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ORIGINAL PAPER

Supercritical Carbon Dioxide Extraction and Characterization of Argentinean Chia Seed Oil

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Abstract Extraction of chia seed oil was performed with supercritical carbon dioxide (SC-CO_2). To investigate the effects of pressure and temperature on the oil solubility and yield, two isobaric (250 and 450 bar) and two isothermal (40 and 60 °C) extraction conditions were selected. The global extraction yield of chia oil increased with pressure enhancement, but temperature had a little influence on it. The maximum oil recovery using SC-CO_2 at a mass flow rate of 8 kg/h was 97%, which was obtained at 60 °C, 450 bar for a 138-min extraction. The results showed that solubility changed from 4.8 g oil/kg CO_2 at 60 °C–250 bar to 28.8 g oil/kg CO_2 at 60 °C–450 bar. The final extract obtained by SC-CO_2 under different conditions and Soxhlet extraction contained mainly α-linolenic (64.9–65.6%) and linoleic (19.8–20.3%) acids. SC-CO_2 extraction is an interesting alternative methodology because it is possible to achieve a chia oil yield close to that obtained by conventional extraction with a similar fatty acid composition using an environmentally friendly process.

Keywords Antioxidants · Chia seed oil · Fatty acids · Omega-3 · Supercritical extraction

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Introduction

With the growing body of evidence that not all fats and oils are equivalent in relation to health benefits, interest in specific fatty acid (FA) composition has emerged. Among the polyunsaturated FA (PUFA), the most important families are the ω-3 and ω-6 fatty acids [1]. These compounds are considered essential because the human body is unable to synthesize them, so incorporating them into our diet plays a crucial role with regard to health. The consumption of oils rich in ω-3 FA is very important because they play a fundamental role in the prevention and treatment of coronary artery diseases, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer [2].

Dietary sources of ω-3 PUFA include fish oils rich in eicosapentaenoic and docosahexaenoic acids and plants oils rich in α-linolenic acid (ALA) [3]. Chia (*Salvia hispanica* L.) seeds comprise about 25–38% oil by weight, with the highest proportion of ALA (~60%) compared to other known vegetable species, and a high content of linoleic acid (LA) (~20%) [4]. Dubois et al. [5] have considered chia seed oil as an interesting source of these two essential FA with a good equilibrium between them. Chia seeds are consumed in Mexico, the southwestern United States and South America, but they are not widely used in

other parts of the world. They have been investigated and recommended for their oil, protein, antioxidant and dietary fibre content [4–6]. Nowadays, chia seed oil is receiving increased attention, since it can improve human nutrition by providing a natural, plant-based source of ω-3 FA and antioxidants.

Similar to other commercial vegetable oils, chia seed oil is produced by either cold pressing or organic solvent extraction. Cold pressing, results in a partial recovery of the oil seeds whereas organic solvents such as hexane pose safety risks and health and environmental hazards and its replacement is being sought by the oil industry.

The supercritical fluid extraction (SFE) technique has been studied extensively as an alternative to conventional methods of extraction. The SFE is a mass transfer process based on the use of fluids at temperatures and pressures above their critical values. This separation technique offers extraction yields comparable to those obtained by conventional extraction processes using liquid solvents, but it requires a certain combination of operating parameters. Supercritical carbon dioxide (SC-CO_2) has been the most frequently used fluid for oil extraction. In processing terms, carbon dioxide has a low critical temperature and pressure (31.1 °C and 73.8 atm, respectively), which makes it the ideal solvent for natural products, which would not suffer thermal degradation reactions during the process [7].

SC-CO_2 extraction of various oil seeds, such as soybean, safflower, cottonseed, canola, millet bran, and rice has been reported [8, 9]. Extraction of oils rich in ω-3 FA, such as fish and flaxseed oil has been also investigated [10]. Even though there are numerous reports on SC-CO_2 extraction of oil from various seeds, the literature related to chia seed oil extractions using SC-CO_2 is limited [11].

The objectives of this work were to investigate SC-CO_2 oil extraction from chia seed using a pilot-scale extraction and to study the influence of pressure and temperature on oil solubility and recovery. The quality of the SC-CO_2 extracted oil was also studied in terms of its FA composition, tocopherols, polyphenolic compounds and oxidative stability and then compared to those factors in oil obtained by hexane extraction.

Experimental Procedures

Materials

Commercial chia seeds (*S. hispanica* L.) were purchased from Functional Products S.A., Argentina. Liquid CO_2 (food grade) from PRAXAIR (Córdoba, Argentina) was used as the solvent in the SC-CO_2 extraction process. All reagents used for the analysis were of analytical reagent grade.

Methods

Sample Preparation

Chia seeds were manually cleaned to separate the extraneous matter (dust, vain (empty) seeds and straw from threshed seeds). Cleaned seeds were homogenized and packed in hermetic plastic vessels and stored at 5 °C until further use. The initial moisture content was determined according to the AOAC approved vacuum oven method [12].

Seeds used for SC-CO_2 were dehydrated by heating at 40 °C until constant weight, ground using a coffee mill (Braun, Type 4041, Mexico) for 60 s, and fractionated using a sieve (ASTM # 18; mesh opening 1 mm).

Oil Extraction by Soxhlet

In order to determine the oil content, ground seed samples (40 g) were extracted with *n*-hexane (boiling point 68–72 °C) in a Soxhlet apparatus by thermal cycles at 80 °C for 16 h, following the IUPAC Standard Method 1.122 [13]. The solvent was removed using a rotary vacuum evaporator at 40 °C (Büchi, Flawil, Switzerland), under a nitrogen stream. The amount of oil was estimated gravimetrically; this value will be referred as the initial oil content of chia seeds. Reported values are means of three determinations. Relative concentrations (%) of triacylglycerols identified in Argentinean chia (*Salvia hispanica* L.) seed oil obtained by solvent extraction are reported in Table 1 [14].

Oil Extraction by Supercritical Carbon Dioxide

The extraction trials were carried out on a pilot plant system (extractor volume: 1.5 L) with a single step separation

Table 1 Relative concentrations (%) of triacylglycerols identified in Argentinean chia (*Salvia hispanica* L.) seed oil obtained by solvent extraction (Ixtaina et al. 2010) [14]

Triacylglycerol	%
LnLnLn	32.8
LnLnL	20.3
LnLL	13.8
LnLnP	7.7
LnLO	7.0
LnLP	5.3
LnOO + LnOP	8.3
LnPP	0.8
LLS	1.1
LnOS	2.1
LnSP	1.0

Ln alpha linolenin, *L* linolein, *O* olein, *P* palmitin, *S* stearin

and solvent recycle capacity. It can be operated at pressures up to 500 bar and flow rates up to 20 kg CO_2/h. A detailed description of this plant can be found in Ruetsch et al. [15]. Figure 1 shows the schematic layout of the supercritical extraction plant. Extraction experiments were done at two pressures (250 and 450 bar) and temperatures (40 and 60 °C) with a CO_2 mass flow rate of 8 kg/h which was measured with a mass flowmeter (Rheonik, Germany). The extracts were collected in the separator vessel at 60 bar and 40 °C. In each experiment, about 500 g of ground chia seeds were used. At approximate time intervals of 5 min, the extracts were collected in dark glass vessels and the amount of oil was determined gravimetrically, recording the corresponding total mass of CO_2 used. The end of the extraction was set when the difference between two consecutive measurements of oil extracted was ≤0.001 g oil/g dry seeds. The oil yield was defined as the ratio between the amount of oil recovered during the extraction and the amount of oil initially-containing in chia seeds (determined by Soxhlet). Initially, the samples were fractionated into four fractions of about 40–50 g each, as a function of time, i.e. the first fraction consisted of the first 40–50 g oil obtained from extraction followed by the second fraction containing the second 40–50 g oil, and so on. After the determination of their FA composition, the four fractions were combined in one lot to obtain the final extract. In all cases the oils were stored at 4 °C.

Oil Analytical Determinations

Fatty Acid Composition

The FA composition expressed as fatty acid methyl esters (FAME) was determined in each of the four fractions obtained from the extract at different operative conditions and in the final extract, as follow: 100 μL oil plus 1 mL 10% KOH in methanol were heated for 45 min at 85 °C. Non-saponifiable lipids were extracted with petroleum ether (b.p. 30–40 °C). After acidification with HCl, saponified FAs were extracted from the methanolic phase with petroleum ether. Fatty acids were methylated with 1 mL of a boron trifluoride-methanol-complex (20% solution in methanol) (Merck) and 1 mL of methanol for 45 min at 60 °C, and then extracted from the methanolic phase with petroleum ether. For the GC analysis, 1 μL of hexane solution of FAME was injected in a HP 6890 (Hewlett Packard, USA) gas chromatograph equipped with a Supelco 11090-02A Omegawax (30 m × 0.250 mm, i.d. 25 μm) capillary column. The separation was carried out at 175–220 °C (3 °C/min) with helium as the carrier gas at a constant pressure of 25.1 psi and a FID detector at 260 °C. The results were expressed as the relative area percentage of each individual FA present in the sample.

Iodine and Saponified Values

Iodine and saponified values were determined according to AOCS recommended practices Cd 1c-85, Cd 3a-94, respectively [16].

Tocopherol Analysis

Oil tocopherol content was determined by normal phase HPLC using a Hewlett Packard chromatographic system (HPLC Hewlett Packard 1050 Series, Waldbronn, Germany) following the procedures described in IUPAC 2.432 [13] and AOCS Ce8-89 [16]. Approximately 0.25 g of oil in 5 mL of hexane was placed in an ultrasonic bath

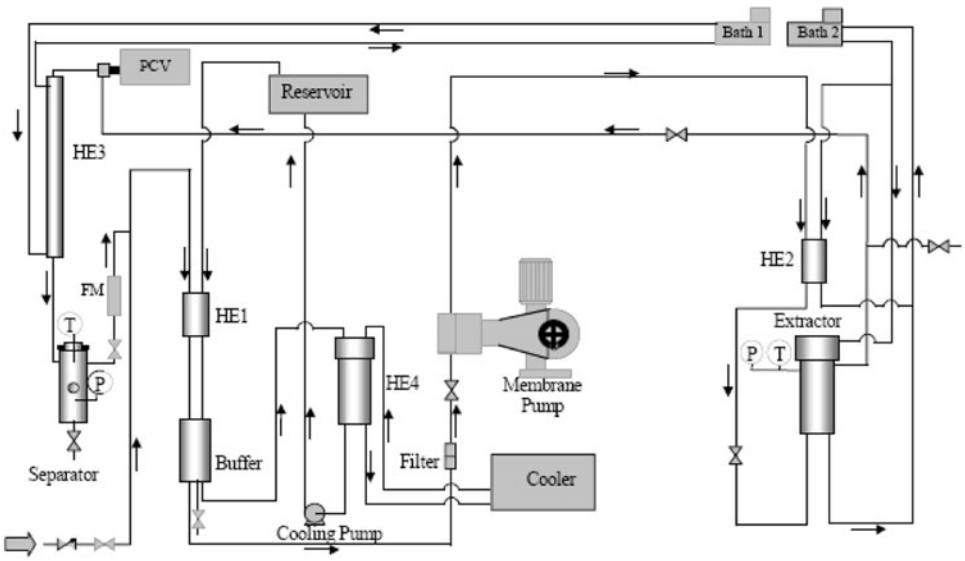

Fig. 1 Schematic layout of the experimental set-up (supercritical extraction plant) (Ruetsch et al. 2003) [15]. *HE* heat exchanger, *PCV* pressure control valve, *T* temperature sensor, *P* pressure gauge, *FM* mass flowmeter

for 2 min and protected from light. A 20-μL aliquot of this solution was then injected into a LiChrosorb Si 60 column (5 μm, 25 cm × 4.00 mm, Merck, Darmstadt, Germany) using *n*-hexane: isopropanol (99.5:0.5, HPLC solvent, J. T. Baker, Phillipsburg, USA) as mobile phase at a flow rate of 1.5 mL/min. Tocopherols were detected using a fluorescence detector (Agilent Technologies 1100 Series Fluorescence Detector G1321A, Palo Alto, CA, USA) with the excitation/emission wavelength set at 290/330 nm, and quantified using a six-point external standard curve.

Polyphenol Analysis

Oil samples (2 g) were dissolved in 1 mL of hexane. Polyphenolic compounds were extracted by adding 1 mL of acetonitrile and 1 mL of 10% acetic acid. Samples were centrifuged and the bottom phase was collected. This procedure was repeated three times and the pooled extracts were evaporated to dryness by using a SpeedVac evaporator (Heto VR-1). The residue was dissolved in acetonitrile: 10% acetic acid (50:50) and analyzed by HPLC.

HPLC analysis was performed using a Hewlett Packard 1050 system (Avondale, PA, USA), quaternary pump, with Metasil 3 μm ODS 150 × 4.6 mm column from Varian and guard column Widepore C_{18} ODS 3 × 4 mm from Phenomenex (CA, USA). The mobile phase was acetonitrile: 10% acetic acid, in the following gradient: (90:10), 0–5 min; (90:10)–(40:60), 5–15 min; (40:60)–(20:80), 15–16 min; (20:80)–(90:10), 18–20 min. The flow rate was set at 1 mL/min; column temperature was maintained at 35 °C. Peak detection was made by checking UV absorption at 323 nm (0–5 min) and 375 nm (5–20 min).

Polyphenolic compounds were identified by comparing their retention times with those of authentic standards: 2.7 min for chlorogenic acid, 3.6 min for caffeic acid, 9.8 min for myricetin, 11.8 min for quercetin and 13.3 min for kaempferol. These compounds were quantified using external standard curves of concentration that ranged from 10^{-3} to 10^{-7} M.

Oxidative Stability

Oil oxidative stability was evaluated by the Rancimat (Mod 679, Metrohm) method, using a 5-g oil sample warmed at 98 °C with air flow of 20 L/h. Oil stability was expressed in terms of induction time (h).

Statistical Analysis

$SC\text{-}CO_2$ extraction of chia seeds at each temperature and pressure condition was carried out in duplicate. Oil analytical determinations of each extract were performed in duplicate and the mean values were reported. ANOVA of the results was performed using Statgraphics Plus statistical package (Version 4.0 for Windows, Manugistics Inc., USA). Multiple comparisons of the means were performed by a Tukey test at a significance level (α) of 0.05.

Results and Discussion

Moisture and oil content of chia seeds were 7.0 ± 0.4% (d.b.) and 0.34 g oil/g dry seed, respectively.

$SC\text{-}CO_2$ Extraction

Figure 2 presents the accumulative extraction curves for oil removal by $SC\text{-}CO_2$ at four operative conditions assayed: 40 °C–250 bar, 60 °C–250 bar, 40 °C–450 bar and 60 °C–450 bar, at a CO_2 mass flow rate of 8 kg/h. $SC\text{-}CO_2$ extraction of chia seed oil.

Similarly to the other oil seeds, the extraction can be divided into two periods as fast (an initial linear and a transition period) and slow extraction periods (second linear period). Most of the extraction of chia seed oil mainly occurred in the fast extraction period as can be deduced from the curves. The apparent solubility of chia seed oil, determined from the initial linear portion under each set of processing conditions when the oil yield is plotted as a function of time is shown in Table 2. As can be seen, the largest extraction of chia seed oil in $SC\text{-}CO_2$ occurred mainly in the initial fast period. For example, at 40 °C–250 bar, 0.27 g oil/g dry seed was obtained during the fast extraction period whereas only 0.01 g oil/g dry seed (3% of the initial oil content) was removed during the last 88 min of extraction. A similar trend exists for the other extraction conditions. Similar results were found for Mexican chia

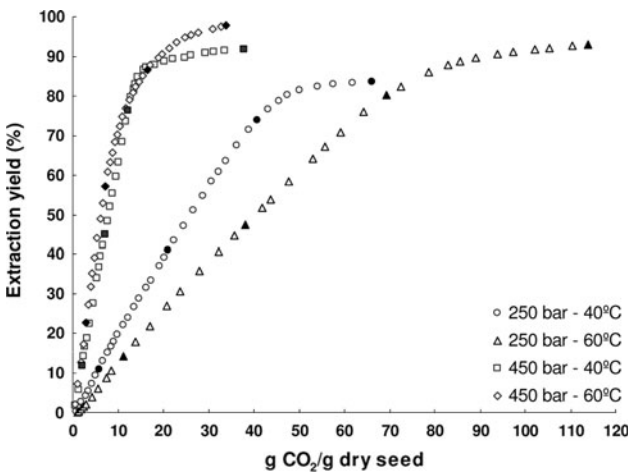

Fig. 2 Extraction yield (g extracted oil/g dry seeds) as a function of specific CO_2 mass (g CO_2/g dry seeds) during $SC\text{-}CO_2$ extraction of chia seed oil. Full points delimit the four fractions collected

and flaxseed oil extractions [11, 17]. According to Özkal [17], in the fast extraction period, the oil released from the oil cells is extracted from the surface of the particles; however, in the slow extraction period, the unreleased oil from the intact cells is removed. In the fast extraction period, the mass transfer rate is determined by the solubility of the oil in SC-CO_2, while in the slow extraction period, it is controlled by the diffusion of the oil in the particles. Özkal et al. [18] reported for apricot kernel oil that the mass transfer rate was low and the oil yield was insignificant in the slow extraction period compared to those corresponding to the fast extraction period. During the fast extraction period the relationship between extraction time and yield was linear. The correlation coefficients values obtained for the fast period at 40 °C–250 bar, 60 °C–250 bar, 40 °C–450 bar and 60 °C–450 bar were 0.995, 0.995, 0.996 and 0.963, respectively ($p \leq 0.0001$).

The total oil yield ranged from 82 to 97%, without any significant differences ($p > 0.05$) between the different operating conditions assayed. Ixtaina et al. [11] reported an oil yield of 92.8% after 300 min of extraction time at 80 °C–450 bar for Mexican chia seeds. Similar results have been found in other vegetable oils, such as Sacha Inchi seeds from which Follegatti-Romero et al. [7] reported a 92.3% recovery at 60 °C–400 bar.

Significant differences ($p \leq 0.05$) were found between the different operative conditions in the duration of the fast extraction period, the total extraction time and the solubility of the oil. At isothermal conditions, an increase of pressure from 250 to 450 bar caused a diminution in the extraction time of about 53% (40 °C) and 67% (60 °C) (Table 2; Fig. 2).

With regard to the extraction temperature, a different effect on the total extraction time, was observed. At 250 bar, a rise in the extraction temperature increased the length of the fast extraction period and the total extraction time whereas at 450 bar no significant effect of temperature was observed (Table 2). Similar trends were reported by Salgin and Salgin in walnut kernel oil [19].

Concerning solubility, the results showed that it changed from 4.8 g oil/kg CO_2 at 60 °C–250 bar to 28.8 g oil/kg CO_2 at 60 °C–450 bar. Changes of the solubility of chia seed oil in SC-CO_2 depend on CO_2 solvent capacity, which is mainly related to solvent density and viscosity. The density and viscosity of CO_2 for each temperature and pressure studied in this work are presented in Table 2. At constant temperature, CO_2 has a higher density in the experiments carried out at 450 bar than at 250 bar, and as a consequence the solubility of the oil is higher as showed in Table 2. An increase in temperature at constant pressure reduces the density of CO_2 but also increases the solute vapor pressure. These opposite effects lead to the well-known crossover phenomenon [20] and it has been observed for the extraction with CO_2 of similar oils like Sacha Inchi oil [7]. In this work an increase in temperature at 250 bar leads to a decrease in the solubility, while the same increase at 450 bar causes an increase in solubility of the oil in CO_2. Although the solubility results could be attributed to the crossover effect more experiments at different pressure and temperature conditions would be necessary to prove it. Solubility of chia seed oils under all the operating conditions studied (Table 2) was higher than that reported for Sacha Inchi oil [7] (4.4, 1.7, 14.7 and 16.7 g oil/kg CO_2 at 40 °C–200 bar, 60 °C–200 bar, 40 °C–400 bar and 60 °C–400 bar, respectively). These differences can be attributed to the different operating pressure and fatty acid composition in the oils, e.g. the chia seed oil contains higher amounts of linolenic acid (~65%) than Sacha Inchi oil (~45%).

Table 2 Extraction yield, time and solubility of chia seed oils obtained by SC-CO_2

	SC-CO_2 extraction			
	40 °C–250 bar	60 °C–250 bar	40 °C–450 bar	60 °C–450 bar
Amount of oil extracted by the end of the fast extraction (g oil/g dry chia seed)	0.27 ± 0.01[a]	0.30 ± 0.03[a]	0.29 ± 0.07[a]	0.29 ± 0.07[a]
Amount of oil extracted at the end of the extraction (g oil/g dry chia seed)	0.28 ± 0.01[a]	0.31 ± 0.03[a]	0.31 ± 0.07[a]	0.33 ± 0.07[a]
Oil yield by the end of the fast extraction period (%)	79 ± 3[a]	88 ± 5[a]	85 ± 0.2[a]	85 ± 2[a]
Oil yield at the end of the extraction (%)	82 ± 3[a]	91 ± 5[a]	91 ± 2[a]	97 ± 2[a]
Fast extraction period (min)	197 ± 2[b]	317 ± 3[c]	56 ± 3[a]	70 ± 2[a]
Slow extraction period (min)	88 ± 2[b]	106 ± 3[c]	79 ± 2[ab]	68 ± 2[a]
Total extraction time (min)	285 ± 2[b]	423 ± 3[c]	135 ± 2[a]	138 ± 2[a]
CO_2 density (kg/m^3)	879.6	786.8	974.6	913.4
CO_2 kinematic viscosity ($\times 10^{-7}$ m^2/s)	0.9897	0.8869	1.159	1.053
Solubility (g oil/kg CO_2)	7.0 ± 0.1[a]	4.8 ± 0.1[a]	22.3 ± 0.9[b]	28.8 ± 3.6[b]

Mean values ± standard deviation ($n = 2$) followed by different letters differ at $p \leq 0.05$, according to Tukey (HSD) test

In order to calculate the effective diffusivity coefficient of the oil from the chia matrix, the simple extraction model presented by Catchpole et al. [21] was used to fit the experimental data already presented in this work. Following the procedure proposed by these authors, when the extraction rate is controlled by the intraparticle diffusion and the solubility of the extract is very high compared with the initial concentration of the extract in the herb material, a very simple solution can be obtained from Eq. 1 with only the effective diffusivity coefficient as parameter.

$$E = 1 - \exp\left[-\frac{15D_e(t - z\varepsilon/U)}{R^2}\right] \quad (1)$$

where E is the yield of extract, D_e is the intraparticle effective diffusivity coefficient, t is the extraction time, z is the extraction bed height; ε is the bed porosity, U is the interstitial velocity, and R is the particle radius.

Diffusivity coefficients have been correlated to experimental extraction data by minimization of the difference between experimental and calculated extraction yield, according to the objective function presented in Eq. 2.

$$\text{AARD } \% = \frac{100}{n}\sum_{i=1}^{n}\frac{|y_{i\,\text{exp}} - y_{i\text{calc}}|}{y_{i\,\text{exp}}} \quad (2)$$

where n is the number of data points, and, $y_{i\text{exp}}$ and $y_{i\text{calc}}$ are the experimental and calculated extraction yield, respectively.

Since Eq. 1 is only valid for when the extraction is controlled by intraparticle diffusion, only experimental data where the extraction yield was over 90% were used. In this region, the model was able to reproduce the experimental data with a good accuracy obtaining average absolute relative deviations (AARD%) lower than 1% for every experimental temperature and pressure conditions. The intraparticle diffusion coefficients obtained with this methodology and the corresponding average absolute relative deviation of the fitted data are presented in Table 3. The values obtained for the diffusion coefficient are similar to those reported by several authors for the oil extraction from similar matrices with supercritical CO_2, such as coriander seed (2.57×10^{-11} m^2/s at 40 °C/250 bar) [21] and sunflower (9.18×10^{-10} m^2/s at 40 °C/400 bar) [22].

Table 3 Intraparticle diffusion coefficients fitted according to Catchpole et al. 1996 [21]

Temperature (°C)	Pressure (bar)	Diffusion coefficient (m^2/s)	AARD%
40	250	5.81×10^{-12}	0.21
60	250	3.04×10^{-12}	0.53
40	450	1.19×10^{-11}	0.09
60	450	9.25×10^{-12}	0.52

Under an isothermal condition, the D_e increased with pressure, whereas at a given pressure, D_e decreased with temperature. Similar behavior was observed by Salgin et al. [22] in SC-CO_2 sunflower oil extraction.

Analytical Oil Determinations

Based on the FA compositions (Table 4), the final extract of chia oil obtained by SC-CO_2 under different conditions and Soxhlet extraction contained mainly α-linolenic acid (64.9–65.6%), linoleic acid (19.8–20.3%), palmitic acid (6.2–6.7%), oleic acid (5.0–5.5%) and stearic acid (2.7–3%). These results are similar to those previously reported for Argentinean and Mexican chia seed oils [4, 11, 23]. The high content of polyunsaturated fatty acids (~85%) and the low proportion of saturated fatty acids (~9%) make chia seed oil a very appropriate vegetable oil for incorporation in the development of functional foods. In addition, the high amount of α-linolenic acid in this oil enhances its nutritional value.

Table 4 presents the FA composition corresponding to the final extracts of oils obtained by SC-CO_2 and hexane. In general, no significant differences were observed ($p > 0.05$) in the FA composition of oils obtained by the different methodologies. Only the linoleic acid content was slightly but significantly higher ($p \leq 0.05$) in oils obtained by SC-CO_2 than in the oil extracted by hexane. Also significant differences ($p \leq 0.05$) were found for this fatty acid in oils obtained at different operative conditions. Bozan and Temelli [10] reported that the FA composition of SC-CO_2 extracted oils was different from that of oils obtained by Soxhlet extraction using petroleum ether. These authors reported that SC-CO_2 extracted oil presented a higher content of PUFAs than that of solvent-extracted oil. Martínez et al. [24] reported minor differences in individual fatty acid content among the walnut oils obtained by SC-CO_2 at different pressure and temperature conditions.

The properties of chia seed oil extracted by SC-CO_2 and those obtained by conventional extraction with hexane were not significantly different in terms of the main physicochemical parameters, such as the saponification index and iodine value (Table 4). Similar results were found by Follegatti-Romero et al. [7] for Sacha Inchi oil. The high iodine value is related to the fatty acid composition of chia seed oil, riched mainly in PUFAs.

Figure 3 shows the unextracted amount (%) of each FA during the SC-CO_2 process for different operating conditions. For a proper interpretation of the results, two different phenomenological approaches should be considered. The first one is related to the mechanisms involved in the extraction of oils from herb matrices with supercritical fluids previously explained. It is well known that extraction

Table 4 Fatty acid composition, iodine and saponified values and induction time of the final extract of chia seed oils obtained by SC-CO_2 and solvent extraction

Fatty acids (%)	SC-CO2 extraction				Solvent extraction
	250 bar–40 °C	250 bar–60 °C	450 bar–40 °C	450 bar–60 °C	
Palmitic acid (16:0)	6.6 ± 0.4[a]	6.6 ± 0.2[a]	6.7 ± 0.4[a]	6.7 ± 0.4[a]	6.2 ± 0.4[a]
Stearic acid (18:0)	2.7 ± 0.1[a]	2.8 ± 0.1[a]	3.0 ± 0.3[a]	3.0 ± 0.2[a]	3.0 ± 0.7[a]
Oleic acid (18:1)	5.2 ± 0.1[a]	5.5 ± 0.3[a]	5.2 ± 0.6[a]	5.0 ± 0.1[a]	5.3 ± 1.1[a]
Linoleic acid (18:2)	20.0 ± 0.0[b]	20.2 ± 0.0[c]	20.1 ± 0.1[b,c]	20.3 ± 0.1[c]	19.8 ± 0.0[a]
α-Linolenic (18:3)	65.5 ± 0.3[a]	64.9 ± 0.4[a]	64.9 ± 0.7[a]	65.0 ± 0.4[a]	65.6 ± 0.8[a]
SFA	9.3 ± 0.5[a]	9.4 ± 0.1[a]	9.8 ± 0.1[a]	9.7 ± 0.2[a]	9.3 ± 0.3[a]
PUFA	85.4 ± 0.4[a]	85.1 ± 0.4[a]	85.0 ± 0.7[a]	85.3 ± 0.3[a]	85.4 ± 0.8[a]
PUFA/SFA	9.2 ± 0.5[a]	9.0 ± 0.1[a]	8.7 ± 0.2[a]	8.8 ± 0.2[a]	9.2 ± 0.2[a]
ω-6/ω-3 FA ratio	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.3 ± 0.0[a]
Iodine value (g I_2/100 g oil)	210.4 ± 1.0[a]	209.5 ± 0.7[a]	209.1 ± 1.3[a]	209.4 ± 0.8[a]	210.5 ± 1.1[a]
Saponified value (mg KOH/g oil)	194.14 ± 0.05[a]	193.14 ± 0.04[a]	193.14 ± 0.03[a]	193.16 ± 0.05[a]	193.1 ± 0.07[a]
Induction time (h)	1.12 ± 0.29[a]	1.22 ± 0.08[a]	1.60 ± 0.22[a]	1.53 ± 0.10[a]	2.37 ± 0.06[b]

Mean values ± standard deviation ($n = 2$) followed by different letters differ at $p \leq 0.05$, according to Tukey (HSD) test

SFA saturated fatty acids, *PUFA* polyunsaturated fatty acids; *ω6/ω3 FA ratio* (linoleic acid/α-linolenic acid)

processes have three characteristic steps, a first step where the available oil in the surface of the particles is extracted and the extraction rate is determined by the solubility of the compounds in the supercritical fluid; a last step where the less accessible oil is extracted and the interaction between the solute, solvent and particle controls the overall extraction rate; and a transitional region where both mechanisms are involved [25]. In this work, different fractions of the extract were collected, the first two from the first extraction step, the third one from the transition zone, and the fourth fraction from the last step, which can be delimited by full points in the corresponding extraction curves (see Fig. 2). The second consideration is related to the diverse sources of each FA in the extracts, because these compounds are present in different species of TAG of chia seed oil previously cited (see Table 1). Chia seed oil contains mainly three TAG such as LnLnLn, LnLnL and LnLL, with a similar molecular weight and a related chemical structure which are associated with their solubility in SC-CO_2. These three compounds would be the major ones responsible for the extraction rate observed in the extraction process. Several authors [26–28] reported that the solubility of some types of FA present in certain oils increases with pressure at the same temperature, and decrease with a rise of temperature at a constant pressure. This behavior was observed in the first step of the extraction curves of chia seed oil presented in Fig. 3.

The evolution of the extraction curve corresponding to the different FA gives useful information for understanding the extraction mechanisms and suggest conditions for fractionating the different FA. In virtue of the results obtained, the extraction rate for the unsaturated FA is close related to that corresponding to the overall oil, and the fractionation of these compounds seems to be difficult for the conditions assayed. In contrast, a different behavior was observed for saturated FA (palmitic and stearic acids). Palmitic acid is present in the TAG of the lowest molecular weight (see Table 1) and exhibited a fast extraction rate during the first step (two-first fractions), due to its solubility in SC-CO_2 which is mainly dependent on this characteristic.

For stearic acid, a much more complex behavior was observed with two different trends depending on the extraction pressure. At 250 bar, there is a different extraction rate between the first and second fractions (first extraction step). This behavior would be related to the differences between the stearic acid sources such as LLS, LnOS and LnSP, the last one being more soluble in SC-CO_2 than the others. The fast extraction of LnSP would be responsible for the high initial extraction rate of stearic acid, and when this TAG was removed, a decrease in the extraction rate was recorded. This trend was observed for both temperatures, being more pronounced at 60 °C. Finally, stearic acid is the lowest extracted FA at low pressure conditions and a fractionation between stearic and palmitic acid can be achieved under these conditions, especially at 60 °C.

Figure 4 shows the levels of tocopherol concentration in chia seed oil obtained using hexane and by SC-CO_2 extraction at different temperatures and pressures. The total tocopherol concentration corresponding to chia oil obtained by SC-CO_2 varied between 36 and 95 mg/kg oil, with significant differences ($p \leq 0.05$) as a function of the operative conditions assayed. The total tocopherol content in oils obtained at 450 bar was higher than oil extracted at

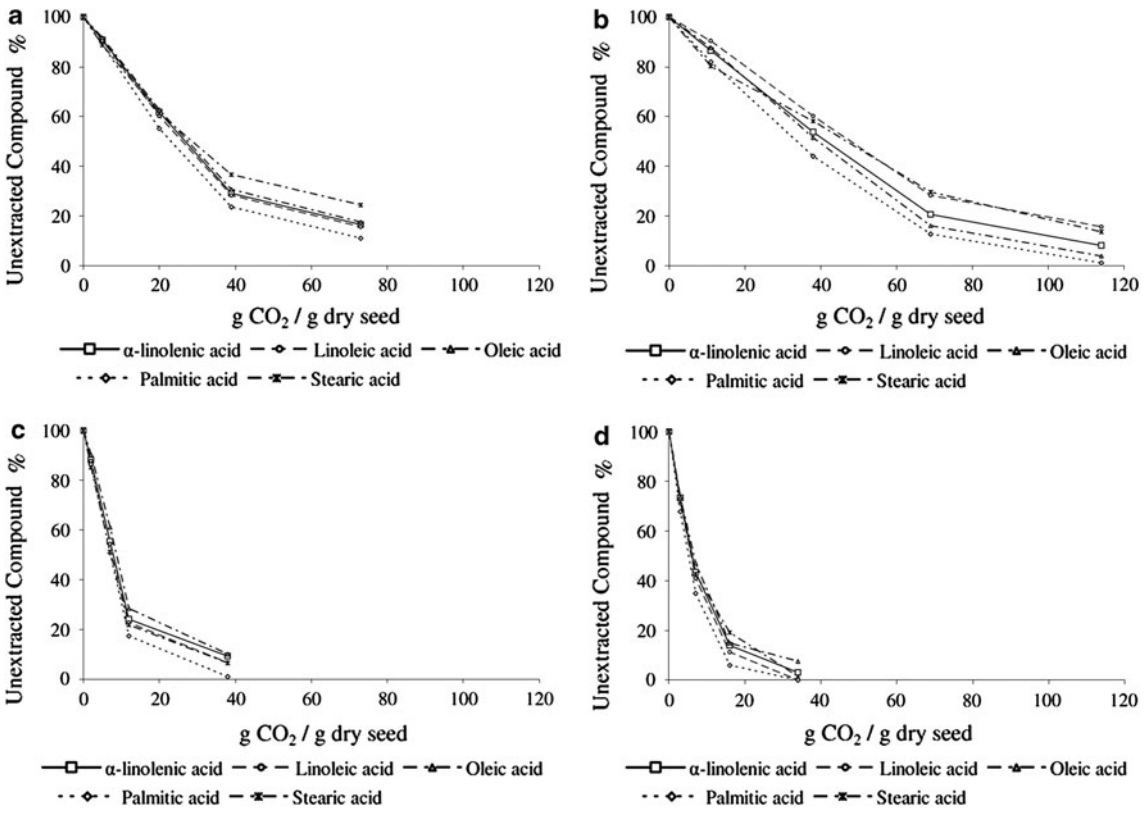

Fig. 3 Unextracted amount (%) of each fatty acid (*FA*) (Unextracted FA(%) = ((Total FA_x in chia oil − Extracted FA_x amount) / Total FA_x in chia oil) × 100) for SC-CO_2 oil extraction: **a** 40 °C 250 bar, **b** 60 °C 250 bar, **c** 40 °C 450 bar, **d** 60 °C 450 bar

60 °C–250 bar, which contained the lowest levels of these compounds. The gamma tocopherol, was the main tocopherol detected in all of the oils obtained by SC-CO_2 (Fig. 4); delta tocopherol was only found at a very low concentration (4.17 mg/kg oil) at 40 °C and 250 bar, the operative conditions in which the lowest oil yield was obtained. Hence this may be due that a low dilution of tocopherol in the oil allowed the detection of delta tocopherol. Authors such as Leo et al. [29] indicated that the great selectivity of the CO_2 for tocopherols means that the concentration of these compounds in the extracted oil is higher during the initial extraction phase than during the subsequent extraction phases. However, other factors could also affect the concentration of tocopherols, such as co-solvent effect of the oil [30], and thermal degradation [31], making it necessary to do further research. Nevertheless, the tocopherol content of the SC-CO_2-extracted oil was significantly lower ($p \leq 0.05$) than that of the hexane-extracted oil. Similarly, studies have indicated that the tocopherol content of SC-CO_2 extracted cottonseed and flaxseed oils was found to be lower than that of solvent-extracted oil [10].

The main polyphenolic antioxidants present in the chia seed oil were: caffeic acid > myricetin ≈ chlorogenic acid ≈ quercetin > kaempferol, with no significant

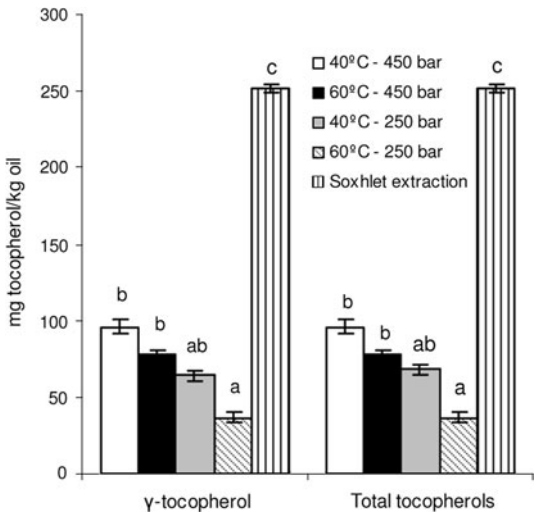

Fig. 4 Tocopherol content of chia seed oil obtained by SC-CO_2 and Soxhlet extraction

differences ($p > 0.05$) in their content between the assayed conditions (Table 5). These compounds are the same substances detected in chia whole seeds [6], although much lower levels were found in the oil than in the chia seeds. This fact is mainly related to the hydrophilic and polar nature of these compounds whose chemical structures

Table 5 Polyphenolic compounds of chia seed oils obtained by SC-CO_2 and solvent extraction

Polyphenolic compound (mol/kg)	SC-CO_2 extraction				Solvent extraction
	40 °C–250 bar	60 °C–250 bar	40 °C–450 bar	60 °C–450 bar	
Chlorogenic acid	$1.31 \times 10^{-5} \pm 2.17 \times 10^{-6a}$	$1.05 \times 10^{-5} \pm 5.95 \times 10^{-8a}$	$9.55 \times 10^{-6} \pm 1.42 \times 10^{-9a}$	$2.58 \times 10^{-5} \pm 2.00 \times 10^{-5a}$	$3.54 \times 10^{-5} \pm 7.16 \times 10^{-6a}$
Caffeic acid	$3.01 \times 10^{-5} \pm 4.61 \times 10^{-6a}$	$2.44 \times 10^{-5} \pm 2.57 \times 10^{-7a}$	$2.15 \times 10^{-5} \pm 4.47 \times 10^{-7a}$	$5.92 \times 10^{-5} \pm 4.59 \times 10^{-5a}$	$3.10 \times 10^{-5} \pm 7.81 \times 10^{-7a}$
Myricetin	$1.35 \times 10^{-5} \pm 6.33 \times 10^{-7a}$	$1.75 \times 10^{-5} \pm 2.31 \times 10^{-6a}$	$1.00 \times 10^{-5} \pm 6.97 \times 10^{-8a}$	$2.57 \times 10^{-5} \pm 2.32 \times 10^{-6a}$	$1.14 \times 10^{-5} \pm 7.44 \times 10^{-6a}$
Quercetin	$1.23 \times 10^{-5} \pm 1.76 \times 10^{-6a}$	$9.73 \times 10^{-6} \pm 3.48 \times 10^{-7a}$	$9.38 \times 10^{-6} \pm 2.02 \times 10^{-7a}$	$2.34 \times 10^{-5} \pm 1.83 \times 10^{-5a}$	$1.07 \times 10^{-5} \pm 2.02 \times 10^{-7a}$
Kaempferol	$3.48 \times 10^{-6} \pm 5.35 \times 10^{-7a}$	$2.89 \times 10^{-6} \pm 1.85 \times 10^{-8a}$	$2.63 \times 10^{-6} \pm 1.56 \times 10^{-8a}$	$7.16 \times 10^{-6} \pm 5.77 \times 10^{-6a}$	$6.69 \times 10^{-6} \pm 1.44 \times 10^{-7a}$
Total phenolic compounds	$7.25 \times 10^{-5} \pm 1.94 \times 10^{-6a}$	$6.50 \times 10^{-5} \pm 5.99 \times 10^{-7a}$	$5.30 \times 10^{-5} \pm 1.47 \times 10^{-7a}$	$1.41 \times 10^{-4} \pm 1.85 \times 10^{-5a}$	$9.15 \times 10^{-5} \pm 7.78 \times 10^{-6a}$

Mean values ± standard deviation ($n = 2$) followed by different letters differ at $p \leq 0.05$, according to Tukey (HSD) test

therefore do not promote their oil solubility. Total polyphenolic compounds were similar to those found in chia seed oil obtained by solvent extraction.

Oxidative stability test using the Rancimat method showed values ranging from 1.12 to 2.37 h; with the oils obtained at 250 bar having the lowest stability (Table 4). The oxidative stability values in SC-CO_2 extracted oils were lower than those obtained from hexane-extracted oils. These results agree with data obtained by Martínez et al. [24] in walnut oil, indicating that chia seed oil obtained by SC-CO_2 is less protected against oxidation. Despite tocopherol content was lower in oils obtained by SC-CO_2 than in hexane-extracted oil, these differences in stability cannot be only attributed to tocopherol content. One possible explanation is related to the lower solubility of the phospholipids in SC-CO_2 and the synergistic effect between tocopherols and phospholipids [32]. Thus, the low level of phospholipids and tocopherols in oils extracted using SC-CO_2 would be one of the factors that could determine the low oxidative stability found in SC-CO_2 extracted oil compared with hexane extracted oil. On the other hand, Calvo et al. [32] reported that there is something intrinsic to the supercritical extraction procedure: these authors demonstrated that the oxidation of sunflower oil extracted by SC-CO_2 appears to result from the presence of trace amounts of oxygen in the extraction solvent. The presence of oxygen and the absence of mass transfer limitations promote oxidation of oil triglycerides in the supercritical phase. Nevertheless, other factors such as free fatty acids, mono- and diacylglycerols, transition metals, thermally oxidized compounds and pigments are involved in the oxidative stability of oils [33] and further studies are necessary to improve the low oxidative stability of chia seed oil by the addition of natural antioxidants, such as phenolic compounds.

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References

1. Alais C, Linden G (1991) Lipids. In: Alais C, Linden G (eds) Biochimie Alimentaire. Masson, Paris, pp 55–73
2. Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54:438–463
3. Harper CH, Jacobson T (2001) The fats of life: the role of omega-3 fatty acids in the prevention of coronary heart disease. Arch Int Med 161:2185–2192

4. Ayerza R Jr (1995) Oil content and fatty acid composition of Chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. J Am Oil Chem Soc 72:1079–1081
5. Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007) Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. Eur J Lipid Sci Tech 109:710–732
6. Taga MS, Miller EE, Pratt DE (1984) Chia seeds as a source of natural lipid antioxidants. J Am Oil Chem Soc 61:928–931
7. Follegatti-Romero L, Piantino C, Grimaldi R, Cabral F (2009) Supercritical CO_2 extraction of omega-3 rich oil from Sacha inchi (*Plukenetia volubilis* L.) seeds. J Supercrit Fluid 49:323–329
8. Stahl E, Schutz E, Mangold HK (1980) Extraction of seed oils with liquid and supercritical CO_2. J Agr Food Chem 28: 1153–1157
9. Friedrich JP, Pryde EH (1984) Supercritical CO_2 extraction of lipid-bearing materials and characterization of the products. J Am Oil Chem Soc 61:223–228
10. Bozan B, Temelli F (2002) Supercritical CO_2 extraction of flaxseed. J Am Oil Chem Soc 79:231–235
11. Ixtaina VY, Vega A, Nolasco SM, Tomás MC, Gimeno M, Bárzana E, Tecante A (2010) Supercritical carbon dioxide extraction of oil from Mexican chia seed (*Salvia hispanica* L.). Characterization and process optimization. J Supercrit Fluids. doi:10.1016/j.supflu.2010.06.003
12. AC AO (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington, DC
13. International Union of Pure and Applied Chemistry (IUPAC) (1992) In: Paquot C, Hautffenne A (eds) Standard methods for the analysis of oils, fats and derivates, 7th edn. Blackwell, Oxford, UK
14. Ixtaina VY, Martínez ML, Spotorno V, Maestri DM, Diehl BW, Nolasco SM, Tomás MC (2010) Characterization of chia (*Salvia hispanica* L.) crude seed oils obtained by pressing and solvent extraction. J Food Compos Anal (in press)
15. Ruetsch L, Daghero J, Mattea M (2003) Supercritical extraction of solid matrices. Model formulation and experiments. Lat Am Appl Res 33:103–107
16. AOCS (1998) Official Methods and Recommended Practices of the AOCS, 5th edn. AOCS Press, Champaign
17. Özkal SG (2009) Response surface analysis and modeling of flaxseed oil yield in supercritical carbon dioxide. J Am Oil Chem Soc 86:1129–1135
18. Özkal SG, Yener ME, Bayindırli L (2005) Mass transfer modeling of apricot kernel oil extraction with supercritical carbon dioxide. J Supercrit Fluid 35:119–127
19. Salgin S, Salgin U (2006) Supercritical fluid extraction of walnut kernel oil. Eur J Lipid Sci Tech 108:577–582
20. King JW (1993) Analysis of fats and oils by SFE and SFC. INFORM 4:1089–1098
21. Catchpole OJ, Grey JB, Smallfield BM (1996) Near-critical extraction of sage, celery, and coriander seed. J Supercrit Fluid 9:273–279
22. Salgın U, Döker O, Çalımlı A (2006) Extraction of sunflower oil with supercritical CO2: experiments and modeling. J Supercrit Fluid 38:326–331
23. Álvarez-Chávez LM, Valdivia-López MA, Aburto-Juárez ML, Tecante A (2007) Chemical characterization of the lipid fraction of Mexican chia seed (*Salvia hispanica* L.). J Food Prop 11:687–697
24. Martínez ML, Mattea MA, Maestri DM (2008) Pressing and supercritical dioxide extraction of walnut oil. J Food Eng 88:399–404
25. Eggers R (1996) Supercritical fluid extraction of oilseeds/lipids in natural products. In: King JW, List GR (eds) Supercritical fluid technology in oil and lipid chemistry. AOCS Press, Champaign, pp 35–64
26. Hassan MN, Rahman NN, Ibrahim MH, Omar AK (2000) Simple fractionation through the supercritical carbon dioxide extraction of palm kernel oil. Sep Purif Technol 19:113–120
27. Nilson WB (1996) Supercritical fluid extraction and fractionation of fish oils. In: King JW, List GR (eds) Supercritical fluid technology in oil and lipid chemistry. AOCS Press, Champaign, pp 180–212
28. Zaidul ISM, Nik Norulaini NA, Mohd Omar AK, Smith RL Jr (2006) Supercritical carbon dioxide (SC-CO_2) extraction and fractionation of palm kernel oil from palm kernel as cocoa butter replacers blend. J Food Eng 73:210–216
29. Leo L, Rescio L, Ciurlia L, Zacheo G (2005) Supercritical carbon dioxide extraction of oil and α-tocopherol from almond seeds. J Sci Food Agric 85:2167–2174
30. Jachmanián I, Margenat L, Torres A, Grompone MA (2006) Estabilidad oxidativa y contenido de tocoferoles en el aceite de canola extraído con CO_2 supercrítico. Grasas Aceites 57:155–159
31. Bostyn S, Duval-Onen F, Porte C, Coïc JP, Fauduet H (2008) Kinetic modeling of the degradation of the α-tocopherol in biodiesel-rape methyl ester. Bioresour Technol 99:6439–6445
32. Calvo L, Cocero MJ, Díez JM (1994) Oxidative stability of sunflower oil extracted with supercritical carbon dioxide. J Am Oil Chem Soc 71:1251–1254
33. Choe E, Min DB (2006) Mechanism and factors for edible oil oxidation. Compr Rev Food Sci Food Saf 5:169–186