INFLUENCE OF TEMPERATURE ON THE SUPERCRITICAL EXTRACTION OF LINSEED OIL (*Linum usitatissimum* L.).

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This study investigated the effect of temperature on the extraction process yield of linseed oil obtained with supercritical CO_2 and its influence on α-linolenic acid (18:3n-3) concentration. The experiments were performed isobarically (at 250 bar) for three extraction temperatures (T = 50, 60 and 70°C) in a fixed bed extractor operating at a constant solvent flow of 1.5gCO_2/min. Experimental extraction time was fixed at 4h. The oil samples were collected at predetermined intervals (to obtain the kinetic extraction curves) and analyzed using gas chromatography coupled to mass spectrometry. The results showed no significant alteration in α-linolenic acid concentration for the temperature range tested. Process yield varied from 5.9 to 8.3%.

INTRODUCTION

Linseed (*Linum usitatissimum*) is an oleaginous seed widely used in industrial oil production. Industries that work with the extraction process of this oil currently use conventional techniques such as cold pressing or organic solvents to remove the extract contained in the seed. Although cold pressing is the most commonly used technique, it only partially recovers the oil present in the seeds and is generally followed by extraction with solvent (usually hexane) at relatively high temperatures in order to increase oil recovery [1].

In the case of linseed, whose oil is rich in essential fatty acids, mainly α-linolenic (18:3n-3), the use of high temperatures is not adequate, since 18:3n-3, isolated or as a component of the oil, is highly susceptible to heating [2]. It is therefore, essential to obtain it under low temperature conditions.

Supercritical technology can be used in these cases, given that this technique enables the use of lower extraction temperatures and has proven to be ecologically more attractive than conventional extraction methods. Supercritical fluid extraction (SFE) has been widely studied in some parts of the world and successfully used for extracting the oil of various seeds, such as canola, corn, sunflower and soybean [3,4]. The present study investigated the effects of operational temperature on the extraction process yield of linseed oil obtained with supercritical CO_2 and its influence on α-linolenic acid (18:3n-3) concentration.

MATERIALS AND METHODS

Raw Material Preparation

The samples of brown linseed (*Linum usitatissimum* L.) used in the experiments were obtained from 3 supermarkets in Natal (Brazil), homogenized, stored in vials and maintained below -20ºC until use. Moisture content of the samples was determined according to AOCS Method 2-54 [5]. To prepare the particle bed, the seeds were ground in a domestic multiprocessor for 20 seconds and separated in a Tyler sieve shaker. The granulometry of the samples was composed of a particle mixture with 30% of 24 mesh, 30% of 28 mesh, 20% of 32 mesh and 20% of 48 mesh. For each experiment, samples of approximately 140 g of linseed (capacity of the extractor column) were introduced into the extraction cell. The experiments were performed in duplicate for each experimental condition tested.

SC-CO_2 Extraction

For this extraction technique, the linseed sample is placed in an extractor column, forming a fixed bed of particles through which the supercritical CO_2 drains. Thus, the desired components (linseed oil) are transported from the solid phase (seed) to the supercritical fluid phase.

The extraction conditions were:

- Condition 1: T = 50ºC, P = 250 bar, mean flow of 1.5gCO_2/min.
- Condition 2: T = 60ºC, P = 250 bar, mean flow of 1.5gCO_2/min.
- Condition 3: T = 70ºC, P = 250 bar, mean flow of 1.5gCO_2/min

Extraction time was fixed at 4h for each experiment.

A schematic drawing of the extraction unit used in the experiments is shown in figure 1.

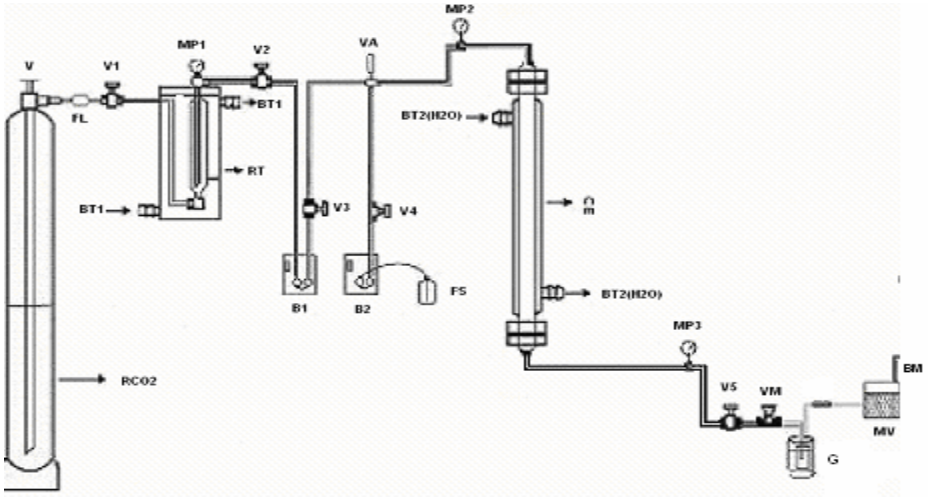

Fig. 1. Experimental unit

RCO2 = CO_2 containing a siphon with capacity of 23 kg (99.5% pure White Martins Gases Industriais); **FL** = Stainless steel line filter (HOKE); **RT =** Jacketed cylinder to keep the solvent

as a subcooled liquid with capacity of $0.4x10^{-3}$ m^3; **MP1 =** Bourdon gauge (Record, 004-99, with capacity of 100 ± 1 kgf/cm^2, Brazil); **MP2 and MP3 =** Gauges (ZÜRICH, Class A1, ABNT, 0-600 bar) **; BT1 and BT2 =** Thermostatic bath (TECNAL, model TE 184); **B1 and B2** = HPLC pumps (Constametric 3200, LDC Analytical, EUA); **FS**- Co-solvent vial; **CE =** Jacketed equilibrium cell (stainless steel extractor, length of 0.60 m, diameter of 0.0216m and wall thickness of 0.028 m); **VM =** Micrometric valve (AUTOCLAVE ENGINEERS, stainless steel 316 1/8" OD 15.000 psi REF. 10VRMM2812) with heating system (NOVUS, model N480D, Brazil); **G =** 5 mL glass vial, and glass recipient with ice cubes and water; **MV =** Flow totalizer (LAO, model G1, Brazil); **BM** = Glass bubble meter; **VA**- Relief valve (SWAGELOK); V1,V2,V3,V4,V5- Needle valves (AUTOCLAVE ENGINEERS, stainless steel 316, 15.000 psi REF. 10V2071).

The pilot extraction unit has a carbon gas reservoir (RCO_2) that supplies solvent to the system; a stainless steel extractor column (CE), where the sample is introduced, forming a fixed bed; a 400 cm^3 stainless steel jacketed tank (RT) cooled by a thermostat bath (BT1); pressure pumps (B1 and B2), valves (VA, V1, V2, V3, V4, V5 and VM), pressure gauges (MP1, MP2 and MP3) and flow gauges (MV and BM). The RT tank is responsible for cooling the HPLC pump heads and for maintaining the solvent (CO_2) in a liquid state. The operational temperature of the system is fixed in the BT2 thermostat bath that controls the temperature in the extractor column CE. The pressurization of the system is achieved by activating pumps B1 (pumping of CO_2) and B2 (pumping of co-solvent). The system allows for the nonuse of co-solvents by closing valve V4 and not using pump B2. Working pressure is monitored by gauges MP2 and MP3, located before and after the extractor column. Gauge MP1 measures the pressure in the CO_2 before system pressurization. Micrometric valve VM, responsible for fine adjustment of the outlet flow, is heated by a temperature heater-controller (NOVUS, model N480D) to avoid product freezing in the outlet line. Flow is monitored by two systems: a flow totalizer (MV) and a glass bubble meter (BM). The fractions of extracted oil were collected in 5mL glass vials (G) at previously established intervals, determined gravimetrically, combined in a single vial and stored at -20ºC for subsequent analysis.

Extract Analysis

The extracts were sterifed and the samples were prepared to obtain fatty acid methyl esters and subsequent chromatographic analyses.

Obtaining Fatty Acid Methyl Esters. Hartman and Lago's [6] method was used to prepare the fatty acid methyl esters. The methyl esters obtained were resuspended in 1 mL of hexane. This solution was injected into the chromatograph.

Gas Chromatography. The analyses were performed by capillary gas chromatography – CGC AGILENT 6850 SERIES GC SYSTEM. An AGILENT DB-23 capillary column (50% cyanopropyl-methylpolysiloxane, 60m x 0.25 mm x 0.25 μm) was used. The chromatographic conditions were the following: 1) Temperature gradient: initial temperature was 110°C for 5 min, and heat was increased by 5°C/min up to 215°C, remaining at this temperature for 24 minutes; 2) Vaporizer temperature: 250°C; 3) Detector temperature: 280°C; 4) Carrier gas: He (1.0 μL) with

flow rate of 1mL/min. The identification of fatty acids in the samples was compared to the spectra of fatty acid patterns determined under the same conditions.

RESULTS

Process yield

The yield values for each temperature condition tested in the supercritical CO_2 extraction assays varied from 5.9% (at 60°C) to 8.3% (50°C), as can be seen in Figure 2. At a temperature of 70°C, was found an oil recovery rate of 7.1%. The yield of each extraction was calculated from the ratio between the mass of oil obtained in each assay and the mass of linseed introduced into the extractor.

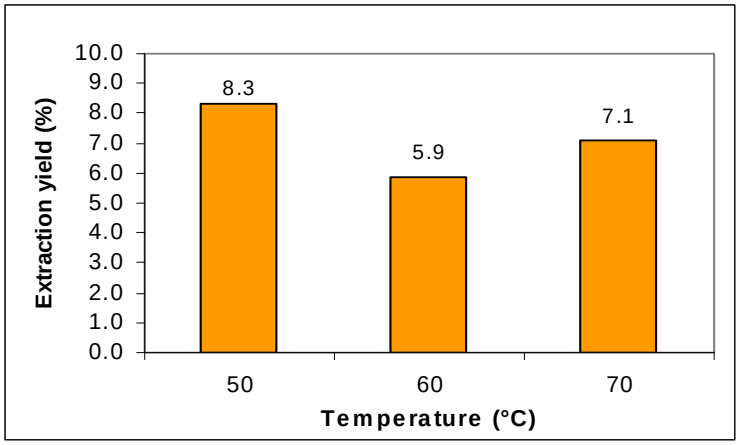

Figure 2. Influence of operational temperature on SFE process yield of brown linseed oil. (P = 250 bar, mean flow rate of 1.5gCO_2/min).

According to Figure 2, the highest brown linseed oil recovery was obtained at 50°C, with a yield of 8.3%. These results corroborate the values reported by Bozan and Temelli [1] for the solubility of golden linseed oil in supercritical CO_2. These researchers worked with temperatures of 50°C and 70°C at pressures of 210, 350 and 550 bar. They observed increased oil solubility as temperature increased for the high pressures of 350 and 550 bar and the occurrence of a crossover in the solubility isotherms for the working pressure of 210 bar. The present study, where a pressure of 250 bar was used, showed similar behavior for temperatures of 50°C and 70°C, that is, an increase in temperature caused a reduction in linseed oil recovery. However, at 60°C, where an intermediate yield value was expected, a lower oil recovery percentage (5.9%) was observed. Thus, based on these results, it is not possible definitely confirm that a reduced temperature results in increased linseed oil solubility. The temperature and pressure range used must be widened and the phase equilibrium behavior of the linseed oil/CO_2 system must also be investigated.

FA Composition

The main fatty acids found in the brown linseed oil were palmitic (6.42-6.57%), stearic (5.24-5.91%), oleic (21.96-22.09%), linoleic (13.75-13.91%) and α-linolenic (51.19-51.54%). Mean FA concentration values as a function of extraction temperature used in the experiments are shown in Table 2.

Table 2. Composition of brown linseed oil fatty acids (% m/m) obtained at different extraction temperatures.

Fatty acid	50°C	60°C	70°C
Palmitic (C16:0)	6.51	6.42	6.57
Stearic (C18:0)	5.91	5.27	5.24
Oleic (C18:1)	21.96	22.02	22.09
Linoleic (C18:2n-6)	13.86	13.75	13.91
Linolenic (C18:3n-3)	51.49	51.54	51.19
Unidentified	0.99	1.00	1.00

Table 2 shows that the percentage of each fatty acid found in linseed oil did not vary significantly with extraction temperature. Brown linseed oil had a mean value of 65.25% of polyunsaturated fatty acids (C18:2n-6 and C18:3n-3), 22.02% of unsaturated fatty acids (C18:1) and 11.73% of saturated fatty acids (C16:0 and C18:0).

Figure 3 shows the percentage of the main fatty acids found for each temperature condition tested (50°C, 60°C and 70°C) in the assays with supercritical CO_2, with prominence for the α-linolenic acid (C18:3n-3).

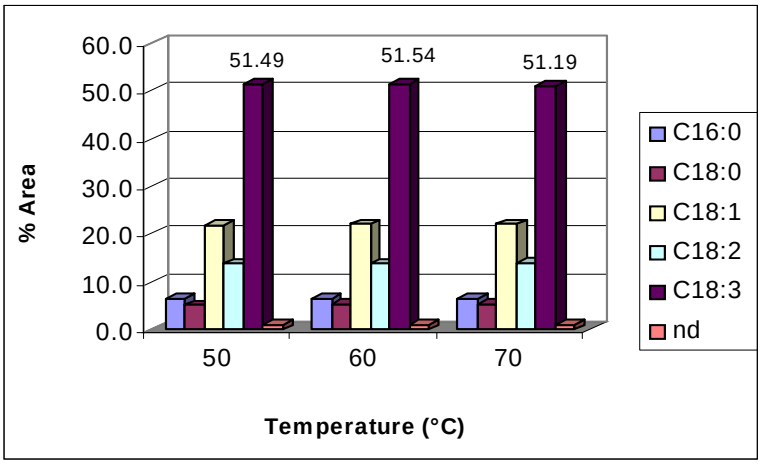

Figure 3. Influence of operational temperature on the recovery of the main fatty acids from brown linseed oil obtained by SFE.
(P = 250 bar, mean flow rate of 1.5gCO_2/min).

For the operational temperatures studied, no significant variation was found in the percentage recovery of α-linolenic acid, which for this oil was approximately 51%.

CONCLUSIONS

The temperature range used in the supercritical extraction of linseed oil resulted in yield percentages of 5.9%, 7.1% and 8.3% (at 60°C, 70°C and 50°C respectively) for a total extraction time of 4h at a pressure of 250 bar and mean solvent flow rate of 1.5gCO_2/ min. Although the highest linseed oil recovery occurred at 50°C, based only on these results, we cannot affirm that a reduction of operational temperature leads to an increase in linseed oil recovery. Therefore, the temperature range and pressures studies must be widened and the phase equilibrium behavior of the linseed oil/CO_2 system must also be investigated. As to oil composition, no significant differences were observed between FA concentration values as a function of the extraction temperature used in the experiments.

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