

Supercritical carbon dioxide extraction of oil and minor lipid compounds of cake byproduct from Brazil nut (*Bertholletia excelsa*) beverage production

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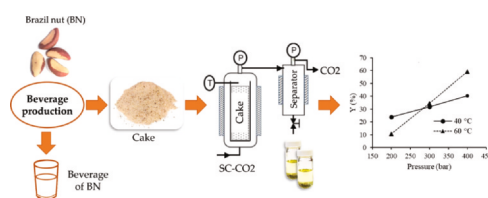
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HIGHLIGHTS

- SC-CO₂ can recover oil from the by-product of Brazil nut beverage production.
- At 400 bar and 60 °C the oil recovery was c.a. 100%.
- Extraction rate was greatly dominated by oil solubility in SC-CO₂.
- High concentration of squalene and β -sitosterol were obtained at 200 bar and 60 °C.

GRAPHICAL ABSTRACT



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ABSTRACT

Supercritical carbon dioxide extraction (SC-CO₂) was performed to recover the lipid fraction of the cake by-product from Brazil nut beverage (BNM) production. Extraction kinetics and the effect of pressure and temperature on the yield, oil recovery, fatty acid (FA) profile and minor lipid compounds were evaluated. The modeling of the kinetic assays indicated that most of the oil is readily accessible and the extraction velocity is dominated by the oil solubility. High yield with c.a. 100% oil recovery was achieved at 400 bar and 60 °C. While the FA profile of extracts did not present relevant variations with the extraction conditions, higher content of squalene and β -sitosterol (590.8 and 283.9 mg/g extract) were obtained at the lowest pressure and highest temperature assessed (200 bar and 60 °C). Therefore, SC-CO₂ extraction is a suitable technique to recover the oil and other minor lipid bioactive compounds of the cake by-product from BNM production, with a potential application in foods, cosmetics or nutraceuticals.

1. Introduction

Brazil nut (BN) is the fruit of *Bertholletia excelsa* HBK nut tree, which has received increasing interest in the last decade for the potential positive effects on human health. The consumption of BN is related with the reduction of risk factors for cardiovascular disease [1–3], cancer prevention [4] and the improvement of cognitive functions [5]. The BN

biological properties, for example the antioxidant and anti-inflammatory activities, have been attributed to its nutritional characteristics and bioactive compounds, such as a high level of unsaturated fatty acids, biological valued proteins, dietary fiber, minerals, phenolic compounds, tocopherols, phytosterols and squalene [6–9].

In this sense, several food products from BN have been developed and studied in recent years with special interest, being one of them a

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beverage popularly known as “Brazil nut milk” (BNM). This product is a plant-based beverage obtained from BN processing, i.e. BN milling and extraction with water, followed by the separation by filtration or centrifugation of the aqueous extract, known as the “milk”, which is then homogenized, standardized and packaged. Most of the work done on BNM is focused on improving the technological processes and/or product quality [10–13] with little attention to the main by-product generated, the “cake” resulted after the separation of the milk. In the recent study carried out by De Oliveira Sartori et al. [14], partially defatted BN by pressing was used to produce BNM, obtaining a cake with important levels of macronutrients (50% lipids, 25% proteins and 6% minerals). Particularly, the fatty acid profile reported for the lipid fraction of the cake was rather similar to that of BN, with a predominance of unsaturated fatty acids (39% linoleic acid and 32% oleic acid). This suggests the opportunity for studying the extraction of oil and phytochemical compounds from the cake of BNM and its potential use.

Supercritical fluid extraction with carbon dioxide (SC-CO₂) is an alternative technique to conventional oil extraction methods, which also allows fractionation and refining steps [15,16]. The kinetic modeling of SC-CO₂ extraction is useful to understand the mechanisms involved in this process, necessary for its scaling and design [17]. Most of the modeling used in plant materials and oilseeds are based on the model proposed by Sovová, who considers that the extractable material is formed in a structure of broken and intact cells [18,19].

Santos et al. [20] evaluated the extraction of BN oil using SC-CO₂ and obtained the highest yield (67%) at 300 bar and 60 °C. The oil product showed acid (0.615% free fatty acid) and peroxide (2.4 mEq/Kg) index values within the quality standards. Also, some works in the literature reported the SC-CO₂ extraction to recover oil remnants, present in the press cake, by-product of oil seeds after a pressing process. Several of those studies reported better performance at pressure conditions in a range of 300–600 bar and temperature of 40–60 °C, as applied in the case of press cake of walnut and hazelnut [21–23]. Nevertheless, no studies have been reported about the SC-CO₂ extraction of the residual cake from BNM production.

The present work presents for the first time a study concerning the SC-CO₂ extraction of the cake by-product from the production of BNM, in a perspective of agrifood residues valorization by recovering nutritional and bioactive ingredients. The cake by-product was produced at pilot scale, following the conventional method of Brazil nut beverage production. The kinetic behavior and different conditions of the SC-CO₂ process were evaluated, together with their effect on the fatty acid profile of the lipid fraction obtained and minor lipid compounds. Even though further studies would be necessary for any particular industrial BNM-cake in order to define the precise characteristics of the oil recovered, this study provides data validating that SC-CO₂ extraction of BNM-cake is a feasible and promising industrial application.

2. Materials and methods

2.1. Samples and chemicals

The BN used were from the 2019 harvest. They were purchased under presentation for export (without shell, dry, vacuum-packed in plastic bag) from a local producer “Asociación de Castañeros de la Reserva de Tambopata Los Pioneros – ASCART”, of Puerto Maldonado, Perú. The samples were kept at a temperature of 4 °C until use.

CO₂ (99.98% purity) was purchased from Carburos Metálicos (Madrid, Spain). The reagents chloroform, methanol, anhydrous sodium sulfate (Na₂SO₄), sodium hydroxide in methanol and n-hexane were purchased from Panreac (Barcelona, Spain). N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and the standards for GC analysis (DL- α -tocopherol, squalene, oleic acid, CI-stearoyl-RAC glycerol and β -sitosterol) were purchased from Sigma-Aldrich (Missouri, USA).

2.2. Methods

2.2.1. Production of the cake by-product from BNM processing

The cake was obtained as a by-product of the BNM production, according to the method of Felberg et al. [10] with some modifications. Briefly, BN was ground in a blender to reduce their size and then homogenized for 3 min in hot water (75 °C) at a ratio 7:1 (vol/wt) and 11,000 rpm (Ultraturrax IKA T25 basic). It was then filtered with a stainless mesh (≤ 2 mm) and retained the non-soluble particles, which was the cake. Subsequently, the cake was frozen to -80 °C, then freeze-dried (LyoBeta 15, Azbil Telstar SL, Terrasa, Spain), vacuum packed and stored at -20 °C until use.

The moisture and particle size of the freeze-dried cake was characterized. The moisture was calculated by the gravimetric method (dried in oven at 105 °C to constant weight) and the particle size was determined by an electromagnetic sieve shaker equipment (model BA 200N, CISA).

2.2.2. Supercritical CO₂ extraction

Extractions were performed in a pilot-plant scale supercritical fluid extractor (Thar Technology, Pittsburgh, USA, model SF2000) which include a cylinder extraction cell of 273 ml and two different separators (0.5 L capacity each) with independent control of temperature and pressure. The extraction device also includes a recirculation system, where CO₂ is condensed and pumped up to the desired extraction pressure. Fig. 1 shows a scheme of the SC-CO₂ device used. The extraction vessel has a height/diameter ratio of 4.4 (0.188 m height, 0.043 m internal diameter). For each experiment, the cell was completely filled using about 0.6 kg of cake.

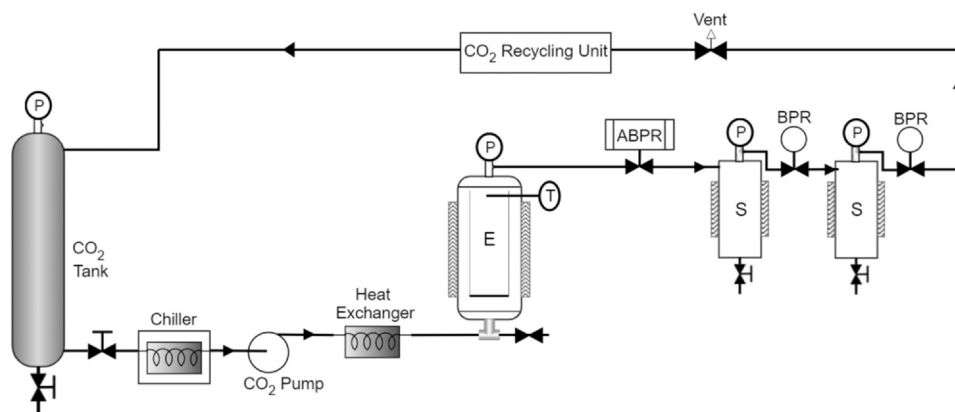


Fig. 1. Schematic diagram of supercritical fluid extractor used in the present study. ABPR: back pressure regulator; BPR: Back pressure regulator; S1: Separator 1; S2 Separator 2; P: Manometer; T: Temperature control.

2.2.2.1. Extraction kinetics. Overall Extraction Curves (OEC) were studied varying the CO₂ flow rate (20, 40 and 80 g/min) at 300 bar and 40 °C. These values of pressure and temperature were selected as suitable conditions according to the extensive data available in the literature about the extraction of vegetable oils and the solubility of triglycerides in SC-CO₂ [20,24–26]. Furthermore, the kinetic behavior of the extraction process was analyzed using sea sand (50% vol/vol) to expand the packed bed and betting for an enhancement of extraction velocity. All extractions were carried out for a time of 180 min and collecting the extract from the separator every 20 min. According to the kinetic study, the CO₂ flow rate was selected for the next stage of experimental extraction.

The OEC were represented following a simplified version of the model of broken and intact cells [27], in which the kinetic behavior is described by two different extraction periods [19]. In the first period ($t < t_c$) it is considered that the mass extracted depends on the equilibrium conditions and thus, the global yield (Y) is related to the solute solubility (S) according to the following equation:

$$Y = Sqt \quad 0 < t < t_c \quad (1)$$

Where q is the ratio between the CO₂ flow rate (Q_{CO_2}) and the mass of cake (M) placed in the extraction vessel, t is the extraction time, and S is the solute weight fraction in the SC-CO₂.

In the second extraction period, the overall yield is described by the equation:

$$Y = X_u[1 - C_1 \exp(-C_2 qt)]t \quad t > t_c \quad (2)$$

In Eq. (2), C_1 and C_2 are adjustable parameters, and X_u is the ratio between the mass of solute and the mass of insoluble solids in the cake, given by,

$$X_u = \frac{X_s}{1 - X_s} \quad (3)$$

Where X_s is the weight fraction of the solute in the cake.

2.2.2.2. Global yield and oil recovery. Experimental assays were performed at constant CO₂ flow rate and varying extraction pressure (200, 300 and 400 bar) and temperature (40 °C and 60 °C).

Global yield (Y) was calculated according to the following equation:

$$Y(\%) = \left(\frac{W_{SFE}}{W} \right) \times 100 \quad (4)$$

where W_{SFE} is the weight of total material extracted by SC-CO₂ and W is the weight of the cake placed in the extraction vessel.

Oil recovery was determined according to:

$$R(\%) = \left(\frac{W_{oilSFE}}{W_{oil}} \right) \times 100 \quad (5)$$

where W_{oilSFE} is the weight of total lipids in the extracted material by SC-CO₂ and W_{oil} is the weight of total lipids determined in the cake, analyzed by Folch method.

The material extracted in each assay was stored under refrigeration (4 °C) until analysis.

2.2.3. Determination of total lipid content

The amount of total lipids in the different samples (material extracted by SC-CO₂ and cake-control) was assessed by the method of Folch, Lees & Sloane Stanley [28], as follows. Briefly, 1 g of sample was mixed with 20 ml of chloroform-methanol (2:1, vol/vol) to be homogenized in Ultraturrax at 7000 rpm for 3 min, followed by sonication by ultrasound bath for 3 min and then centrifuged at 3000 rpm for 10 min. The supernatant was collected and stirred with 4 ml of distilled water Milli-Q by vortex for 2 min and then centrifuged again at 3000 rpm for 10 min. The upper aqueous methanol phase was removed and the bottom organic phase (non-polar compounds) was filtered using filter

paper with a bed of anhydrous sodium sulfate to allow the retention of impurities and water traces. The solvent was removed under vacuum at 40 °C and the samples were kept at 4 °C until further analysis. This procedure was carried out in duplicate.

2.2.4. Analysis of fatty acid profile

The fatty acid profile of lipid samples previously extracted was analyzed after derivatization to fatty acid methyl esters (FAMES). Briefly, 20 mg of samples were mixed with 0.5 ml of chloroform/methanol (2:1; vol/vol) and 1 ml of 0.1 M NaOH in methanol. This mixture was heated at 60 °C for 30 min and then the reaction was stopped with the addition of 0.2 ml of distilled water. The FAMES formed were extracted with the addition of 1 ml of hexane. After stirring, it was decanted and the isolated upper phase was collected. The procedure was repeated with the lower phase to recover remaining FAMES. Sodium sulfate was added to the collected solution of FAMES to eliminate aqueous traces, and it was shaken and left to stand for 30 min. Finally, hexane was removed under nitrogen.

The analyses of FAMES were performed at a concentration of 1 mg/ml in hexane with a gas chromatograph (Agilent Technologies), GC 7890A model, equipped with a flame-ionization detector, and a triple-axis mass spectrometer detector, a split-splitless injector and an auto sampler. FAMES were separated by using a HP-5MS column (30 m length, 0.25 mm internal diameter and 0.25 µm film thickness). Helium was used as carrier gas at 2 ml/min. Injection temperature was 260 °C, splitless mode, 1 µl, and the mass spectrometer ion source and interface temperatures were 230 and 280 °C, respectively. The chromatographic analysis starts at 50 °C and increases at 20 °C/min until 210 °C, it remains for 18 min, and then it goes up to 230 °C at 20 °C/min and stays for 13 min. The total running time is 40 min. The mass spectra were obtained by electronic impact at 70 eV. The scan rate was 1.6 scans/s at a mass range of 30–700 amu. Identification of fatty acids was performed by the NIST MS Data library. The analytical determinations were carried out in duplicate and the results were expressed as g of fatty acid/100 g of FAMES.

2.2.5. Analysis of minor lipid compounds

This analysis involved the identification and quantification of free fatty acids, tocopherol, β-sitosterol and squalene present in the lipid fraction of the SC-CO₂ extracts and cake-control oil. Samples were previously derivatized using BSTFA according to [29]. Briefly, 5 mg of sample was mixed with 1 ml of BSTFA and then submitted to 75 °C for 1 h, shaking every 15 min. After cooling, samples were analyzed by the previously described chromatograph equipment. The separation procedure started at 50 °C and was held for 3 min, increased to 310 °C at a rate of 15 °C/min and was held for 25 min. Identification of compounds was performed by the NIST MS Data library and commercial standards. For quantification, calibration curves were obtained from the standards α-tocopherol, squalene, oleic acid, and β-sitosterol, which were also previously derivatized following the same procedure described for the samples. The analytical determinations were carried out in duplicate.

3. Results and discussion

3.1. Effect of CO₂ flow rate on the extraction kinetic

The results obtained were based on the extraction of a solid matrix (cake) that had the following characteristics: particle size < 1 mm, average moisture content of 0.25% w/w and total lipid content of 40.2% wt/wt. CO₂ flow rate was varied from 20 to 80 g/min, and temperature and pressure were kept constant for all kinetic assays at 40 °C and 300 bar, respectively.

Fig. 2 shows the overall extraction curve (OEC) obtained for the different experiments, representing the kinetic behavior of the yield (Eq. (1)) at 20, 40 and 80 g CO₂/min during 180 min of extraction. As can be observed in the figure, the highest CO₂ flow rate (80 g/min)

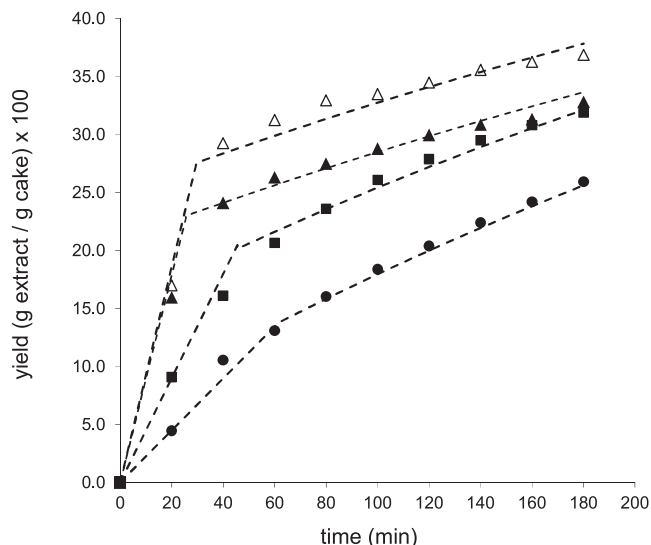


Fig. 2. Experimental yield and kinetic modeling of the OEC obtained in the SC- CO_2 of cake at 40 °C and 300 bar: (•) OEC1, 20 g/min CO_2 ; (■) OEC2, 40 g/min CO_2 and (▲) OEC3, 80 g/min CO_2 without sea sand in the cake packed bed; (△) OEC4, 40 g/min CO_2 with sea sand (50% v/v) in the cake packed bed. Dotted lines: model fitting.

allows a fast extraction, with about 25% of yield at 40 min (representing 76% of the overall extraction yield), while at the lowest CO_2 flow rate (20 g/min) the yield is considerably lower (10.5%) at 40 min.

Since the high oil content of the cake (40.2% wt/wt) could promote particle agglomeration or formation of preferential channels that affects the CO_2 diffusion and reducing extraction efficiency, a test was performed with a mixture of cake and sea sand (50%, vol/vol) and a CO_2 flow rate of 40 g/min (OEC4 in Fig. 2). In this experiment, the ratio between the CO_2 flow and the mass of cake was very similar to that of OEC3 (see Table 1). According to the kinetic behavior depicted in Fig. 2, the use of sand to expand the solid sample enhanced the extraction process in comparison to previous assays (without sand), reaching the highest yields of 29.2% and 36.8% at 40 and 180 min, respectively. The effect of using sea sand to improve SC- CO_2 diffusion in the solid bed was previously observed and reported in the literature [30–32]. Nevertheless, although the use of sand improves the efficiency of oil extraction, its use is not a recommended strategy for large scale production because the defatted cake retains large amounts of proteins and carbohydrates, which may be recovered and exploited for other food applications.

3.1.1. Modeling of the extraction kinetic

Fig. 2 shows the results of the kinetic modeling. The corresponding model parameters are given in Table 1, together with the mean standard deviation (MSD) obtained for each kinetic curve:

Table 1

Model parameters and mean standard deviation (MSD %) of the experimental OEC obtained in the SC- CO_2 of cake at 40 °C and 300 bar.

	OEC1	OEC2	OEC3	OEC4
Q_{CO_2} (g min ⁻¹)	20	40	80	40
M (g)	62.5	62.3	62.7	30.1
Q_{CO_2}/M (min ⁻¹)	0.32	0.64	1.28	1.33
C_1	0.9055	0.7737	0.6890	0.6262
C_2	0.0066	0.0034	0.0014	0.0015
t_c (min)	61.5	45.5	25.5	29.5
Y_c (%)	13.78	20.45	22.78	27.44
k_s^* (min ⁻¹)	0.0039	0.0027	0.0020	0.0020
MSD %	1.7522	1.4848	1.4765	1.3935

$$MSD = \frac{1}{N} \sqrt{\sum_{i=1}^N \left(\frac{Y_{exp} - Y_{cal}}{Y_{exp}} \right)^2} \quad (6)$$

The X_u value was calculated according to the quantified concentration of oil in the cake ($X_s = 0.402$) and resulted to be $X_u = 0.672$. The oil solubility was approximated to the solubility of triolein at the extraction temperature and pressure conditions. Triolein was selected to represent the solubility of Brazil nut oil, due to the large content of C18 fatty acids in the oil (>85% mass). Furthermore, accurate solubility data of this triglyceride are available in the literature, being $S = 0.007$ g/g at 30 MPa and 313.15 K, as reported by Gupta and Shim [26]. It is highlighted that this solubility data has to be considered an approximated value, since the extracted material is a multicomponent mixture comprising saturated and unsaturated triglycerides, together with other minor compounds. In this respect, Rodrigues et al. [33] reported solubility data of Brazil nut oil with values of 7.1 g/kg and 6.1 g/kg, respectively, at 50 °C and 72 °C. No data were reported at 40 °C. Taken into account these values and the fact that the oil used by Rodrigues et al. comprised less amounts of C18 fatty acids, the solubility value selected for the mathematical modeling is reasonably.

Both X_u and S values were considered constant for the mathematical modeling of all OEC. Then, the C_1 and C_2 parameters were adjusted to the experimental data using Excel software and minimizing the objective function (Mean Standard Deviation) given by Eq. (6) by the method of least squares. The t_c values were calculated for each OEC equating Eqs. (1) and (2).

Satisfactory representation of all kinetic curves was obtained using Eqs. (1) and (2) with MSD lower than 2%. In the first period, it is assumed that the external mass transfer resistance is negligible and thus, yield depends only on the solute solubility. Then, the extraction yield is represented by a straight line, and the extraction proceeds very fast. Furthermore, the t_c values decrease when the CO_2 flow rate increase, and the equilibrium period is reduced from 61.5 min at 20 g/min CO_2 to 25.5 min at 80 g/min CO_2 .

That is, increasing the amount of supercritical solvent feed to the extraction vessel, the saturation of the solvent with the oil resulted in a shorter equilibrium period. Additionally, it can be observed that with the same CO_2 flow rate (OEC2 and OEC4), the addition of sea sand in the cake packed bed reduce significantly the first extraction period, obtaining lower t_c values in OEC4 than in OEC2. In fact, t_c of OEC4 (29.5 min) is rather similar to the one of OEC3 (25.5 min), since in these experiments almost the same Q_{CO_2}/M ratio was maintained.

The extraction yields obtained at t_c (Table 1) indicate the percentage of the extractable oil that was obtained during the equilibrium period. When t_c decrease with increasing CO_2 flow rate, the Y_c values increase more than 6% and the Q_{CO_2}/M ratio is doubled (OEC1 and OEC2). Nevertheless, although the Q_{CO_2}/M ratio was also doubled in OEC3 in comparison with OEC2, the extraction yield in the equilibrium period only increased 2.33%. The addition of sea sand in the packed cake enhanced bed porosity and thus, Y_c is higher in OEC4 than in OEC3 even though the Q_{CO_2}/M ratios are rather similar.

The C_2 parameter can be related with the slope of the OEC curve in the second extraction period, and resulted in lower values as the CO_2 flow rate was increased. In the second extraction period, the model stands that the kinetic behavior is affected by the mass transfer resistance and thus, the shape of the OEC changes and the straight line of the first period becomes curved. According to the model equations, the mass transfer capacity in this second period is proportional to the following term [19]:

$$k_s^* = C_1 \exp \left(-\frac{C_2 q t_c}{2} \right) Q_{\text{CO}_2} \frac{C_2}{(1 - X_s)M} \quad (7)$$

Table 1 gives the values obtained for k_s^* which follow the same tendency observed for the C_2 values. Higher Q_{CO_2}/M ratios implicate lower amounts of oil accessible for extraction after the equilibrium

Table 2

Overall extraction yield (Y), lipid content in the extract (according to Folch method) and oil recovery (R) obtained by SC-CO₂ of cake at 40 g/min SC-CO₂ and different extraction temperature and pressure.

Exp.	Temperature (°C)	Pressure (bar)	CO ₂ density (kg/cm ³)	Extraction yield (%)	Lipid content (%)	Oil recovery (%)
1	40	200	839.81	23.8	77.8	46.1
2	40	300	909.89	31.9	79.5	62.9
3	40	400	956.07	40.3	77.0	77.2
4	60	200	723.68	10.9	76.9	20.8
5	60	300	829.71	34.5	78.6	67.5
6	60	400	890.14	59.2	78.9	116.3

period and then, the extraction become more difficult, the velocity is delayed (mass transfer resistance is not negligible in this period) and the slopes of the OEC in the second period are lower with higher Q_{CO_2}/M ratios. Furthermore, in the case of similar Q_{CO_2}/M ratios (OEC3 and OEC4) very similar C_2 values were obtained, and the yield enhancement obtained in OEC4 is more effected by the equilibrium period than by a reduction of mass transfer resistance in the second period.

3.2. Effect of temperature and pressure on extraction yield and oil recovery

Table 2 shows the overall extraction yield (Y) and the recovery of lipid compounds (R) obtained from the cake, at the different pressure and temperature conditions studied, and constant CO₂ flow rate (40 g/min) during 180 min. Also are included in the table, the SC-CO₂ density corresponding to the different extraction conditions assessed.

Lipid compounds were the major substances extracted by SFE. According to Folch method, the lipophilic substances present in the extracts are in the range 76.8–79.5% of the total material extracted, indicating that small amounts of non-lipid compounds were co-extracted. Table 2 reports the result of Folch method for each experimental assay, from which it can be calculated that c.a. 1.5–7.5 g of compounds not assessed by Folch method were extracted. In respect to the temperature and pressure extraction conditions, the co-extraction of these substances presented the same tendencies as those observed in the oil extraction.

The highest yield and oil recovery with values of 59.2% and 116.3%, respectively, were obtained to density 890.14 kg/cm³ (at condition of 60 °C and 400 bar). The value somewhat higher than 100% attained for the oil recovery may be attributed to the own experimental error of the method of determination of total lipid content, but indeed is suggesting a high extraction efficiency with SC-CO₂ to extract lipid substances from nut-type vegetal materials. In agreement, Santos et al. [20] reported an oil yield from BN extracted with SC-CO₂ close to 67% at 300 bar and 60 °C, which is very similar to the oil recovery obtained in this work at the same process conditions (Table 2).

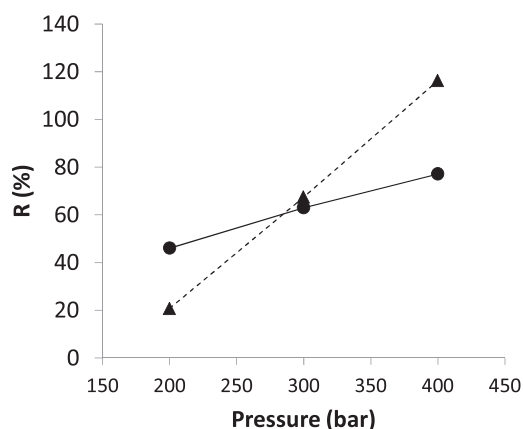


Fig. 3. Oil recovery (R) obtained by SC-CO₂ in different pressures (200, 300 and 400 bar) and temperatures of (●) 40 °C and (▲) 60 °C.

Fig. 3 shows the extraction yield at 40 and 60 °C as a function of the extraction pressure. As expected, at both temperatures, yield increases when the pressure increases. This effect is attributed to the increase of density and solvation power of the SC-CO₂ (see Table 2), which favors the solubility of the solutes in the supercritical solvent [18].

Furthermore, it is observed in Fig. 3 that the extraction yield isotherms exhibit an intersection point near 300 bar, in a phenomenon known as “cross-over or inversion pressure point” [18]. At a pressure of 200 bar (pressure lower than the cross-over point) a higher yield was obtained at 40 °C (23.8%) than at 60 °C (10.9%), so the yield has an inverse correlation with the temperature, explained by the dominant effect of the supercritical fluid density (higher density at lower temperature, Table 3). However, at 400 bar the yield was higher at 60 °C (59.2%) than at 40 °C (40.3%), i.e. at pressures above the cross-over pressure (300 bar) the temperature positively affects extraction yield, and this is attributed to the dominant influence of the solutes volatility in comparison with the effect of the supercritical fluid density [18,34]. Similar cross-over behavior can be found in the literature at pressures in the range 200 and 300 bar [20,34,35], depending on the temperatures applied, solvent flow rate, composition of solutes of the solid matrix, among others.

3.3. Fatty acid profile

Fig. 4 shows the fatty acid profile of cake-control oil and SC-CO₂ extract collected every 20 min for 3 h at 40 °C, 300 bar, 40 g/min CO₂, and cake conditioned with sea sand (50% vol/vol) in the packed bed (OEC4). In general, the fatty acid profile remained constant throughout the extraction period and was similar to that of the cake-control oil, indicating a negligible influence of extraction time on the oil fatty acid profile. Similar results were obtained in other studies, such as the supercritical extraction of oil from walnut and hazelnut by SC-CO₂. In both studies, no significant differences were found in the fatty acid profile of samples collected during extraction, in comparison to the oil extracted with a liquid solvent (n-hexane) from the initial feed material [36,37]. Similarly, when Özkal et al. [23] extracted hazelnut oil at 30, 70 and 120 min (300 bar and 40–60 °C) they found no significant differences in the fatty acid profile during extraction.

Furthermore, the influence of the extraction pressure and temperature conditions on the fatty acid profile of the extracts was evaluated. Table 3 shows that within the group of saturated fatty acids (SFA) and unsaturated fatty acids (UFA) there were no significant differences between samples, but from an individual perspective of each fatty acid, minor variations could be noted. The sample extracted at conditions of 200 bar and 60 °C (treatment 4) showed a slight increase in palmitic acid (16.95%) in comparison with the rest of SC-CO₂ extracts and cake-control oil, but the contrary occurred with stearic acid, which registered a decrease (7.96%). In fact, this last effect of slight decreasing levels of stearic acid respect to the cake-control oil was observed for all the conditions of extraction. In case of linoleic acid there was not a clear difference between the SC-CO₂ extracts and cake-control oil, except for samples from treatment 6, which showed a significant slight decrease.

Therefore, despite these slight differences, in general, the fatty acid profile of all SC-CO₂ extracts and the cake-control oil were similar,

Table 3Fatty acid profile of SC-CO₂ extracts and cake-control oil.

Fatty acid (%)	Cake-control oil	SC-CO ₂ extracts					
		1	2	3	4	5	6
C16:0	14.55 ± 0.03 ^a	15.34 ± 0.57 ^a	15.32 ± 0.68 ^a	15.21 ± 0.39 ^a	16.95 ± 0.26 ^b	15.27 ± 0.25 ^a	15.29 ± 0.14 ^a
C16:1	0.22 ± 0.00 ^b	0.16 ± 0.02 ^a	0.17 ± 0.01 ^a	0.16 ± 0.01 ^a	0.19 ± 0.02 ^a	0.16 ± 0.01 ^{ab}	0.17 ± 0.01 ^a
C18:0	10.05 ± 0.05 ^d	8.77 ± 0.09 ^a	8.63 ± 0.06 ^{ac}	8.90 ± 0.07 ^{acf}	7.96 ± 0.02 ^b	8.89 ± 0.05 ^{acf}	9.10 ± 0.00 ^c
C18:1 ω9	34.72 ± 0.07 ^a	35.80 ± 0.29 ^a	35.35 ± 0.50 ^a	36.35 ± 0.53 ^a	34.72 ± 0.66 ^a	36.17 ± 0.02 ^a	36.40 ± 0.76 ^a
C18:2 ω6	40.45 ± 0.00 ^b	39.92 ± 0.39 ^{ab}	40.54 ± 0.13 ^b	39.39 ± 0.07 ^{ab}	40.18 ± 0.40 ^{ab}	39.51 ± 0.29 ^{ab}	39.05 ± 0.61 ^a
ΣSFA	14.77 ± 0.03 ^a	15.50 ± 0.59 ^a	15.49 ± 0.69 ^a	15.37 ± 0.39 ^a	17.14 ± 0.28 ^a	15.43 ± 0.26 ^a	15.45 ± 0.15 ^a
ΣUFA	75.18 ± 0.07 ^a	75.73 ± 0.68 ^a	75.88 ± 0.63 ^a	75.74 ± 0.60 ^a	74.90 ± 1.07 ^a	75.68 ± 0.31 ^a	75.45 ± 1.36 ^a
PUFA/SFA	2.74	2.58	2.62	2.56	2.34	2.56	2.53

Data represent the mean ± standard deviation of duplicate. Different letters in the same row indicate statistical significant difference ($p < 0.05$).

indicating that there was not a substantial effect of temperature and pressure parameters, in the range of conditions tested, on the fatty acid profile of the samples. In agreement, similar results were observed in other works who used other vegetable sources such as walnuts, hazelnuts, flaxseed or chia seeds [21,23,35,38,39].

Hence, all the extracts obtained had a fatty acid profile with about 75% of UFA, comprising mainly linoleic and oleic acids, and about 24% of SFA, mainly palmitic and stearic acids. Additionally, the fatty acid profile of the SC-CO₂ extracts or cake-control oil are similar to previous reports at BN [40] and its oil extracted with SC-CO₂ [20].

3.4. Minor lipid compounds

Table 4 shows the concentration of free fatty acids (FFA), squalene, tocopherols and β-sitosterol present in cake-control oil and extracts obtained with SC-CO₂ under the different pressure and temperature conditions. With respect to the concentration of FFA there were no significant differences ($p < 0.05$) between the samples. In general, for all samples, the concentration of FFA (about 0.5%) was within the standards equivalent to virgin or cold-pressed vegetable oil ($\leq 2\%$ FFA (oleic acid), Codex Alimentarius [41]). Additionally, similar results were reported in the work of Santos et al. [20], who extracted BN oil with SC-CO₂ at 300 bar and 60 °C, and obtained a FFA concentration of 615 mg/100 g oil (expressed as oleic acid).

On the other hand, Fig. 5A shows that at 60 °C the extraction pressure affected the FFA concentration of the extracts, while at 40 °C the FFA concentration was kept almost constant with pressures. The variation of the FFA content in oils extracted by SC-CO₂ is well related with the solubilities of the different lipid-type solutes in the

supercritical solvent which, in turn, are influenced by pressure and temperature. In general, the reduction of pressure and increase of temperature₂ enhances the solubility of FFA in SC-CO₂, as well as that of monoglycerides and diglycerides, in comparison with the triglycerides solubility [42]. This effect is commonly used as a strategy to refine oils, removing undesirable compounds such as FFA and monoglycerides, which are considered pro-oxidant agents. Some examples can be found in the deacidification of fish oil [43], rice bran oil [44] and olive oil [45].

In general, several bioactive compounds can be found as part of the minor lipid compounds present in edible vegetal oils. In this study, squalene, tocopherol and β-sitosterol were considered, since these are compounds that have been previously described in BN oil [40,46]. According to Table 4, the extract obtained at 200 bar and 60 °C (treatment 4) was the only one that had a higher concentration of these bioactive compounds, with a total value of 947 mg/100 g extract, in comparison with the cake-control oil (532 mg/100 g oil). This result was mainly due to significant higher concentrations of squalene and β-sitosterol, up to 2.0 and 2.5 fold increases, respectively. Therefore, this extract obtained with SC-CO₂, possess higher concentrations of these bioactive compounds than most other plant sources, such as nuts or oilseeds, which normally contain levels of squalene in the range of 8–170 mg/100 g oil and of β-sitosterol around 100–140 mg/100 g oil [40,46,47].

Fig. 5B–D shows an inverse relationship between pressure and the concentration of bioactive compounds for both temperatures studied, since with higher pressures the concentration of all minor bioactive compounds decreased. Similar behavior was observed in a study of amaranth oil SC-CO₂ extraction at constant temperature of 40 °C and

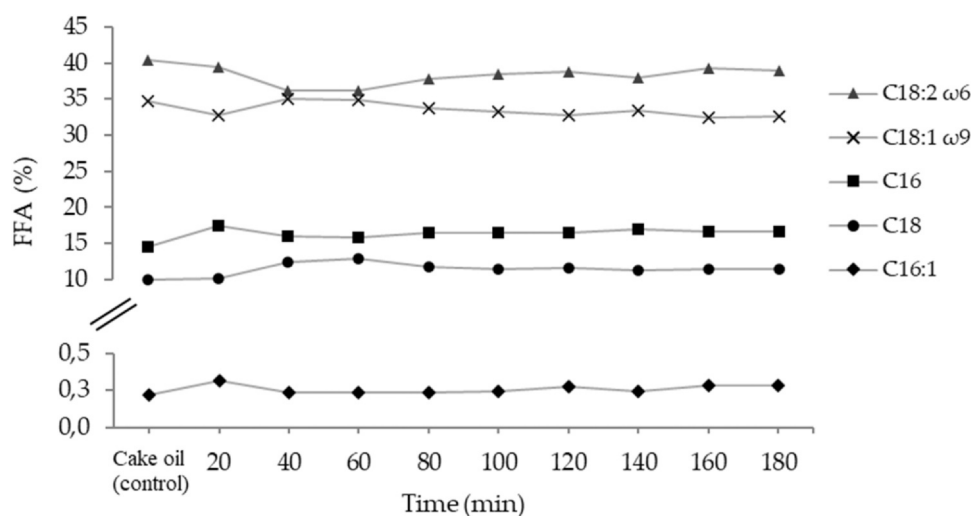
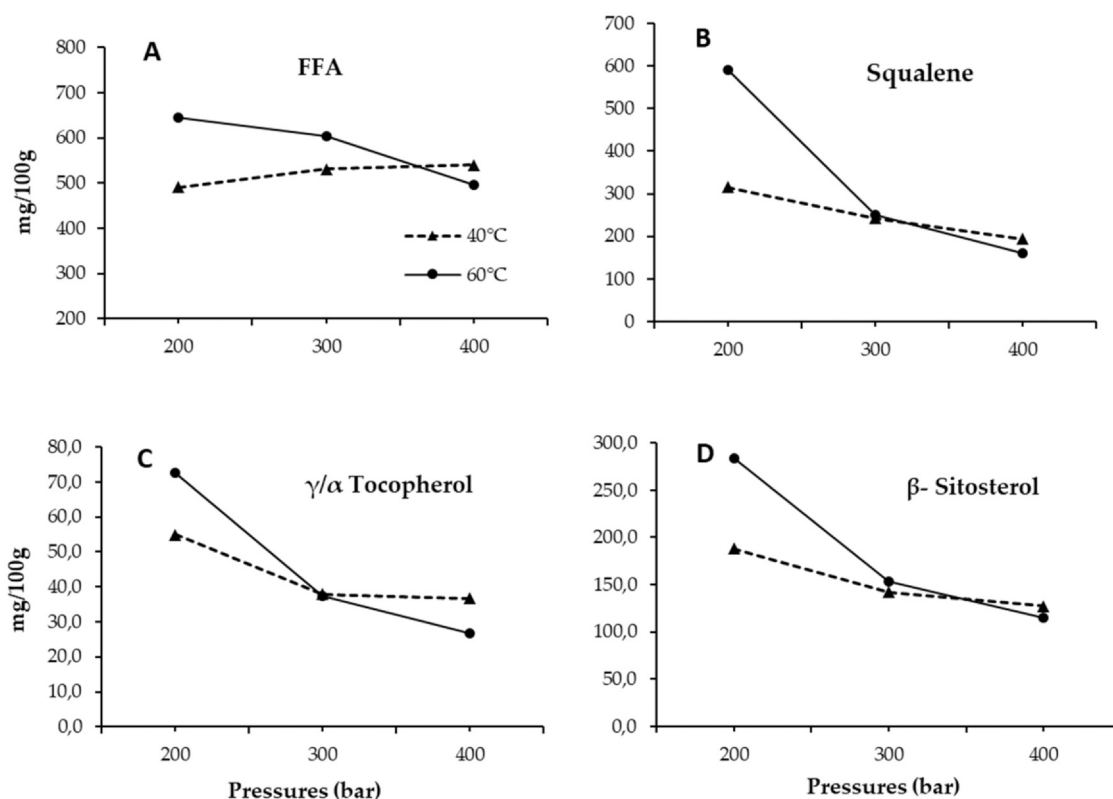


Fig. 4. Fatty acid profile of the SC-CO₂ extract collected at different extraction times, at 40 °C, 300 bar, 40 g/min SC-CO₂, and sea sand (50% vol/vol) in the cake packed bed.

Table 4Content of minor compounds (mg/100 g) in SC-CO₂ extracts and cake-control oil.

Exp. Assay (Samples)	FFA	Bioactive compounds			
		Squalene	Tocopherol	β-Sitosterol	Total
1	491.0 ± 101.8 ^a	315.1 ± 2.7 ^b	55.0 ± 7.7 ^{abc}	188.2 ± 17.6 ^b	558.4 ± 28.0 ^b
2	531.5 ± 156.0 ^a	241.9 ± 23.1 ^{bcd}	37.9 ± 0.8 ^{bcd}	142.0 ± 1.7 ^{bcd}	421.8 ± 25.6 ^{bc}
3	540.1 ± 207.5 ^a	193.5 ± 32.5 ^{cd}	36.7 ± 4.1 ^{cd}	127.3 ± 10.9 ^{cd}	357.6 ± 47.5 ^c
4	645.6 ± 125.8 ^a	590.8 ± 17.3 ^a	72.7 ± 5.4 ^a	283.9 ± 5.4 ^a	947.4 ± 28.1 ^a
5	603.7 ± 146.6 ^a	249.5 ± 35.9 ^{bcd}	37.4 ± 2.7 ^{bcd}	152.9 ± 18.7 ^{bcd}	439.9 ± 57.3 ^{bc}
6	495.9 ± 206.9 ^a	161.0 ± 26.6 ^d	26.6 ± 0.9 ^d	114.6 ± 8.1 ^d	302.2 ± 35.6 ^c
Cake-control oil	487.3 ± 3.8 ^a	291.2 ± 22.3 ^{bc}	56.6 ± 7.6 ^{ab}	183.9 ± 26.6 ^{bc}	531.7 ± 56.5 ^b

Data represent the mean ± standard deviation of duplicate. Different letters in the same column indicate statistical significant difference ($p < 0.05$).**Fig. 5.** Effect of pressure and temperature on the concentration of FFA (A), squalene (B), tocopherol (C) and sitosterol (D) of SC-CO₂ extracts.

pressures from 150 to 650 bar, obtaining decreasing squalene concentrations from 148 to 63 mg/g oil, respectively [48].

Considering the extraction at 200 bar, it was also clearly observed that the concentrations of all the bioactive compounds were considerably higher at 60 °C than at 40 °C (Fig. 5B–D). This effect was also observed by Dąbrowski et al. [38], who optimized the SC-CO₂ oil extraction of flaxseed, in a temperature range of 40–80 °C, concluding that the highest concentrations of tocopherols, sterols and squalene were mainly obtained at high temperatures.

It is interesting to note that, despite the highest concentration of bioactive compounds was obtained at 200 bar and 60 °C, at these conditions the lowest global yield and oil recovery were obtained (Table 2). When the extraction pressure increased, the global yield was increased, but the concentration of bioactive compounds decreased, which can be explained by the triglyceride dilution effect. The observed increased concentration of squalene, β-sitosterol and tocopherols at low CO₂ densities (low pressures and high temperatures) is often used as a strategy to attain the enrichment of minor bioactive lipids present in oils. For example, Kraujalis and Venskutonis [47] increased the tocopherol and squalene concentration in amaranth oil, using a cascade decompression system to fractionate the oil recovered from solid

amaranth seeds supercritical extraction. Furthermore, also in the case of countercurrent packed SC-CO₂ columns, this strategy was used to the concentration of bioactive lipids, for example as demonstrated by Dunford and King [48] studying the phytosterol enrichment of rice bran oil. Indeed, SC-CO₂ extraction conditions and the physicochemical properties of the extractable compounds in the plant matrix determine the concentration of certain compounds of interest in the extracts [37,49]. As was confirmed in the present work, minor lipid compounds, such as squalene, phytosterols and tocopherols, are generally extracted more easily and quickly than other large molecular weight lipid compounds, such as triglycerides.

4. Conclusions

In this study de supercritical fluid extraction of the by-product (cake) of the Brazilian nut beverage production is presented for the first time. The effect of different process parameters on yield and oil composition of the extract is evaluated. The analysis of the extraction kinetics indicates that most of the oil is readily accessible for extraction and the extraction velocity is greatly dominated by the oil solubility and thus, by the amount of supercritical solvent per unit of cake mass feed

into the extraction vessel. Furthermore, enhancing bed porosity by using sea sand results in a substantial increase of yield at the end of the period controlled by the solute solubility, but does not decrease significantly the mass transfer resistance. By increasing extraction pressure and temperature higher yields are obtained, allowing 100% oil recovery at 400 bar and 60 °C.

About the chemical composition of the extracts, the fatty acid profile of the oil does not present relevant variations with respect to the extraction time, or pressure and temperature conditions. However, the concentration of minor lipid compounds exhibits important variation as an effect of the extraction conditions, since the highest levels of bioactive compounds (mainly squalene and β -sitosterol) are obtained at the lower pressure and higher temperature assessed (200 bar and 60 °C).

Therefore, it can be concluded that SC-CO₂ extraction is a suitable technique to recover the large amounts of oil present in the cake residue of the Brazil nut beverage production, allowing high yield and oil recovery, including minor high valued lipid compounds present in the nut fruit. This result suggests the useful of SC-CO₂ extraction to revalorize a by-product of the Brazil nut beverage production, recovering a high-valued oil enriched in bioactive compounds with potential application in the food, cosmetic or nutraceutical sector.

Declaration of Competing Interest

There are no conflicts to declare.

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