STATISTICA® 8.0 (StatSoft, Inc., Tulsa, OK 74104, USA) software to evaluate the effects of the independent variables (pressure and temperature) on the dependent variables (extraction yield, antioxidant activity, profiles of fatty acids and phytosterols) in the process of extraction using CO_2 and LPG, considering a 95% confidence level for all the variables.

3. Results and discussion

3.1. Chemical composition of perilla seeds

Moisture content found in perilla seeds was 6.15 g/100 g, very similar to the figures found by Sargi et al. [15] where the values have