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# Supercritical $CO_2$ extraction of oil and omega-3 concentrate from Sacha inchi (*Plukenetia volubilis* L.) from Antioquia, Colombia

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**SUMMARY:** Sacha inchi *(Plukenetia volubilis* L.*)* seeds were employed for oil extraction with supercritical $CO_2$ at laboratory scale. The supercritical extraction was carried out at a temperature of 60 °C, pressure range of 400–500 bars and $CO_2$ flow of 40–80 g/min. The maximum recovery was 58% in 180 min, favored by increasing the residence time of $CO_2$ in the extraction tank. Subsequently, the process was evaluated at pilot scale reaching a maximum recovery of 60% in 105 min, with a temperature of 60 °C, pressure of 450 bars and $CO_2$ flow of 1270 g/min. The fatty acid composition of the oil was not affected for an extraction period of 30–120 min. The Sacha inchi oil was fractionated with supercritical $CO_2$ to obtain an omega-3 concentrate oil without finding a considerable increase in the proportion of this compound, due to the narrow range in the carbon number of fatty acids present in the oil (16–18 carbons), making it difficult for selective separation.

***KEYWORDS****: α-linolenic acid; Omega-3; Sacha inchi; Supercritical extraction*

**RESUMEN:** ***Extracción con $CO_2$ supercrítico de aceite y un concentrado de omega-3 a partir de Sacha inchi (*Plukenetia volubilis* L.) proveniente de Antioquia, Colombia.*** Semillas de Sacha inchi fueron empleadas para la extracción de su aceite con $CO_2$ supercrítico a escala de laboratorio, a una temperatura de 60 °C, entre 400–500 bares de presión y un flujo de $CO_2$ entre 40–80 g/min, obteniendose una recuperación máxima del 58% en 180 min favorecida por el aumento en el tiempo de residencia del $CO_2$ en el tanque de extracción. Posteriormente, se evaluó el proceso a escala piloto, alcanzando una recuperación máxima del 60% en 105 min de extracción, a una temperatura de 60 °C, presión de 450 bares y flujo de $CO_2$ de 1270 g/min, sin afectar la composición de los ácidos grasos del aceite durante un periodo de extracción entre 30–120 min. El aceite de Sacha inchi fue fraccionado con $CO_2$ supercrítico para la obtención de un aceite concentrado de omega-3, sin encontrar aumento considerable en la proporción de este compuesto debido al estrecho rango en el número de carbonos (16–18 carbonos) de los ácidos grasos presentes en el aceite, lo que dificulta su separación selectiva.

***PALABRAS CLAVE****: Ácido α-linolénico; Extracción supercrítica; Omega-3; Sacha inchi*

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## 1. INTRODUCTION

Sacha inchi (*Plukenetia volubilis* L.) is a native plant of the Amazon jungle and is considered an oleaginous with protein levels between 27–30% and lipid levels between 40–60%. Its oil content is higher than the oil content in soybean seeds (16.5–17.5%), chia seeds (26.7–35%) and safflower seeds (27.5%) and it is within the range registered for flaxseed (33.6–44.8%). Sacha inchi oil is of great interest in the pharmaceutical and food industry due to its high content of unsaturated fatty acids (approximately 90% of total lipids). It is composed of oleic acid (omega-9) (8.7–9.6%), linoleic acid (omega-6) (33.4–36.2%) and α-linolenic acid (omega-3) (46.8–50.8%), highlighting nutritional and protective benefits against cardiovascular disease provided by linoleic and α-linolenic essential fatty acids. In addition, it has a lower proportion of saturated fatty acids (6.1–7.2%) than canola, sunflower, flax, corn, olive and cotton oils, ranging between 8.5–25.2% (Guillén *et al.,* 2003; Gutiérrez *et al.,* 2011; Maurer *et al.,* 2012; Chirinos *et al.,* 2013; Pereira De Souza *et al.,* 2013; Ruiz *et al.,* 2013).

Sacha inchi oil can be obtained by cold pressing or extraction with toxic and flammable solvents which generate quality deterioration of the oil by high processing temperatures and solvent residues in the product. An alternative process is extraction with supercritical $CO_2$, an environmentally friendly method that avoids the risk of thermal degradation and the presence of toxic residues in the product, since $CO_2$ is a non-toxic and non-flammable solvent which is easily separated from the extract and ideal for obtaining thermally labile compounds for its moderate supercritical conditions. This technique uses the solvation and transport properties of the supercritical $CO_2$ to favor the separation of further components by changes in temperature and pressure that modify $CO_2$ density and solute solubility in the $CO_2$ (Shahidi, 2005; Follegatti-Romero *et al.,* 2009; Rubio-Rodríguez *et al.,* 2010).

Omega-3 concentrates are usually obtained from animal sources which are limited and under threat such as fish. Other abundant and varied vegetable sources of omega-3 such as Sacha inchi oil have not been used extensively, and its extraction has not been performed at pilot scale to evaluate the influence of extraction time on fatty acid composition, which establishes it as a fatty acid source with important cardio-protective properties. Therefore, the present study evaluated the supercritical $CO_2$ extraction of Sacha inchi oil and the Sacha inchi oil fractionation with supercritical $CO_2$ to obtain an omega-3 concentrate.

## 2. MATERIALS AND METHODS

### 2.1. Pre-treatment of seeds and Sacha inchi oil

Sacha inchi seeds used for the oil extraction were provided by a producer whose crop is located at Santa Rosa de Osos, Antioquia, Colombia. They were peeled and milled in a food processor (Black&Decker, model FP1336).

Sacha inchi oil to obtain an omega-3 concentrate was provided by Colombiana de Biocombustibles S.A from crops located at Bajo Cauca region (Colbio, Antioquia, Colombia), and by the producer mentioned above (Antioquia, Colombia). The oil was transesterified to ethyl esters by the addition of 40 mL of a sodium hydroxide solution (Sigma-Aldrich) in ethanol (Panreac) (0.5 M) to 100 g of oil. This mixture was put in reflux for 1 h at 60 °C. Subsequently, 40 mL of aluminum chloride solution (Panreac) in ethanol (Panreac) (3.5 w/w) were added to the mixture and the reflux was maintained for 1 h. Then, 100 mL of hexane (Merck) were added and the mixture was cooled to room temperature. Finally, 30 mL of a saturated solution of sodium chloride (Merck) was added, and the superior phase that contained the ethyl esters was removed and stored in a dark container at 5 °C.

### 2.2. Characterization of the almond and Sacha inchi oil

The moisture content of milled almonds was determined by a moisture analyzer (Mettler Toledo, model HB43-S). The residual material was sieved for 20 min using a sieve arrangement with meshes of 4, 8, 10, 16, 30, 50, 60 and collector of the Tyler series to determine the average particle size (Dp), according to Follegatti-Romero *et al.,* (2009). Likewise, the bulk density of the material was determined (Gardco, model WG-3006) and its oil content was estimated by Soxhlet extraction for 20 h (Follegatti-Romero *et al.,* 2009; Gutiérrez *et al.,* 2011).

For the determination of the fatty acid composition, the oil and the extracts obtained were transesterified to methyl esters according to the AOCS method Ce 2–66 (American Oil Chemists' Society, 1997). Samples were analyzed by gas chromatography using a Perkin Elmer Autosystem XL equipment (Perkin Elmer, USA), with a flame ionization detector (FID) and a capillary column Omegawax 250 fused silica 30 m x 0.25 mm x 0.25 µm (Sigma-Aldrich, Supelco, reference 24136). The following operation conditions were also considered: injection volume of 1 µL, hydrogen as carrier gas at 12 PSI, vent flow of 25.2 mL/min, injection and detection temperature of 250 °C, oven temperature range of 200 to 250 °C with increases of 4 °C/min and holding the final temperature for 3 min.

Finally, the following physicochemical properties of the Sacha inchi oil were determined: density using pycnometer at 20 °C, acidity according to ISO 660:2009 standard (International Organization for Standardization, 2009b) and iodine value according to ISO 3961:2009 standard (International Organization for Standardization, 2009a).

### 2.3. Equipment of supercritical CO2 extraction

$CO_2$ liquid with 99.9% purity (Cryogas, Antioquia, Colombia) was employed as solvent during the extraction and fractionation of Sacha inchi oil.

Sacha inchi oil fractionation was carried out in Thar Technologies model P200 1998 equipment at laboratory scale. It has an extraction tank of 1 L and two separators of 500 mL, and it also has an electric heating adapted externally to the pressure tanks (Roker, Argentina). The equipment components can be seen in the flow diagram in Figure 1. In the same way, Sacha inchi oil extraction was achieved in the same Thar Technologies equipment at laboratory scale and in a Guangzhou Masson New Separation Technology Co Ltd model 12L-SFE 2007 equipment at pilot scale. It contains two extraction tanks of 12 L and two separators of 6 L. The equipment configuration is similar to that shown in Figure 1.

### 2.4. Experimental procedures

Oil extraction from Sacha inchi almonds was evaluated according to Follegatti-Romero *et al.,* (2009). A sample of 200 g was employed in the extraction tank of 1 L at a temperature of 60 °C, pressure range of 400–500 bars, $CO_2$ flow of 40–80 g/min and extraction time range of 120–180 min. All experiments were carried out in dynamic operation mode and the final extract corresponded to separator discharge. Subsequently, the test that yielded the highest and operation limits of the equipment at pilot scale were considered to scale up the system maintaining the relation between $CO_2$ flow and mass of raw material constant (Mezzomo *et al.,* 2009; Prado *et al.,* 2012). The overall extraction curve to this scale was done in duplicate using 6.050 g of milled almonds at a temperature of 60 °C, pressure of 450 bars and $CO_2$ flow of 1270 g/min.

At the same time, Sacha inchi oil fractionation to obtain an omega-3 concentrate was evaluated through the addition of 80 g of oil distributed in an inert material that comprised 80% of the extraction tank to improve the solvent-raw material contact. The inert material was deposited into a metal basket disposed within the extraction tank.

Initially, a factorial experiments design was carried out in duplicate based on the operation limits of the equipment and the reports about solubility and supercritical $CO_2$ extraction (Chrastil, 1982; Nilsson *et al.,* 1991; Guclu-Ustundag and Temelli, 2000; Soares *et al.,* 2007; Follegatti-Romero *et al.,* 2009; Martínez and De Aguiar, 2014). This experimental design employed a fixed bed of glass beads of 4 mm and raschig rings at a temperature of 40 and 60 °C, pressure of 180 and 350 bars and $CO_2$ flow of 40 and 200 g/min for 30 min. Subsequently, based on experimental design, the results were evaluated using four replicates at temperatures of 40, 60 and 80 °C using a bed of glass beads of 4 mm, pressure of 180 bars, $CO_2$ flow of 40 g/min for 120 min,

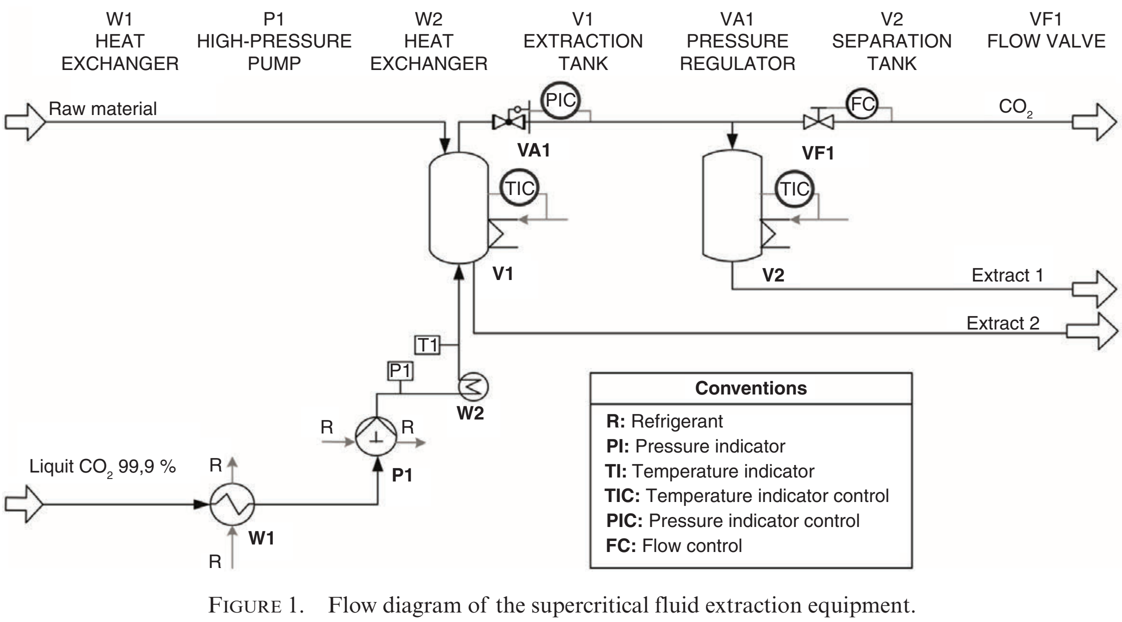


FIGURE 1. Flow diagram of the supercritical fluid extraction equipment.

highlighting the increase of extraction time according to Uquiche *et al.,* (2012).

Finally, ethyl ester fractionation was done in duplicate using a bed of glass beads of 4 mm at temperature of 80 °C, pressure of 180 bars, $CO_2$ flow of 40 g/min for 120 min based on the evaluation results of the extraction temperature.

## 3. RESULTS AND DISCUSSION

### 3.1. Sacha inchi oil extraction

Sacha inchi oil was extracted from its milled almond (Figure 2) using supercritical fluid extraction Thar Technologies model P200 1998 equipment. The test material supplied by a local producer (Antioquia, Colombia) was milled and presented an average particle size in the range of 1.51–2.01 mm and a humidity of 2.36 ± 0.48%. The oil was extracted at a temperature of 60 °C, pressure of 400 and 500 bars, $CO_2$ flow of 40 and 80 g/min, extraction time of 120 and 180 min.

Physicochemical properties such as iodine value, which measures the unsaturation degree of the oil, and the acidity, a measurement of the free fatty acid proportion in the oil which affects oil quality and generates bad odors and flavors (Staunton *et al.,* 1969), were determined in each oil extracted. These oils presented an increase in acidity (Figure 3a) and a decrease in the iodine value (Figure 3b) on residence time. It was calculated through the multiplication of the extraction tank volume and $CO_2$ density by the temperature and pressure evaluated (National Institute of Standards and Technology, 2011), then the result was divided by $CO_2$ flow. Variations in acidity and iodine value were caused by changes in pressure and mainly in the $CO_2$ flow because a low $CO_2$ flow generated an increase in acidity of up to 0.2% and a decrease in iodine value of 33 g $I_2$/100 g of oil, while a major pressure generated an increase in acidity of 0.1% and a decrease in iodine value of 15 g $I_2$/100 g of oil.

The values of these physicochemical properties affect the oxidative stability of oil showing a favorable quality of the oil to shorter residence time that generated the lowest acidity. All extracted oils presented an acidity lower than the acidity of 1.0–1.5%, a range in which oils are commercially rejected (Maurer *et al.,* 2012). Moreover, the high iodine value of the Sacha inchi oil caused by the high polyunsaturated fatty acid (PUFAs) proportion such as omega 3 and 6 provide a better nutritional value to the oil of great interest in the food industry. However, the extraction conditions (60 °C, 400 bar, 80 g/min, 180 min) to the shortest residence time achieved a low yield of 19.81 g of oil/100 g of almonds and the maximum yield in oil extraction was achieved at 60 °C, 500 bar, 40 g/min, 180 min with a value of 23.48 g of oil/100 g of almonds.

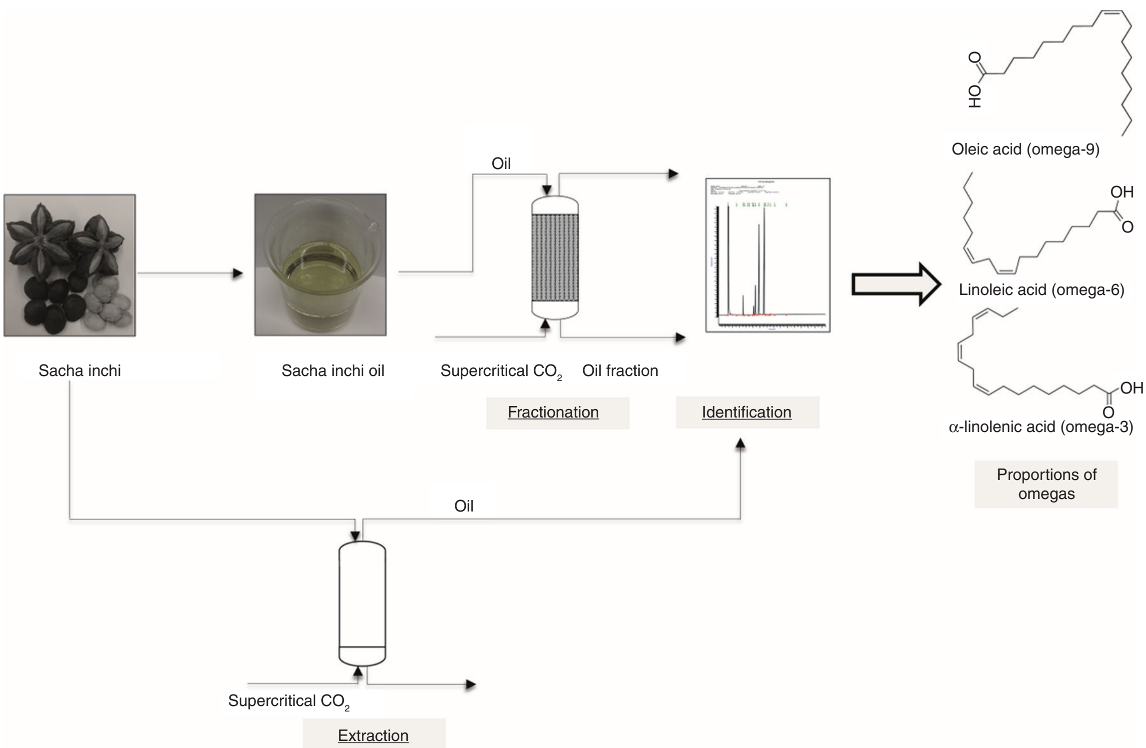


FIGURE 2. Experimental evaluation of the extraction and fractionation processes with supercritical $CO_2$.

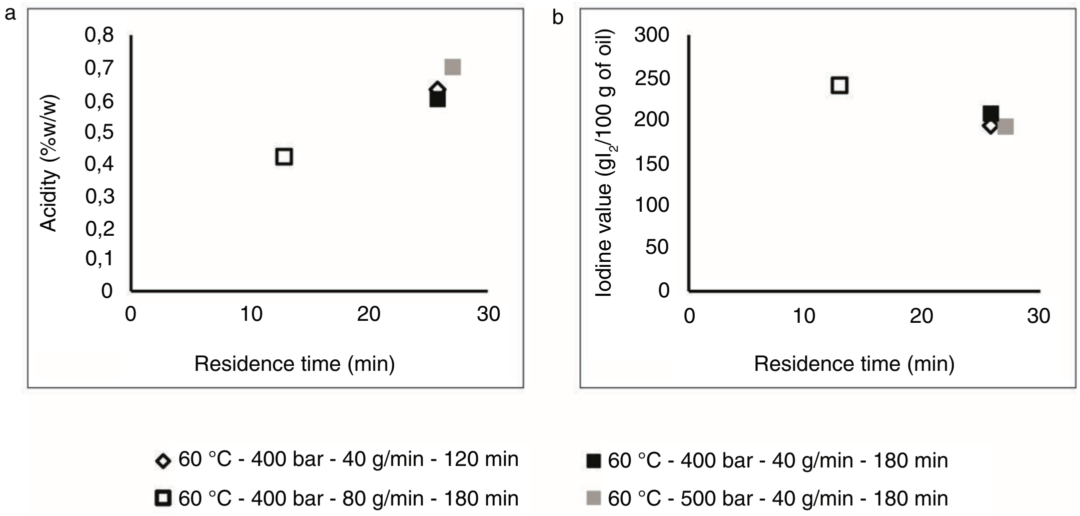


FIGURE 3. Variation in the physicochemical characteristics of the Sacha inchi oil with residence time. a) Acidity expressed as oleic acid. b) Iodine value.

The fat content of the Sacha inchi almonds was determined by soxhlet extraction with a value of 40.55 ± 0.51 g of oil/100 g of almonds, which is within the range of 40–60% registered for this oleaginous (Chirinos *et al.,* 2013) and it is close to the value reported (42.0 ± 1.1%) by Gutiérrez *et al.,* (2011) for seeds grown in Caquetá, Colombia. Based on this parameter, 400P.80F.180t oil (60 °C, 400 bars, 80 g/min, 180 min), 400P.40F.180t oil (60 °C, 400 bars, 40 g/min, 180 min), 400P.40F.120t oil (60 °C, 400 bars, 40 g/min, 120 min) and 500P.40F.180t oil (60 °C, 500 bars, 40 g/min, 180 min) whose P, F and t suffixes denote pressure, $CO_2$ flow, and extraction time, presented recoveries of 48.87, 54.41, 46.93 and 57.91% to residence times of 13, 26, 26 and 27 min, respectively. These oil extraction tests showed that longer extraction and residence time to low $CO_2$ flow increased the process recovery by 9.04% (conditions 400P.80F.180t and 500P.40F.180t) because it allowed for greater $CO_2$ saturation and oil extraction.

Subsequently, the process of Sacha inchi oil extraction was scaled up to a volume of 12 L keeping the ratio between $CO_2$ flow and raw material mass constant (Mezzomo *et al.,* 2009; Prado *et al.,* 2012), using the extraction conditions of the 500P.40F.180t oil that had the highest recovery and its acidity was inferior to the limit to which the oil is rejected. For scaling, the temperature was kept constant, pressure was adjusted to the upper limit of operation of the equipment (450 bars), and $CO_2$ flow was determined with a value of 1270 g/min through the ratio described. The established conditions (60 °C, 450 bars, 1270 g/min) were employed to make the extraction curve of Sacha inchi oil (Figure 4) to observe the variation in the oil yield during the extraction time. This extraction was carried out in Guangzhou Masson New Separation Technology Co Ltd model 12L-SFE 2007 equipment, using milled almonds of Sacha inchi with an average particle size of 1.64 mm, humidity of 3.31 ± 0.26% and bulk density of 0.63 ± 0.01 g/mL.

In Figure 4a a rapid extraction period was observed until 105 min achieving a yield of 24.55 g of oil/100 g of almonds. During this period, the easily accessible oil covering the particles' surface was extracted at a rate of 0.1431 g of oil/100 g of almond x min. After that, the rapid extraction period was continued by a long slow extraction period that generated an increase of 9.2% in the oil yield for an extraction time from 105 to 270 min, by predominating oil diffusion into the material (Mezzomo *et al.,* 2009). Finally, an overall yield of up to 26.81 g of oil/100 g of almonds to an extraction time of 270 min was achieved and approximately 40% of the oil present in the Sacha inchi almonds was extracted at a lower rate.

Also, the extraction time in the first stage (rapid extraction period) affected neither the fatty acid composition in the oil (Figure 4b) nor the oil density (Figure 5a) to an extraction time of up to 120 min. It was confirmed by the statistical analysis in the R-project software version 3.1.2 (R Core Team, 2014) of the iodine value for samples collected in the same extraction period, using a non-parametric Kruskal-Wallis test due to non-compliance of the assumption of variance homogeneity. This test reported a p-value of 0.4355 which confirms the non-variation of unsaturation degree of the oil during the rapid extraction period, where the proportion of each fatty acid was similar to those reported by Follegatti-Romero *et al.,* (2009) except the omega-3 proportion which was 2.14% higher than the proportion obtained in this work (48.31%). It is possibly due to changes in crops that affect the PUFAs biosynthesis in plants.

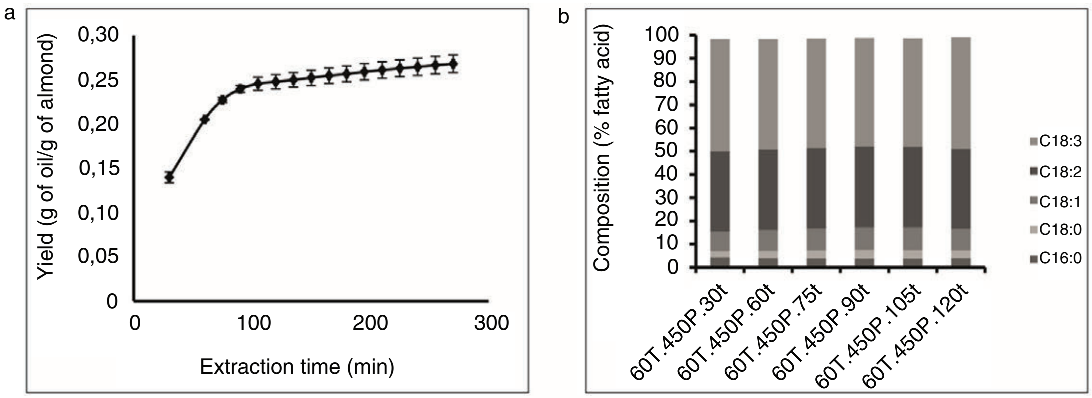


FIGURE 4. Yield and fatty acid composition of the Sacha inchi oil obtained at 60 °C, 450 bars and 1270 g/min. a) Extraction curve at pilot scale. b) Fatty acid composition from an extraction time of 30 min to 120 min.

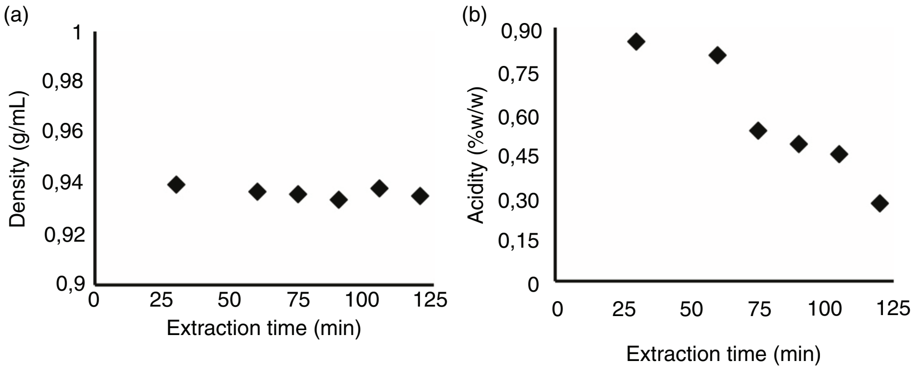


FIGURE 5. Variation in the physicochemical characteristics of the Sacha inchi oil over the extraction time at 60 °C, 450 bars and 1270 g/min. a) Density. b) Acidity expressed as oleic acid.

During the extraction time of up to 120 min (Figure 5b), acidity showed a decrease because the oil initially extracted corresponded to the external layer covering the particles (Mezzomo *et al.,* 2009) which is in direct contact with the environment. It shows the importance of proper storage of the Sacha inchi almonds in order to avoid deterioration of oil quality by exposing the almonds to environmental conditions.

Finally, the Sacha inchi oil recovery at pilot scale was 60.54% in an extraction time of 105 min. This recovery was higher and used a shorter extraction time than the recovery achieved under the conditions when the process was scaled up (57.91% in 180 min), obtaining better process yield by increasing the scale of 1 L to 12 L.

### 3.2. Sacha inchi oil fractionation with supercritical $CO_2$

Sacha inchi oil was fractionated with supercritical $CO_2$ to obtain an omega-3 concentrate as shown in Figure 2. First, temperature, pressure and $CO_2$ flow effect on omega-3 concentration were evaluated under the conditions listed in Table 1, using a fixed bed of glass beads or raschig rings and the Sacha inchi oil provided by Colbio. It presented a density of 0.9211 ± 0.0021 g/mL, acidity of 0.41 ± 0.02%, iodine value of 149 g $I_2$/100 g of oil and fatty acid composition: palmitic acid (C16:0) of 3.92 ± 0.11%, stearic acid (C18:0) of 2.41 ± 0.11%, omega-9 (C18:1) of 9.06 ± 0.03%, omega-6 (C18:2) of 35.28 ± 0.59% and omega-3 (C18:3) of 47.34 ± 0.83%. Subsequently, based on the results of this

TABLE 1. Preliminary experimental design to obtain omega-3 concentrate from Sacha inchi oil fractionation

| Treatments | Temperature (°C) | Pressure (bars) | $CO_2$ flow (g/min) |
|---|---|---|---|
| 1 | 40 | 180 | 40 |
| 2 | 60 | 180 | 40 |
| 3 | 40 | 350 | 40 |
| 4 | 60 | 350 | 40 |
| 5 | 40 | 180 | 200 |
| 6 | 60 | 180 | 200 |
| 7 | 40 | 350 | 200 |
| 8 | 60 | 350 | 200 |

evaluation, the extraction temperature effect was estimated using temperatures of 40, 60 and 80 °C and a fixed bed of glass beads at a pressure of 180 bars and $CO_2$ flow of 40 g/min. In this case Sacha inchi oil provided by a local producer was used (Antioquia, Colombia). It presented a density of 0.9490 ± 0.0113 g/mL, acidity of 0.77 ± 0.30%, iodine value of 189 g $I_2$/100 g of oil and fatty acid composition: C16:0 of 4.21 ± 0.01%, C18:0 of 2.60 ± 0.01%, omega-9 of 9.14 ± 0.02%, omega-6 of 33.91 ± 0.07% and omega-3 of 49.33 ± 0.08%.

Figure 6 shows the variation in the omegas of the Sacha inchi oil of their content in the initial oil in both the extraction tank fraction (x-axis) and the separator fraction (y-axis). The data have been grouped according to pressure and $CO_2$ flow limits evaluated so that each point of the same series corresponds to the two temperature limits (40–60 °C). This means that, at the end of fractionation process, each composition of the omegas 3, 6 and 9 obtained in both the extraction tank fraction and the separator fraction, were subtracted from their proportions present in the initial oil. After that, they were grouped into the same pressure and $CO_2$ flow. Figure 6d corresponds to the difference between treatments with a $CO_2$ flow of 40 and 200 g/min in

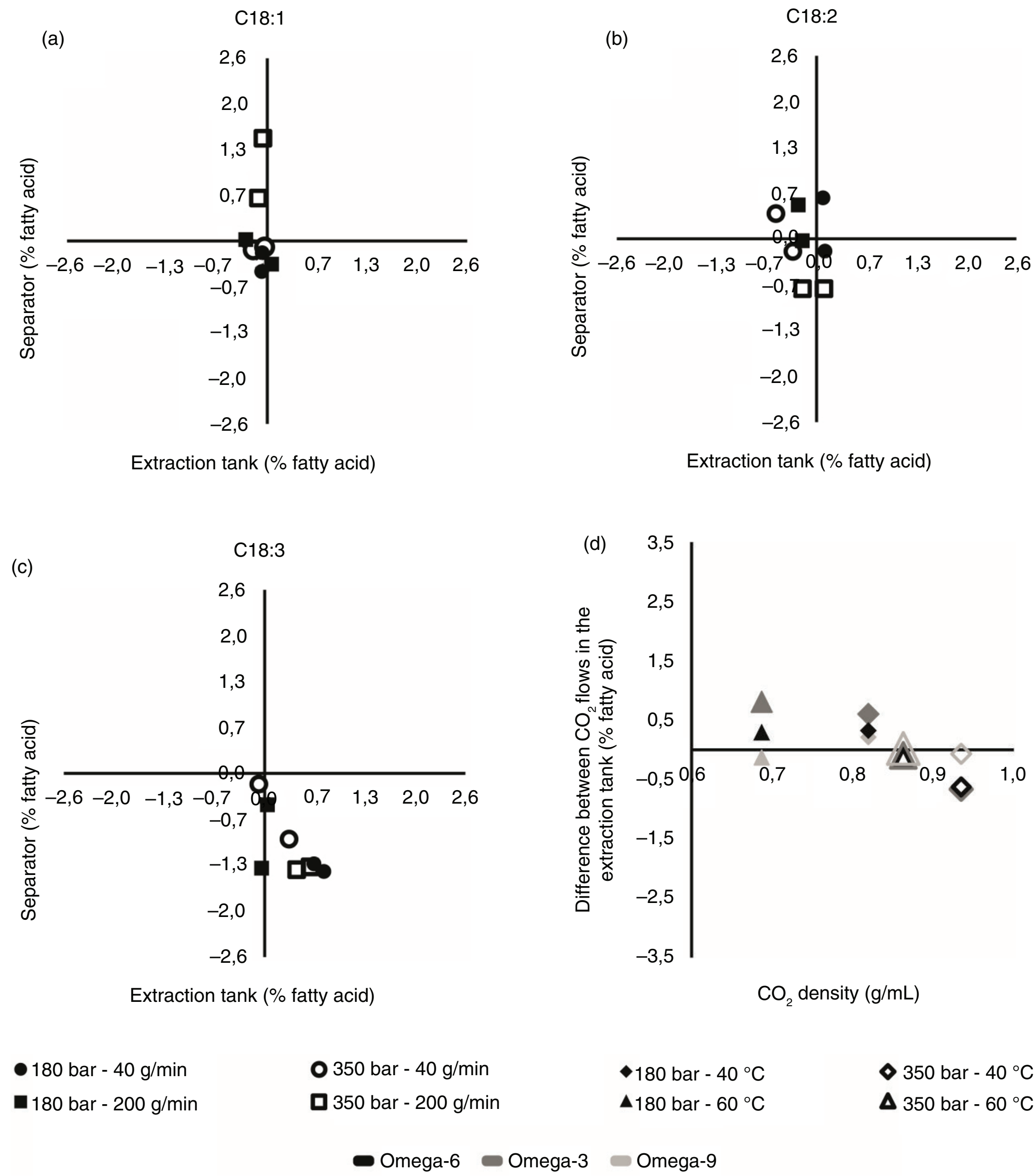


FIGURE 6. Temperature, pressure and $CO_2$ flow effect on the omega composition using a fixed bed of raschig rings. a) Omega-9. b) Omega-6. c) Omega-3. d) Variation in the difference between $CO_2$ flows evaluated (40 and 200 g/min) in each omega with respect to $CO_2$ density.

each pressure and temperature group in the extraction tank. This means that the proportion of the omegas 3, 6 and 9 obtained with a $CO_2$ flow of 40 g/min were subtracted from the proportions of these omegas obtained at a $CO_2$ flow of 200 g/min.

In Figure 6, it can be observed that the temperature did not affect the omega-3 proportion in the extraction tank because the omega composition presented a minimum variation in each group on the x-axis, and only the omega-3 showed the biggest difference of 0.77% at 180 bars and 40 g/min from the initial oil (Figure 6c). For omega-9, all treatments decreased its concentration in both the extraction tank and the separator except for those carried out at 350 bars and 200 g/min, which favored the increase in omega-9 in the fraction obtained in the separator with a difference of 1.46% from the initial oil (Figure 6a). For omega-6, a temperature of 40 °C favored its increase between 0.36–0.58% in the separator in the treatments at 180 bars-40 g/min, 180 bars-200 g/min and 350 bars-40 g/min (Figure 6b). For omega-3, the proportion obtained in the separator did not increase with any of the applied treatments (Figure 6c). However, a temperature effect in the treatments at 180 bars-200 g/min and 350 bars-40 g/min was observed, with a smaller decrease in the omega-3 proportion in the separator at 40 °C. These conditions favored the increase in omega-6 in the separator which showed the difficulty in the selective separation of omega-3.

In Figure 6d the variation in the omega-3, 6 and 9 concentrations with $CO_2$ flow and $CO_2$ density is illustrated, showing that the omega proportions in the extraction tank at a pressure of 180 bars were favored at a $CO_2$ flow of 40 g/min except for omega-9, which was favored at a pressure of 350 bars with a $CO_2$ flow of 200 g/min. This shows that the omegas present a similar chemical behavior which makes their separation difficult.

Subsequently, the statistical analysis of the extraction parameters on the omega-3 proportion in the extraction tank fraction using a fixed bed of raschig rings was achieved employing the R-project software version 3.1.2 (R Core Team, 2014). This study consisted of an analysis of variance (ANOVA) followed by the means comparison through the t-Student and Tukey tests which resulted in a statistically significant interaction effect between pressure and extraction flow factor (p-value, 0.0474). It showed a higher omega-3 concentration in the extraction tank at a pressure of 180 bars and $CO_2$ flow of 40 g/min. However, there was a low oil fractionation and low process yield between 0.07–5.32 g extract/100 g of oil in all the ranges evaluated.

Sacha inchi oil fractionation using glass beads of 4 mm presented better sample distribution and higher increase in the omega-3 concentration in both the extraction tank fraction and the extract. Moreover, there was a decrease of 0.19% at 180 bars-200 g/min and 1.21% at 180 bars-40 g/min in the omega-6 proportion in the extraction tank and the separator, respectively, as shown in Figure 7.

Figure 7 was prepared in the same manner as Figure 6. Here, the oil fractionation with the fixed bed of glass beads mentioned above generated a variation in the omega proportions in both the extraction tank and separator and it caused a difference between treatments at $CO_2$ flow of 40 g/min and 200 g/min in each group of the same pressure and temperature in the extraction tank. It was also observed that the temperature had little effect on the omega concentrations in the extraction tank on their proportions in the initial oil, showing the largest displacements in the x-axis up to 1.23% at 180 bars-40 g/min for omega-3 (Figure 7c). Moreover, some treatments increased the omega-3 proportion in the separator when a fixed bed of glass beads was employed, presenting the highest increase (1.54%) at 350 bars-40 g/min due to the major contact surface between oil and supercritical $CO_2$ provided by this fixed bed.

The omega-9 proportion mainly increased in the separator (Figure 7a), achieving a difference of up to 1.93% at 180 bars-40 g/min, and a temperature effect is observed on the omega-9 proportion in the separator in the treatments at 180 bars-40 g/min and 350 bars-200 g/min. These last conditions generated an increase of 1.65% at 60 °C in the omega-9 concentration while at 40 °C its proportion decreased by 0.44%. For omega-6, its proportion in the extraction tank was up to 0.29% at 350 bars-40 g/min (Figure 7b). It was higher than the maximum omega-6 proportion achieved of 0.11% at 180 bars-40 g/min using a fixed bed of raschig rings. Moreover, the omega-6 proportion in the separator was favored at 180 bars-200 g/min and 350 bars-40 g/min by using a fixed bed of glass beads. Also, Figure 7d shows that the omega proportions at 180 and 350 bars was favored by a $CO_2$ flow of 40 g/min except for omega-9, whose concentration at 350 bars-60 °C was favored by a $CO_2$ flow of 200 g/min as noted in Figure 7a.

Subsequently, the ANOVA of the extraction parameters on the omega-3 proportion in both the extraction tank fraction and the separator fraction using a fixed bed of glass beads showed a significant effect of the extraction flow on the omega-3 concentration in the extraction tank (p-value, 0.0218) where the omega-3 concentration increased at a $CO_2$ flow of 40 g/min. For the omega-3 proportion in the separator, significant effects of the pressure (p-value, 9.96E-05) and the interaction between pressure and $CO_2$ flow (p-value, 8.69E-05) were observed where the highest omega-3 concentration was achieved at 350 bars and 40 g/min. All experiments presented a process yield between 0.58–9.82 g extract/100 g of oil.

Finally, when a fixed bed of glass beads is employed there was a major effect of the operation

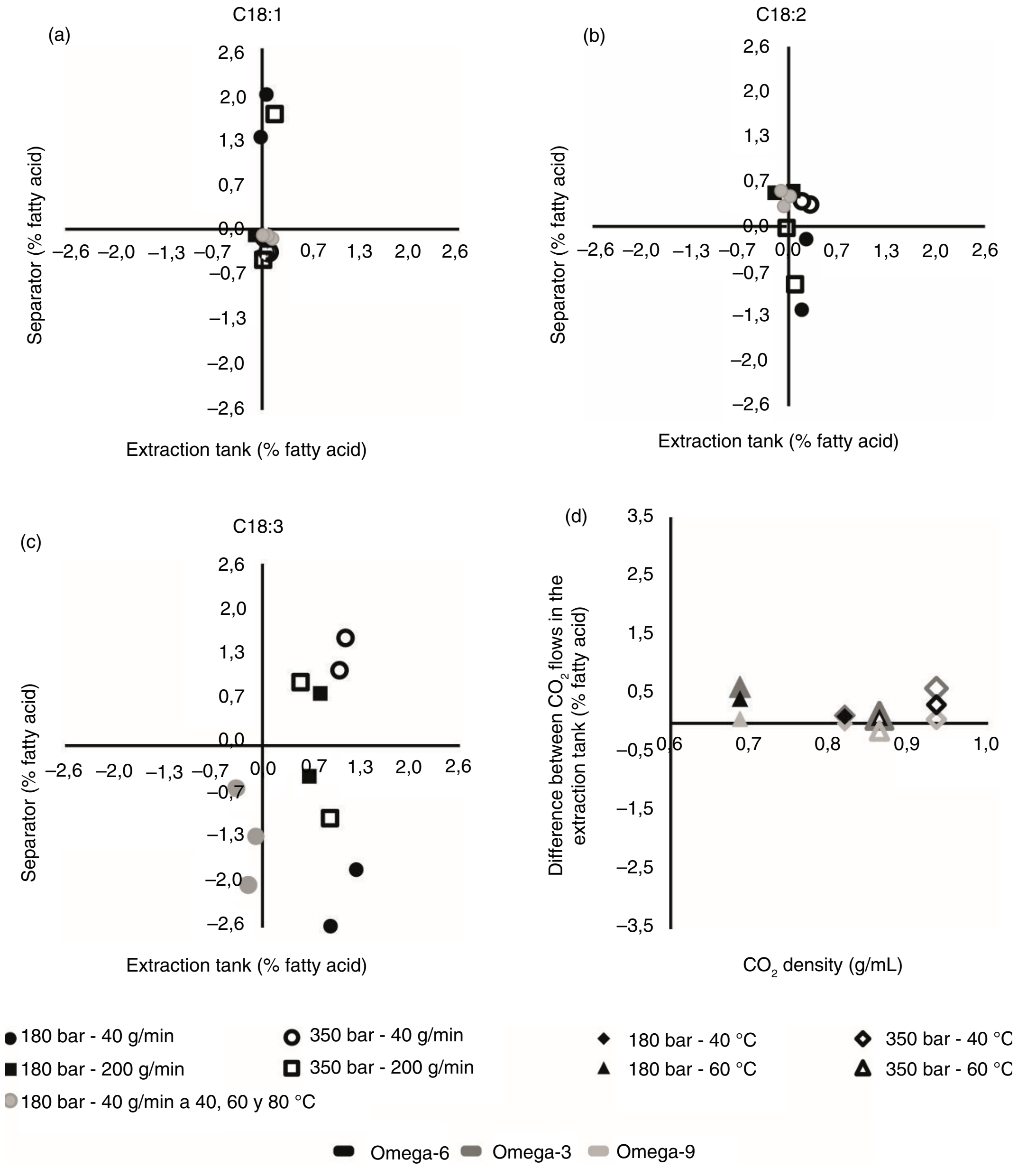


FIGURE 7. Temperature, pressure and $CO_2$ flow effect on the omega composition using a fixed bed of glass beads. a) Omega-9. b) Omega-6. c) Omega-3. d) Variation in the difference between $CO_2$ flows evaluated (40 and 200 g/min) in each omega with respect to $CO_2$ density.

parameters on the omega-3 proportion in the Sacha inchi oil, but the increase in the omega-3 proportion was not significant, neither was the maximum process yield of 5.32 and 9.82 g extract/100 g of oil using a fixed bed of raschig rings and glass beads, respectively. However, the oil fractionation using a fixed bed of glass beads presented a temperature effect on the omega-3 proportion in the separator at 350 bars-200 g/min and 180 bars-200 g/min and in the omega-9 proportion in the separator at 350 bars-200 g/min and 180 bars-40 g/min. This last condition caused the highest increase in the omega-9 proportion in the separator. Therefore, the extraction temperature was evaluated using a bed of glass beads at a pressure of 180 bars, $CO_2$ flow of 40 g/min and temperatures of 40, 60 and 80 °C in order to promote the increase in the omega-3 proportion in the extraction tank fraction, increasing the omega-9 proportion in the separator due to low process yield with respect to the extract obtained.

Figure 7 shows the temperature effect on the proportions of omegas 3, 6 and 9 in the Sacha inchi oil at 180 bars and 40 g/min. This figure shows that the extraction temperature did not have any effect on the omega proportions in the extraction tank fraction. However, there was a statistically significant effect on the omega-3 proportion in the separator (p-value, 0.00295) where all treatments decreased the omega-3 proportion while the omega-6 proportion increased by 0.52% at 80 °C.

The results mentioned above can be caused by the nature of the Sacha inchi oil because approximately 80% of its triglycerides contain at least a fraction of omega-3 that makes the increase in the omega-3 proportion in the oil difficult (Fanali *et al.,* 2011). This means that if triglycerides are removed with other fatty acids, omega-3 fractions can be discarded avoiding the increase in its proportion. Therefore, a test with ethyl esters of the Sacha inchi oil was made to promote the increase in the omega-3 proportion. The sample was fractionated using a fixed bed of glass beads, temperature of 80 °C, pressure of 180 bars, $CO_2$ flow of 40 g/min and extraction time of 120 min and a difference between the omega proportion and the initial ethyl esters of −0.56% for omega-9, 0.36% for omega-6 and 0.46% for omega-3 in the separator was observed along with a difference of −0.95% for omega-9, −0.46% for omega-6 and 2.60% for omega-3 in the extraction tank. Although the increase in the extraction tank of the omega-3 proportion as ethyl ester (2.60%) was higher than the increase in the omega-3 proportion as triglyceride (1.23%), this difference is not significant, so the new conditions evaluated are not viable. It is due to the same carbon number in the omegas which only have different unsaturation degrees and this makes their selective separation difficult (Markom *et al.,* 2001).

## 4. CONCLUSIONS

The Sacha inchi oil extraction with supercritical $CO_2$ was scaled up to a volume of 12 L, keeping the ratio between $CO_2$ flow and raw material mass constant. A scaling process achieved a recovery of 60% at an extraction time of 105 min. This recovery was higher than the recovery achieved at a volume of 1 L at the same time. Therefore, the scaling factor used was appropriate, because it allowed for an increase in recovery and process productivity.

The fatty acid composition of the Sacha inchi oil and the iodine value were not affected during the rapid extraction period. It showed that the oil quality was not affected by prolonged exposure at a temperature of 60 °C and pressure of 450 bars.

This is the first report concerning the scale-up at 12 L of Sacha inchi oil extraction using Supercritical $CO_2$. The results of this work suggest a potential to continue scaling up, designing and evaluating the financial viability of this process.

Due to the similarity in the carbon number of fatty acids in the oil (16–18 C), and the omega-3 presence in the majority of triglycerides present in Sacha inchi oil, the fractionation of this oil was not reached, and an omega-3 significantly rich fraction was not obtained.

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