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Extraction of edible avocado oil using supercritical CO_2 and a CO_2/ethanol mixture as solvents

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ABSTRACT

Avocado oil is similar to olive oil, it can be used as an ingredient in functional foods since it contain high contents of vitamins and phytosterols and its triacylglycerols contain high contents of unsaturated fatty acid. The present study sought to evaluate the technical viability of extraction of avocado oil by supercritical technology using GRAS solvents (Generally Recognized as Safe). Freeze-dried avocado (*Persea Americana)* pulp with 65% lipids was subjected to supercritical extraction in a fixed bed at temperatures of 40, 60, and 80 °C and pressures of 200, 300 and 400 bar, using supercritical carbon dioxide ($scCO_2$) as a solvent in a first step, and a $scCO_2$/ethanol mixture (93/7 w/w) in a second step. Extraction curves (mass of oil extracted versus extraction time) were obtained from the overall extraction yields, and from this curve the solubility of oil in $scCO_2$ was calculated. The extraction appeared to be technically viable to obtain up to 98% oil recovery. The solubility values correlated well with the Chrastil equation, and presented similar order of magnitude to the solubility of oils reported in literature.

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1. Introduction

Avocado is one of the few fruits containing oil as a main component; it possesses high levels of unsaturated fatty acids in its triacylglycerol composition (about 70% of oleic acid) and substantial amounts of compounds with health benefits such as tocopherols, phytosterols, and lutein. The unsaponifiable fraction of avocado oil has regenerative properties for the skin (GUNSTONE, 2006).

Avocado oil is thermally stable, with high glycerol decomposition temperature (smoke point of 255 °C), which allows for its use in frying and baking. Because it is extracted from the fruit pulp, it is similar to olive oil with respect to its physicochemical properties, particularly the fatty acids composition of its triacylglycerols, which may serve as a condiment for fresh salads (Kurlaender, 2004; Berasategi et al., 2012; Canto et al., 1980). The ω-9 fatty acids appear to be the substances responsible for the beneficial effects on human health in preventing cardiovascular diseases (Rebollo et al., 1998). Avocado oil is easily absorbed by the skin, being used as a vehicle for medicinal substances and perfume absorption. It is of great value to the cosmetics industry due to the ease of emulsion formation and for soap manufacture (GUNSTONE, 2006).

Although avocado oil extraction with organic solvents presents high extraction yield, the extraction process can cause environmental impacts. Mechanical extraction by cold pressing or centrifugation procedures are clean technologies, however a low extraction yield is obtained (Kurlaender, 2004). Extraction with supercritical carbon dioxide may be a suitable alternative, since $scCO_2$ is a green solvent suitable for extracting low polarity compounds such as oils and fats, besides producing a product (oil) and byproduct (defatted cake) free of any traces of organic solvents, with high extraction yield.

Mostert et al. (2007) extracted avocado oil from the Fuerte variety using $scCO_2$ as a solvent at 37 °C and 350 atm, and they found overall yields of 62.9% and 66.7% (dry basis). Botha and McCrindle (2008) extracted 94% of the oil within 2 h at 350 atm. In a previous work (Barros et al., 2016), 88% of the oil was extracted at 50 °C and 400 bar from avocado pulp of the Hass variety, which corresponds to an overall yield of 57% from a pulp containing 68% lipids. This oil was used as a co-solvent of the $scCO_2$ for extraction of capsanthin from red bell pepper, and this was performed by simultaneous extraction of oil from avocado and capsanthin from red bell pepper. This process could extract 88% of oil from avocado and up to 50% of the capsanthin from red bell pepper, and still

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produce an avocado cake of commercial value, containing about 25% lipids.

CO_2 is a nonpolar molecule, thus the $scCO_2$ can extract nonpolar and low polarity substances, such as lipids. Co-solvents such as ethanol can also be used in different proportions to modify the polarity of the $scCO_2$/ethanol mixture, and therefore increase the solubility and improve extraction of other substances such as tocopherols. To evaluate the extraction efficiency in terms of yield and extraction time, extraction curves (mass of solvent versus extraction time) were obtained for the different temperature and pressure conditions, which are directly related to the solute concentration in the raw material and the solubility of the solute in the solvent. The extraction curves present three stages: 1) constant extraction rate (CER), characterized by a graphically straight line, which is most soluble fraction of the extract. In this stage, mass transfer by convection is predominant; 2) falling extraction rate (FER), in which failure begins to occur in the solute layers on the surface surrounding the particles or the number of broken cells is not uniform; both convection and diffusion are important at this stage; 3) diffusion controlled stage (DC), in which the surface adsorption sites are exhausted. The extraction process is controlled by diffusion of the solvent inside the particles and diffusion of the dissolved solute from within the particle to the particle surface. The use of a co-solvent together with $scCO_2$, for example a small amount of ethanol, may improve the efficiency of lipids extraction because it increases the solubility and facilitates the extraction of the less nonpolar substances. Scientific literature does not present extraction curves, solubility values and tocopherol contents for avocado oil in all ranges of temperature and pressure, nor extraction values using ethanol as a co-solvent.

In this context, with the aim of obtaining complete extraction of oil from avocado, a two-step process was proposed for extraction of the main compounds and the minor solubility lipidic compounds. This study seeks to evaluate the extraction of avocado oil using supercritical carbon dioxide as the solvent in a first step, followed by a second step using a CO_2/ethanol mixture. Furthermore, technical feasibility was evaluated based on the results of overall extraction yield, oil solubility and tocopherol levels.

2. Materials and methods

2.1. Raw material and its characterization

Ripe avocados from *Persea americana* species and commercial avocado oil (Jaguacy Brasil, Bauru/SP, Brazil) were acquired from local market (Campinas, SP, Brazil). The avocado pulp was separated and frozen at −80 °C in an Ultra-freezer (Glacier NU-9483 Ultra-Low Temperature Freezer, NuAire, Plymouth, MN USA) and subjected to freeze drying (Liobras L101, SP, Brazil). After freeze-drying, the pulp was ground in a domestic blender (Arno, Brazil). The particle size distribution was measured by the ASAE method (ASAE, 1997), using a series of Tyler sieves (Model 1868, Bertel, SP, Brazil), with sizes ranging from 8 to 48 mesh, which are too wide, but because the pulp has a high oil content agglomerate formation is possible, so these sieves were used to avoid this problem. Total volatiles were determined by the gravimetric method (AOAC, 1997), in which a 2.0 g sample was dried in an oven (Marconi, MA-030/12, SP, Brazil) at 105 °C under 600 mm Hg vacuum (Marconi pump, MA-057-13, SP, Brazil) until reaching constant weight. Moisture content was determined by the Karl-Fisher method (KF Metrohm 701 Titrino) (AOCS, 1998), consisting of an oven (832 KF Thermoprep) using a sample of approximately 0.4 g at 105 °C for 30 min, and nitrogen flow rate of 50.0 mL/min (1.01 bar and 0 °C).

Total lipids were determined by the Soxhlet method (AOCS, 1997) in triplicate, using hexane as the solvent (150 mL) (Synth, Brazil, lot 116071) and about 5.0 g of the crushed freeze-dried sample. The solvent was removed from the extracts by evaporation in a rotaevaporator (Marconi, MA-058, Brazil) at 42 °C under vacuum of 600 mmHg in vacuum oven (oven MA 030-12, and pump MA-057-13, Brazil) until reaching constant weight (variation less than or equal to 3.0 mg).

2.2. Reagents

Carbon dioxide 99.5% w/w (White Martins Gases Industrials, Brazil, lot 062 C/13), hexane 98.5% w/w (Synth, Brazil, lot 116071), and ethanol 99.8% w/w (Êxodo, Brazil, lot AE10296RA) were used as solvents in the different extraction steps.

2.3. Extraction procedures

2.3.1. Extraction curves

Avocado samples were subjected to two-step extraction in a fixed bed extractor, the first step using $scCO_2$ and the second step using a mixture of ethanol and $scCO_2$, whose methodology has been discussed in detail elsewhere (Bitencourt et al., 2014; Paula et al., 2013; Garmus et al., 2015).

The bed was packaged with 5.0 g of the sample spread horizontally, where the solvent flowed through bed and the extract was collected at predetermined intervals. The first step lasted about 3 h (9 samples), in which the $scCO_2$ flow rate was 2.5 g/min, while the second step lasted 1 ½ hours (6 samples), in which the first solvent (at the same flow rate) was mixed with ethanol at a flow rate of approximately 0.20 g/min, resulting in a CO_2/ethanol ratio of 93/7 w/w. The pressure and temperature conditions were 200, 300, and 400 bar, and 40, 60, and 80 °C. All extractions were performed in triplicate.

2.3.2. Determination of the global extraction yield

The global extraction yield was used to compare the different extraction steps and different temperature and pressure conditions, obtained by the ratio between the extract mass at the end of the process and the mass of the matrix subjected to extraction. Considering that the experiments were carried out in triplicate, the overall yield was a result of the arithmetic mean of the experimental values.

$$\text{Overall Yield } (\%) = \frac{\textit{mass} \text{ of oil}}{\text{mass of raw material}} \times 100\% = f(T, P) \tag{1}$$

2.3.3. Calculation of solubility

After obtaining the extraction curves and considering the non-extractable solid portion of the matrix as inert, the solubility of avocado oil in $scCO_2$ as a function of the temperature and pressure was obtained from the slope of the extraction curves for the constant extraction rate, as illustrated in Fig. 1. It considered the (mass of oil) *versus* (mass of solvent), calculated from the quotient between the mass of oil sampled and the total mass of carbon dioxide used, according to Eq. (2).

$$\overline{Y} = \frac{\textit{mass} \text{ of oil}}{\text{mass of } CO_2} = f(T, P) \tag{2}$$

2.4. Correlation of oil solubility in $scCO_2$

The values for solubility as a function of temperature and

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