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1 **Title: Production of omega 3-rich oils from underutilized chia seeds. Comparison between**
2 **supercritical fluid and pressurized liquid extraction methods**

5 **Authors:** David Villanueva-Bermejo¹, María V. Calvo², Pilar Castro-Gómez²; Tiziana Fornari¹;
6 Javier Fontecha^{2*}

8 ¹Department of Production and Characterization of Novel Foods
9 ²Department of Bioactivity and Food Analysis
10 Institute of Food Science Research (CIAL UAM-CSIC). C/ Nicolás Cabrera, 9. P.O. Box.
11 28049. Madrid (Spain)

13 David Villanueva-Bermejo (DVB): david.villanueva@uam.es
14 María V. Calvo (MVC): mv.calvo@csic.es
15 Pilar Castro-Gómez (PCG): mpilar.c.g@csic.es
16 Tiziana Fornari (TF): tiziana.fornari@uam.es
17 Javier Fontecha (JF)*: j.fontecha@csic.es

19 **David Villanueva-Bermejo and María V. Calvo contributed equally to this work.**

21 *Corresponding author.
22 Javier Fontecha Alonso j.fontecha@csic.es

29 *Author contribution to the article*

30 The authors' responsibilities were following:

31 **DVB:** Responsible for the current analyses by SFE and participate in the writing of the
32 manuscript;

33 **MVC:** Responsible for the current analyses by PLE and participate in the writing of the
34 manuscript;

35 **PCG:** Initiated the current analyses and supplied valuable knowledge and scientific consultation
36 during the process

37 **TF:** Took part in the funding and design of experiments and participate in the writing of the
38 manuscript;

39 **JF:** Took part in the funding and design of experiments and participate in the writing of the
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41

42 *Statement of author contributions*

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Abstract

Chia seeds constitute a promising source of α -linolenic acid (ALA). In the present work, an underutilized and cheaper set of chia seeds, which were discarded after the harvest according to quality criteria - named in this work as low oil content seeds (LOCS) - have been evaluated as a potential source for obtaining PUFA-enriched oils against the commonly studied high-quality chia seeds denoted as high oil content seeds (HOCS) in this study. Two efficient and environmental friendly techniques, supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE), were evaluated to optimize the extraction process of chia oil. At 60°C, by using pressurized food-grade ethanol, recoveries close to 100% were achieved from both sets of seeds in a short extraction time (10 min). By using SFE, the greatest oil extraction yield (>95%) was attained at the highest pressure and temperature conditions (45 MPa and 60°C) after 240 min. At the early stage of SFE extraction, both LOCS and HOCS exhibited a similar kinetic behavior, reaching oil extraction rates of 0.59 g oil/min and 0.64 g oil/min, respectively. No differences were found between the fatty acid profile of the oils extracted from LOCS and HOCS both by PLE and SFE. The linolenic (ALA) and linoleic acid (LA) concentrations ranged between 65-68% and 17-23% respectively, and a predominance of high molecular weight triglycerides (\geq CN50), was found in all extracted oils. In conclusion, LOCS might constitute a new suitable raw material for the production of ALA-enriched oils. Concerning the extraction methods assayed, the oil was almost entirely recovered by both PLE and SFE at the used conditions.

Keywords:

Chia seed; α -linolenic acid (ALA); supercritical fluid extraction (SFE); pressurized liquid extraction (PLE); triacylglycerol composition; FAME composition

1. Introduction

Currently there is a constant search of natural ingredients whose intake provides benefits for health. One of the most promising sources is chia seeds, which are gaining an immense importance as a new raw material to obtain beneficial, nutritional food ingredients. Chia (*Salvia hispanica* L.) is a plant of the *Lamiaceae* family native to Mexico and Guatemala. Due to its health properties, the seeds have been traditionally used as a food and applied in herbal medicine. Recently a number of studies have been carried out to obtain chia compounds for the food industry in order to improve food formulations (Capitani, Nolasco, & Tomas, 2016; Coorey, Tjoe, & Jayasena, 2014; Julio et al., 2016; Olivos-Lugo, Valdivia-Lopez, & Tecante, 2010) and for the production of functional foods, due to the significant amount of bioactive ingredients contained in these seeds, such as dietary fibre and phenolic compounds (Oliveira-Alves et al., 2017; Reyes-Caudillo, Tecante, & Valdivia-López, 2008). Additionally, chia seeds contain a large amount of oil (20-35% mass of dry seed), which is rich in polyunsaturated fatty acids (PUFA), especially in linoleic acid (LA) (15-20% of total fatty acids) and ω -3 α -linolenic acid (ALA) (60-65%) (Porrás-Loaiza, Jiménez-Munguía, Sosa-Morales, Palou, & Lopez-Malo, 2014). LA and ALA are considered essential to health and because the human body is unable to synthesize them, they are highly demanded ingredients within the food and cosmetic industrial sectors.

Several solvents and extraction techniques have been studied to obtain oil from chia seeds. Ixtaina et al. (2010) studied the effect of temperature, pressure and time on the supercritical CO₂ (SCCO₂) extraction of oil from a Mexican chia variety. Authors obtained an oil recovery of 92.8%, at 45 MPa, 80°C and 300 min. In another contribution (Ixtaina et al. 2011) the authors obtained oil recoveries which ranged from 82% (25 MPa, 40°C and 285 min) to 97% (45 MPa, 60°C and 138 min) from an Argentinian seed variety. Rocha Uribe, Novelo Pérez, Castillo Kauil, Rosado Rubio & Alcocer (2011) evaluated oil extraction using the same temperatures, but lower pressures (13.6-40.8 MPa) and the extractions were carried out in a combined static-dynamic mode. The highest extraction yield (7.2%, expressed as mass of oil extracted/mass of

seeds) was obtained at the highest pressure and temperature and after 40 minutes of extraction time, but these operational conditions were not sufficient to remove the entire amount of oil. Guindani et al. (2016) evaluated the oil extraction from chia seed cakes using SCCO₂ with ethanol and ethyl acetate as co-solvents. The highest extraction yield (10.6%) with pure CO₂ was obtained at 30 MPa and 50°C. The use of co-solvents (2.5% mass) produced an appreciable increase in the extraction yield, especially with ethanol. With regards to the use of organic liquid solvents, de Mello, dos Santos García & da Silva (2015) studied the ultrasound-assisted extraction of oil, achieving extraction yields up to 27.2% by using ethyl acetate as a solvent. Tolentino et al. (2014), Segura-Campos, Ciau-Solís, Rosado-Rubio, Chel-Guerrero & Betancur-Ancona (2014) and Amato et al. (2015) extracted the chia seeds oil using the IUPAC Soxhlet procedure (IUPAC, 1992) with conventional organic solvents, such as petroleum ether or hexane. Zanqui et al. (2015) evaluated the subcritical extraction of oil with propane and Castejón, Luna & Señorans (2017) the oil extraction by PLE, using hexane, ethyl acetate and aqueous ethanol as solvents. Dabrowski, Konopka, Czaplicki & Tanska (2017) evaluated the influence of three techniques, the traditional Soxhlet extraction with hexane and acetone, the SCCO₂ extraction and the use of cold- and hot-pressing methods. In all these studies, independent of what extraction technique and solvents applied, the amount of ALA and LA in the extracts was approximately 60-65% and 20-25% of total fatty acids, respectively.

In recent years, the interest in chia seeds as an alternative source of polyunsaturated oil has risen considerably. Post-harvest the chia seeds are cleaned, classified and selected to be marketed meanwhile those damaged or partially broken are separated and underutilized. Although this damaged by-product can be used for animal feed, its potential as a source of enriched PUFA oil has not been explored until now. Additionally, its low price due to the market surplus, along with its notable oil content would make these underutilized low-quality seeds a material worthy of consideration for the food industry.

In this work, the extraction performance and the composition of oils obtained from two different batches of Mexican chia seeds, high oil content seeds (HOCS) and underutilized or low oil content seeds (LOCS), have been assessed for the first time. The aim of the present work is to

133 evaluate the suitability of LOCS to be used as a raw material for the efficient production of
134 PUFA enriched oils containing high concentrations of ALA. For this purpose, two efficient and
135 green technologies, SFE and PLE, were evaluated to optimize the operational conditions to
136 recover the totality of oil contained in the seeds.

138 **2. Materials and methods**

139 **2.1 Sample and reagents**

140 **2.1.1 Sample preparation**

141 Chia seeds (*Salvia hispanica* L.) from Mexico were generously supplied by Hector Di Luca
142 (Primaria Premium Raw Materials, S.L). Samples denoted as HOCS and LOCS, correspond
143 respectively, to high and low oil content chia seeds. The oil content (value provided by the
144 supplier) contained in each batch of chia seeds was 25.2% (HOCS) and 20.6% (LOCS) on a dry
145 basis. Clean seeds were ground in a knife mill cooled by liquid nitrogen and were sieved in an
146 electromagnetic, digital sieve shaker using mesh sieves, stainless steel with 200 mm diameter,
147 (CISA Cedaceria Industrial S.L. Barcelona, Spain) to obtain a particle size ranging from 250 µm
148 to 500 µm. Samples were kept at 20°C until their use.

150 **2.1.2 Chemicals**

151 All solvents were HPLC grade, and MS grade when available. Dichloromethane, hexane,
152 dimethylformamide, sulphuric acid 98%, methanol and acetonitrile were purchased from
153 Labscan (Dublin, Ireland) and food grade ethanol 96%, v/v (F.C.C.) for PLE was obtained from
154 Alcoholes Montplet (Barcelona, Spain). Absolute ethanol ≥98.5%, employed for SFE, sea sand
155 used as dispersant, sodium sulphate anhydrous and sodium carbonate were obtained from
156 Panreac (Barcelona, Spain). Sodium methoxide 95%, the TAG standards trinanoïn and
157 tridecanoïn, and the FFA standards, were supplied by Sigma-Aldrich (St. Louis, MO, USA).
158 Reference samples with known composition, butterfat BCR-164 and BCR-519 (EU
159 Commissions; Brussels, Belgium) were from Fedelco Inc. (Madrid, Spain).

2.2 Pressurized liquid extraction

Pressurized liquid extractions (PLE) were carried out in an Accelerated Solid Extraction ASE-200 equipment (Dionex Corp., Sunnyvale, CA), using dichloromethane/methanol (D:M) solution (2:1, v/v) and food grade ethanol (EtOH) as extraction solvents. A detailed description of the extraction procedure and equipment employed can be found in the works reported by Castro-Gómez et al. (2014) and Castro-Gómez, Montero & Fontecha (2017). Experimental conditions were the same for both solvents. Briefly, 2 g of chia seeds sample were mixed with 2 g of sea sand, which was used as a dispersant, and loaded into a stainless-steel extraction cell covered with filters on both sides. The extraction was done during one static cycle of 10 min at a pressure of 10.3 MPa. Three different temperatures (40 °C, 60 °C and 80 °C) were assayed. The oil extracts obtained were concentrated by removing the organic solvent in a rotary vacuum evaporator (Strike 202 model; Steroglass S.R.L., Perugia, Italy). In the case of ethanol, to remove all traces of water, the liquid extracts were previously filtered through a Whatman 1-phase separator filter paper (Whatman, Maidstone, UK) fitted with a vacuum pump and containing approximately 3 g of sodium sulphate anhydrous. Finally, all oil extracts were fully evaporated under a gentle stream of nitrogen, weighed, stored in amber vials, and frozen at -35°C until analysis. Each extraction was performed in triplicate.

2.3 Supercritical fluid extraction

Supercritical fluid extractions were carried out using a pilot-plant supercritical fluid extractor (model SF2000; Thar Technology, Pittsburgh, PA, USA), comprising a 0.273 L cylinder extraction cell and two separators (S1 and S2), each of 0.5 L capacity, with independent control of temperature and pressure. A detailed description of the equipment can be found in the work reported by Villanueva-Bermejo, Zahran, Rodríguez-Risco, Reglero & Fornari (2017). A kinetic study was performed using both types of chia seeds. Extractions from LOCS were carried out at two pressures (25 and 45 MPa), two temperatures (40 and 60°C), 40 g/min of CO₂ flow rate and 240 min extraction time. Pressure conditions were selected considering the increased solubility of oil seeds in SCCO₂ at high CO₂ densities. At these conditions, densities

of SCCO₂ varied from 786.6 kg/m³ (25 MPa and 60°C) to 974.7 kg/m³ (45 MPa and 40°C) (NIST Chemistry WebBook, 2018). In the case of temperature, values higher than 60°C were discarded in order to avoid the thermal degradation of the oil.

After that, 45 MPa and 40°C were selected in terms of oil yield and fatty acid composition as the best pressure and temperature to optimize the oil extraction of HOCS. In this sense, several CO₂ flow rates (27, 40 and 54 g/min, which represent a CO₂-to-seeds ratio of 50, 70 and 100, respectively) were studied and the extraction time was set to 240 min. The mass of chia seeds employed for the experiments was 130 g for all runs. All extracts were obtained by depressurization at the system recirculation pressure (5 MPa). The oil mass obtained in each extraction was collected from both separators and mixed in a single fraction. Afterwards, each SFE extract was dissolved in CH₂Cl₂, filtered through a 0.45 µm Millipore filter coupled to a syringe containing approx. 1 g of anhydrous sodium sulphate and was stored at -35°C until chromatographic analysis.

2.4 Chemical analysis

2.4.1 Fatty acid composition

The lipid extracts from chia seeds were derivatized following the method described in Castro-Gómez, Fontecha & Rodríguez-Alcalá (2014). Fatty acid methyl esters (FAMES) were separated using a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm ID, 0.2 µm; Chrompack, Middelburg, The Netherlands) in an Agilent chromatograph (model 6890N; Agilent Technologies Inc., Palo Alto, USA) equipped with an MS detector (Agilent 5973N). Chromatographic conditions were as follows: the column was held at 100°C for 1 min after injection and then temperature increased 7°C/min up to 170°C, held there for 55 min, then increased at 10°C/min up to 230°C and held for 33 min. Total time for chromatographic run was 105 min. The injector temperature was set at 250°C. Helium was used as the carrier gas with a column inlet pressure of 0.2 MPa. The injection volume was 1 µL and a split ratio 1:25 was used. MS detector conditions were as follows: transfer line temperature 250°C, source

temperature 230°C, and quadrupole temperature 150°C. The mass spectrometer operated under electron impact mode (70 eV), it was used in total ion current (TIC) mode and scanned the mass range from 40 to 500 m/z. For peak identification, mass spectra obtained in our analysis were compared with those in the National Institute of Standards and Technology library (NIST, 2.1.0 version; Gaithersburg, MD, USA). For qualitative and quantitative analysis, response factors were calculated using anhydrous milk fat (reference material BCR-164). Tritridecanoine (C13:0-TAG) was used as an internal standard (200 µL; 1.3 mg/mL) and added to lipid extracts before methylation.

2.4.2 Triacylglycerols composition

For triacylglycerols (TAG) analysis, the oil samples from chia seeds were injected (0.5 µL at 30 mg/mL) using a split ratio 1:20 in a CLARUS 400 gas chromatograph (Perkin Elmer Ltd., Beaconsfield, UK) equipped with an automatic split/splitless injector and a flame ionization detector (FID). A RTX-65TAG fused silica capillary column Crossbond™ (30 m × 0.25 mm ID; 0.1µm film thickness; Restek Corp., Bellefonte, PA, USA) was used. The analysis of TAG groups by different carbon number (CN) content was based on the method described by Fontecha, Mayo, Toledano & Juarez (2006). The temperature program was as follows: the column was held at 120°C for 30 s, then temperature increased 10°C/min up to 220°C, held there for 30 s, then increased at 6°C/min up to 350°C and held for 30 min. Total run time was 62.5 min. Injector and FID temperatures were 355°C and 370°C, respectively. Helium (35 mL/min) was used as the carrier gas at 0.2 MPa. For TAG determination and quantification, the reference butterfat BCR-519 of known composition was used and glyceryl trinanoate (100 µL; 1 mg/mL) was added as internal standard to the lipids extracts before the chromatographic analysis.

3. Results and discussion

3.1 Pressurized liquid extraction

As can be observed in Figure 1a, a higher oil extraction yield (g of oil/100 g of seeds) was obtained with D:M as temperature increased. Nevertheless, there were no significant differences

among 60°C and 80°C as the extraction yields achieved were 19.9% and 19.6%, respectively. When food-grade EtOH was used as solvent, the highest oil extraction yield (17.7%) was also obtained at 60°C and an increase in the extraction temperature did not lead to an enhancement in the amount of oil extracted. Based on this, 60°C was selected as optimal. This effect with regards to applying mild temperatures on the extraction of oil was also observed by Zanzi et al. (2015), who assayed temperatures between 30 and 60°C and obtained the highest chia seeds oil recovery (93.2%) at 45°C and 10 MPa by using *n*-propane. The extraction yield obtained with EtOH was comparable to that reached using D:M and with the added advantage of being a green solvent, unlike D:M, which is a pollutant and toxic mixture. Considering that the amount of oil contained in HOCS and LOCS seeds was 25.2% and 20.6%, respectively, on a dry basis (data provided by the supplier), and considering the extraction yield values (see Figure 1b), the total removal of the oil was practically accomplished for both seed batches. The oil recovery (g of oil extracted/100 g of oil in seeds) for LOCS, was slightly lower when using EtOH (85.9%) than when using D:M (96.6%), while for HOCS, the entire oil (100% recovery) was extracted in just 10 min extraction time and independently of the solvent used. Therefore, EtOH demonstrated to be as efficient as D:M, whose efficacy for the extraction of oil has already been thoroughly corroborated in other studies (Araujo et al., 2013; Iverson, Lang, & Cooper, 2001). Furthermore, the use of a moderate temperature (60°C) allows unsaturated fatty acids to be protected from degradation.

3.2 Supercritical fluid extraction

The oil extraction kinetic of LOCS (low oil content seeds) was obtained at two pressures (25 and 45 MPa) and two temperatures (40 and 60°C). Figure 2 represents the extraction curves of oil from this type of seeds, obtained by SCCO₂ at the different operational conditions. As can be observed, the oil extraction yield increased with the pressure and temperature. The same behaviour has been reported for the SFE of oil from chia seeds containing high amounts of oil (Ixtaina et al. 2011; Ixtaina et al. 2010; Rocha Uribe et al. 2011). When studied by Ixtaina et al. (2011 and 2010), the known crossover effect was not produced at the conditions applied.

However, the same effect observed in the present work was reported by Rocha Uribe et al. (2011) at very similar pressures (27.2 and 40.8 MPa) and same temperatures (40 and 60°C). Nevertheless, additional experiments should be applied to corroborate this behaviour. The highest oil recovery (g of oil extracted/g of oil in seeds) was obtained at 45 MPa and 60°C (90.3% oil recovery), while testing at 25 MPa and 40°C led to the lowest amount of oil extracted (64.5%). Considering the influence of the mass of CO₂/mass of seeds ratio employed on yield, an estimated oil recovery of 85% was obtained at 38 g/g in this work, which is considerably higher than reported by Ixtaina et al. (2010) (40.3%), and lower than obtained experimentally under the same conditions (45 MPa, 60°C and 38 g/g ratio) by the same authors, in another contribution (97% recovery) (Ixtaina et al. 2011), however, they were using high oil content seeds at that time. With regards to the kinetics of the extraction, the highest oil extraction rate at the early extraction stage (fast extraction period mainly controlled by the solubility of oil in CO₂) was also achieved at the maximum pressure and temperature, due to the higher density produced when pressure rises and the increase in the vapour pressure of the solutes at high temperatures. The results show that the mass of oil extracted over time ranged from 0.14 g/min (25 MPa and 60°C) to 0.64 g/min (45 MPa and 60°C). Considering that higher oil recoveries were achieved at 45 MPa (Figure 2) and higher concentrations of ALA were obtained at 40°C (section 3.3) from LOCS, the same pressure and temperature were selected to study the oil extraction from HOCS (higher oil content seeds). To optimize the extraction process and remove all the oil contained in the seeds, the influence of different CO₂ flow rates on the chia oil extraction yield was evaluated for the first time: 27 g/min (50 g/g CO₂-to-chia seeds ratio); 40 g/min (74 g/g) and 54 g/min (100 g/g). Figure 3a shows the extraction kinetics plotted as a function of time. As can be observed under these conditions all the oil was extracted (recoveries between 98.5-100%). The extraction rate increased with the flow of CO₂ at the early extraction stage (fast extraction period), at this point, the mass of oil extracted was 0.44 g/min; 0.59 g/min and 0.76 g/min when 27 g/min, 40 g/min and 54 g/min of CO₂ flows were applied, respectively. The value obtained at 40 g/min (0.59 g of oil/min) from HOCS was very close to that obtained with the same conditions (45 MPa, 40°C

and 40 g/min) from LOCS (0.64 g of oil/kg CO₂), therefore, these lower oil content seeds show a kinetic behaviour with the SFE of oil like those obtained for HOCS. Figure 3b shows the recovery of oil plotted as a function of the mass of CO₂/mass of chia seeds ratio used. Based on the amount of oil extracted in the initial fast extraction period, the apparent solubility of chia oil in the SCCO₂ determined at this pressure and temperature was 14.1 g oil/kg CO₂ (the correlation coefficient value was $R^2=0.984$). Although more experiments are necessary to corroborate this value, it was like that obtained at 45 MPa and 40°C with the LOCS (11.1 g oil/kg). Therefore, a similar trend in terms of kinetic and solubility behaviour were observed for both seed batches. Furthermore, the difference in the amount of oil contained in each variety is only 4.6% (the concentration of oil in the HOCS and LOCS is 25.2% and 20.6%, respectively), therefore the LOCS has enough potential to be used as a raw material for the obtaining of high yields of polyunsaturated oil.

3.3 Fatty acid and triacylglycerols composition

The oils obtained by PLE and SFE were analysed to determine their composition in fatty acids and Figure 4 shows comparatively the chromatographic profile obtained for each type of chia seeds. With respect to oils extracted from LOCS by PLE at 40, 60 and 80°C (data not shown), ALA concentrations between 64.2-65.7% were achieved and the n-6/n-3 and of the unsaturated-to-saturated fatty acid (UFA/SFA) ratios ranged between 0.27-0.29 and 7.8-8.8, respectively. Therefore, independently of the organic solvent employed the extraction temperature did not have an influence on the oil fatty acid composition. Table 1 shows the main fatty acids present in the oils extracted from the different types of seeds by PLE at 60°C using EtOH and D:M. As can be observed, within each chia seeds variety, the lipid profile obtained with both solvents was very similar. ALA was the major fatty acid in all oil extracts, followed by LA, palmitic, oleic and stearic acids, thus confirming that chia oil is very rich in PUFA (~ 83%). These findings agree with those reported in previous studies on chia seeds (Zanqui et al. 2015; De Mello et al. 2015; Dabrowski et al. 2017), in which other solvents, extraction techniques and

experimental conditions were applied. Zanqui et al. (2015) achieved ALA concentrations of $\approx 61\%$ and an $n-6/n-3 \approx 0.3$ by using subcritical *n*-propane as a solvent. De Mello et al. (2015) used ultrasound-assisted extraction with ethyl acetate to obtain an oil containing 63% of ALA and having an $n-6/n-3$ of 0.31, while Dabrowski et al., (2017) obtained oils having 60-62% of ALA, 12-13% of SFA and $n-6/n-3$ ranged between 0.32-0.35 by cold and hot pressing, as well as by Soxhlet extraction with hexane and acetone. HOCS exhibited a slightly higher value of UFA/SFA ratio as compared to LOCS (Table 1), which is related to the greater presence of ALA and the lower concentration of palmitic acid in the former. Furthermore, the simultaneous decrease of LA levels resulted in lower $n-6/n-3$ ratios (0.25-0.24 for HOCS and 0.29-0.27 for LOCS).

Table 2 summarizes the fatty acid composition of oils extracted by SFE from LOCS (at the different operational conditions assayed), as well as from the HOCS (45 MPa, 40°C and 40 g/min CO₂ flow rate). As can be observed, an increase in pressure from 25 MPa to 45 MPa at a fixed temperature seemed to exert no effect on the lipid composition of LOCS. Nevertheless, under isobaric conditions, marked differences could be found as the extraction temperature rose. Resulting in a decrease in the amount of ALA observed between both 25 and 45 MPa, with its concentration at 60°C significantly lower ($\sim 55\%$) than that obtained at 40°C ($\sim 67\%$), while the concentration of the remaining fatty acids (particularly LA) increased. Considering that oils are complex mixtures of different lipid classes (fatty acids, mono-, di-, and triglycerides, and fatty acid esters), the intermolecular interactions among them could lead to deviations from pure-component solubility behaviour (Güçlü-Üstündag & Temelli 2000). So, it could be possible that ALA exhibited retrograde solubility behaviour under the experimental conditions studied, while the solubility of the rest of fatty acids or the oil as a whole increased as the temperature rose.

When comparing the lipid profiles of the oils from both seed batches, extracted under identical experimental conditions (45 MPa and 40°C), the concentration of ALA found in LOCS (66.25%) was higher than the observed in HOCS oil (59.17%). In addition, the UFA/SFA was higher (9.20 vs 8.38) and the $n-6/n-3$ ratio was more favourable (0.27 vs 0.36) in the case of LOCS.

When seeds from HOCS were extracted by SFE at 45 MPa, 40°C and different CO₂ flow rates, the oil composition was quite similar for all experimental conditions assayed (data not shown). In this case, the n-6/n-3 ratio ranged between 0.34-0.38 and the concentration of ALA, SFA and PUFA was between 58.7-60.4%; 11.3-11.9% and 80.8-81.5%, respectively.

Comparing both extraction methods (PLE and SFE), the oil composition and the concentration of the main fatty acids obtained by SFE at 40 °C (Table 2) was almost identical to those obtained by PLE in the case of LOCS (Table 1). Therefore, both extraction technologies are suitable to produce high-quality oils from these seeds. On the contrary, at 60°C the concentration of LA was 27-30% higher in the supercritical extracts, at that expense of ALA, where concentration was around 15% lower than in PLE. The results obtained by SFE in this work with LOCS were very similar to those reported in other studies carried out with SCCO₂ and high oil content seeds. Ixtaina et al. (2011) achieved concentrations of ALA around 65% and a value for an n-6/n-3 close to 0.3, at the same pressures and temperatures that were applied in the present work. Likewise, Rocha Uribe et al. (2011) found similar concentrations of ALA (63-66%) and LA (17-18%) in oils which were extracted at pressures higher than 40 MPa, while Dabrowski et al. (2017) reported concentrations between 61-63% and 21-29%, respectively, at 28 MPa and higher temperatures (70 and 90°C) than applied in the present study. Therefore, the oils obtained in this work from LOCS by means of these two green extraction technologies (PLE and SFE) exhibited a similar or even a healthier fatty acid profile than that found in HOCS.

With respect to the TAG molecular species, the profile obtained in PLE extracts from LOCS at the three temperatures explored (40, 60 and 80°C) with both solvents (D:M and EtOH) was very similar, as well as those obtained by SFE from HOCS at different CO₂ flow ratios (data not shown). Table 3 presents the TAG composition obtained from both seed varieties by PLE at 60°C and by SFE at the different pressures and temperatures and a flow rate of 40 g/min CO₂. In results shown, a predominance of TAG with high molecular weight was observed in all the samples, with CN54 being the most abundant molecular species, in agreement with the high concentration of C18 fatty acids (mono- and polyunsaturated) present in all extracts analysed

(Table 2). There is also a noticeable presence of the CN52 molecular species, which probably corresponds to those TAGs having palmitic acid esterified to the glycerol backbone. This distribution is in good agreement with TAG composition previously reported for chia seeds oil from different geographical zones (Ixtaina et al. 2011; Timilsena, Vongsvivut, Adhikari, & Adhikar, 2017). These authors, using HPLC/APCI-MS and reverse phase HPLC, respectively, identified twelve TAG molecular species, with trilinolenin ($\alpha\text{Ln}\alpha\text{Ln}\alpha\text{Ln}$) as the major compound. The presence of TAG molecular species with <CN50 in the isolated extracts could be explained by the co-elution of other lipid classes such as diacylglycerols, monoacylglycerols and polar lipids with these TAGs, as described by Castro-Gómez, Montero & Fontecha (2017). Its concentration in PLE extracts (~ 7.8%) was higher than in SFE oils (~ 3.8%) in the case of LOCS, likely due to the use of organic solvents which extract polar compounds more efficiently than SCCO₂. On the contrary, the concentration of CN54 was slightly higher in SFE extracts for both seeds oil varieties. This can be explained by the fact that CO₂ is classified as a non-polar solvent, and hence is selective for non-polar solutes such as the TAG. Besides, it is known that TAGs with higher unsaturation degree dissolve better in supercritical carbon dioxide than TAGs with lower unsaturation (Davarnejad, Kassim, Zainal, & Sata, 2008). Comparable results were reported by Jokić et al. (2010), who found a higher content of TAG with unsaturated fatty acids (LA and ALA) in soybean oil obtained by supercritical CO₂ compared to the lipid extracts obtained using organic solvents. Even though oils obtained from both types of seeds had, overall, a similar TAG composition, the concentration of CN54 was greater in HOCS oils, especially in the case of SFE extracts. In this regard, the content of this TAG molecular species in the oil obtained at 45 MPa and 40°C from LOCS was 15% lower than in oil collected from HOCS, under the same experimental conditions. In the case of the different CO₂ flow rates experimented on HOCS, the increment in the CO₂/seeds ratio did not produce any effect on the TAG composition of oils.

4. Conclusions

In this work, two batches of chia seeds with different oil content (LOCS vs. HOCS) were compared for their oil extraction yield as well as their lipid composition, by using two environment-friendly techniques (SFE and PLE). At 60°C, pressurized food-grade ethanol allowed to achieve oil recoveries close to 100% in 10 min for both seed types. By SFE, the greatest oil extraction yield was attained under the highest pressure and temperature conditions tested (45 MPa and 60°C) after 150 min. A similar kinetic behavior, in terms of oil extracted, was observed at the early stage period for HOCS and LOCS, whose oil extraction rates were 0.59 g oil/min and 0.64 g/min, respectively.

Regarding the fatty acid profile, no noticeable differences were found between the oils extracted from LOCS and HOCS both by PLE and SFE. The main compound in all extracts was ALA which concentration ranged between 65-68%, except for SFE (45MPa, 60 °C). LA was also present in high concentrations 17-23%. A predominance of TAG molecular species \geq CN50, was found in the oils from both HOCS and LOCS, which agrees with the abundance of C18 fatty acids (in particular, LA and ALA) in the extracts. This circumstance would explain the greater amount of the CN54TAG in the oils obtained by SFE, given the greater solubility of unsaturated TAGs in supercritical CO₂.

In conclusion, LOCS could be efficiently employed for the high-yield production of ALA-enriched oils with a fatty acid composition almost identical to that found in HOCS. Under experimental conditions described in this study, the oil was almost entirely recovered by both PLE and SFE and the quality of the oils obtained was similar as regards their fatty acid composition.

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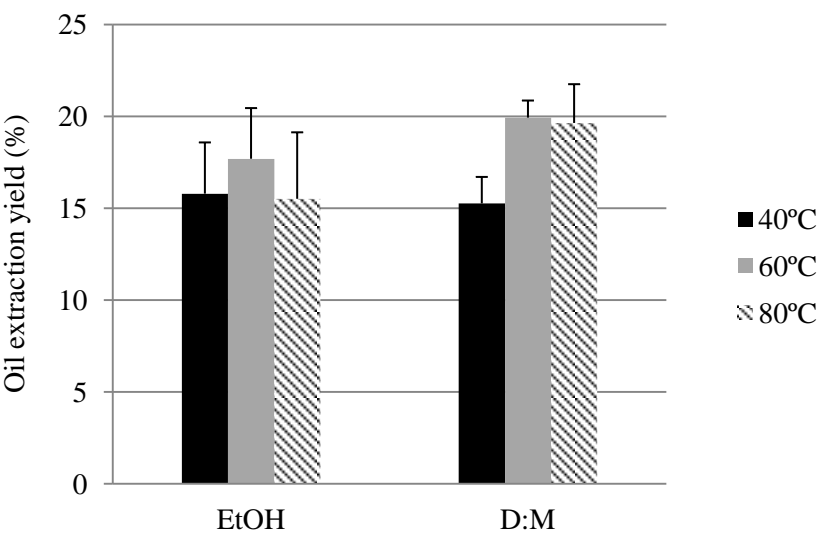
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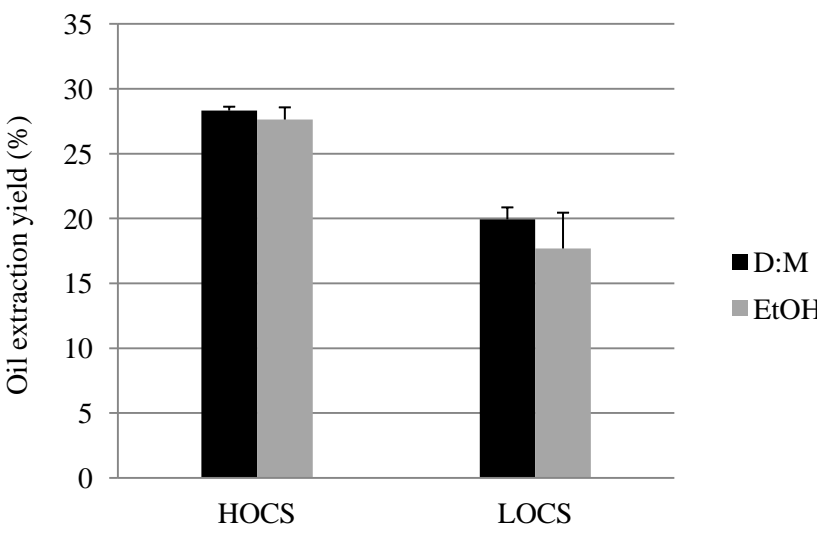
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Figure

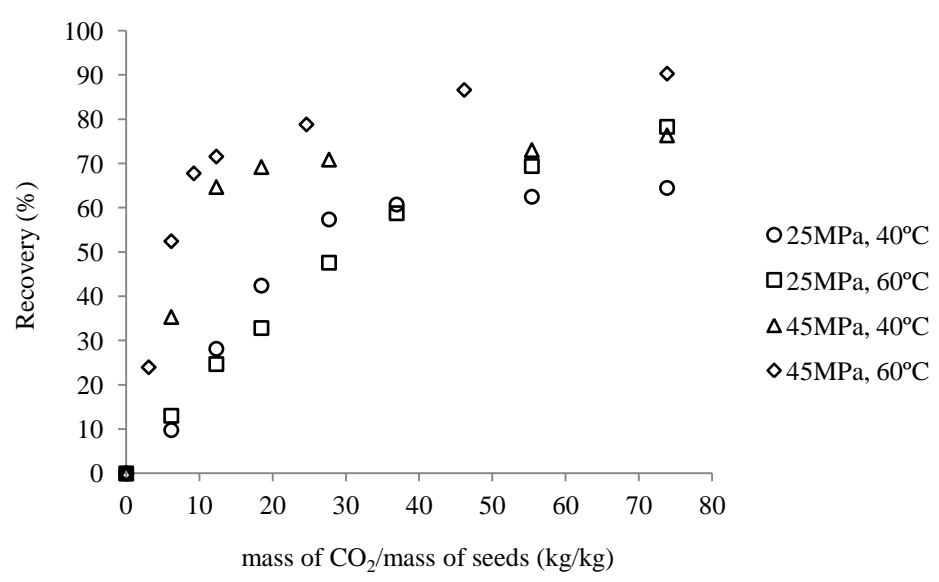
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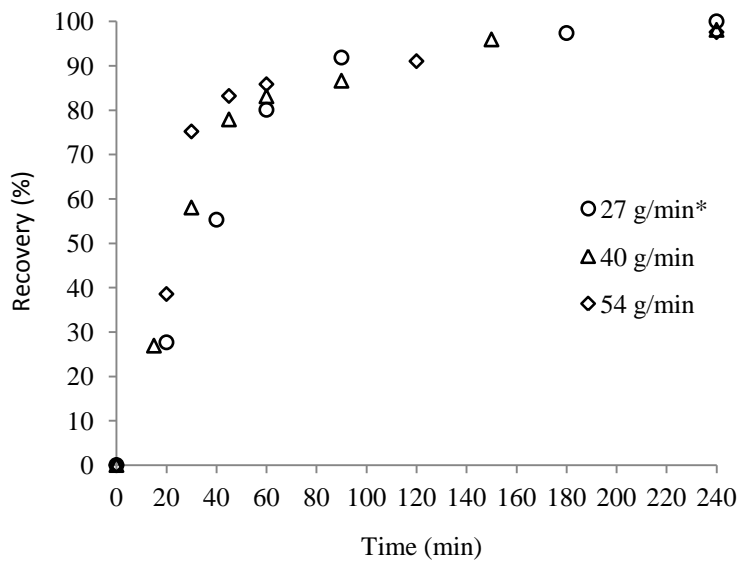


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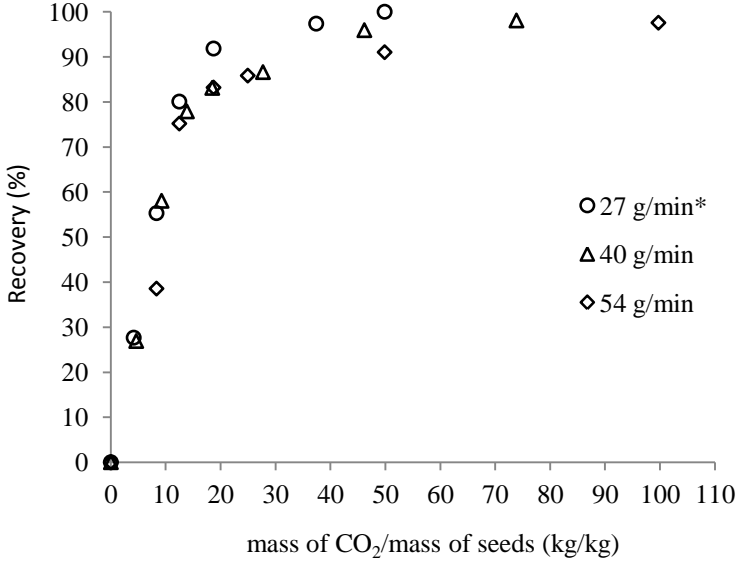


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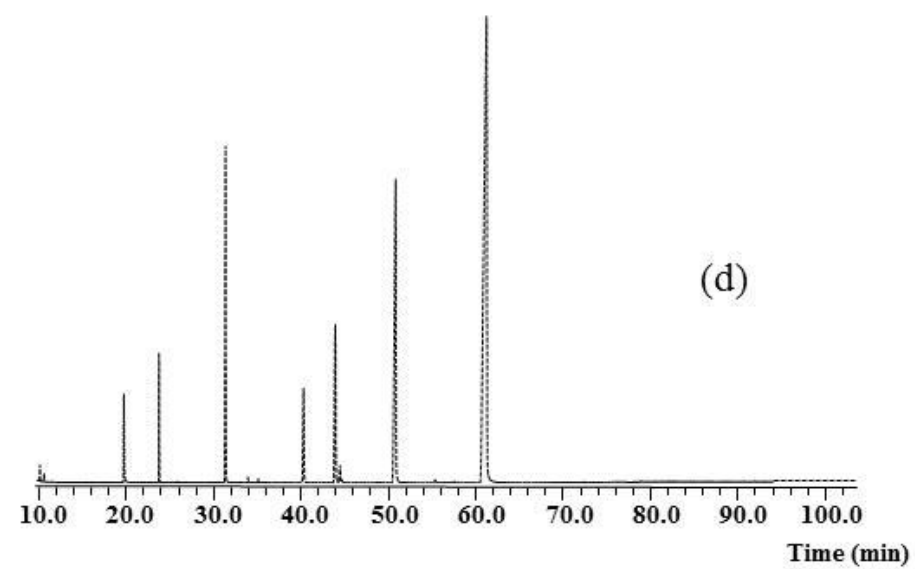
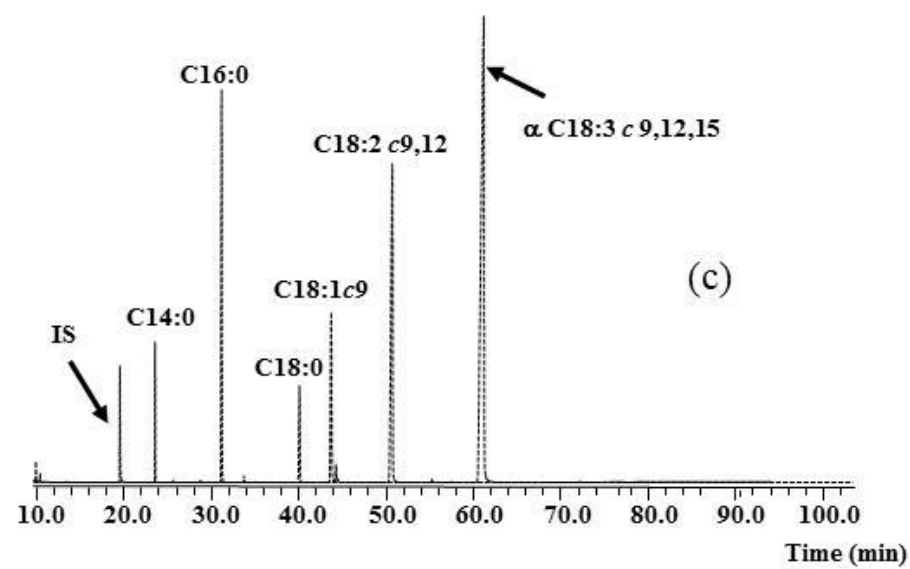
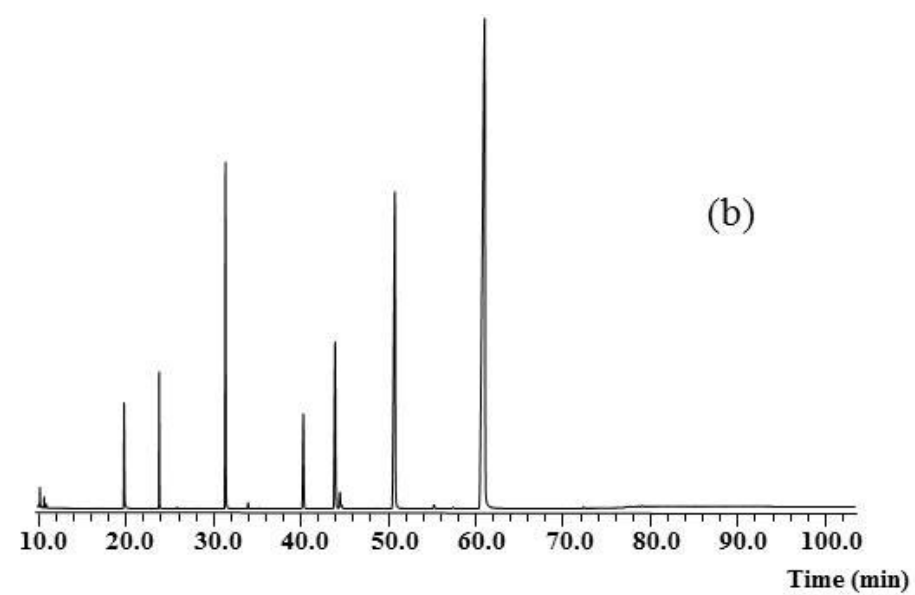
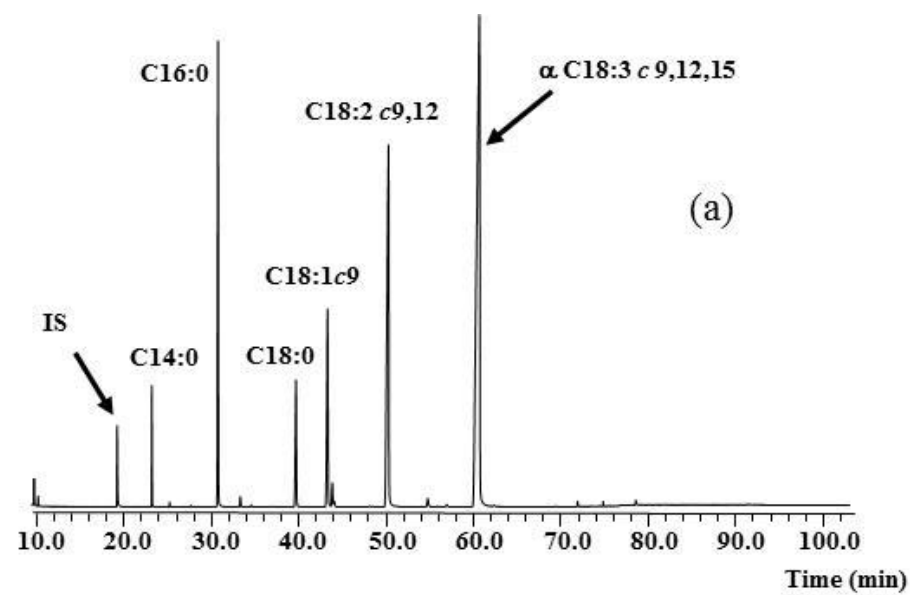


FIGURE CAPTIONS

Figure 1. Oil extraction from chia seeds by PLE using ethanol (EtOH) and dichloromethane/methanol (D:M). (a) Oil extraction yield ($\frac{\text{g of oil}}{\text{g of seeds}} \times 100$) obtained at 40, 60 and 80°C from LOCS. (b) Oil extraction yield obtained at 60°C from HOCS and LOCS.

Figure 2. Overall chia oil recovery curves ($\frac{\text{mass of oil extracted}}{\text{mass of oil in seeds}} \times 100$) obtained by SCCO₂ from LOCS, at 25 MPa and 45 MPa, 40°C and 60°C, and 240 min extraction time.

Figure 3. Overall chia oil recovery curves ($\frac{\text{mass of oil extracted}}{\text{mass of oil in seeds}} \times 100$) obtained by SCCO₂ from HOCS, at 45 MPa, 40°C and 240 min extraction time. (a) Recovery (%) as a function of the extraction time. (b) Recovery (%) as a function of the mass of CO₂/mass of chia seeds ratio. *Extraction carried out by duplicate (the result is plotted as the mean of both extractions. Average relative standard deviation (ARSD) = $\frac{1}{N} \sum \frac{SD_i}{\bar{x}_i} = 6.2\%$).

Figure 4. Comparison of FA profiles, obtained by GC/MS, of the oils extracted from LOCS and HOCS. Experimental conditions: Oil extraction by SFE (45 MPa, 40°C, 240 min) from LOCS (a) and HOCS (b). Oil extraction by PLE (EtOH, 60°C) from chia seeds LOCS (c) and HOCS (d).

1 **TABLES**

2

3 **Table 1.** Fatty acid composition (% of total fatty acids) of oils obtained by PLE at 60°C with
4 dichloromethane/methanol (2:1) (D:M) and ethanol (EtOH) from LOCS and HOCS.

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Fatty acid (%)	LOCS		HOCS	
	D:M	EtOH	D:M	EtOH
C16:0	7.59	7.21	6.43	6.28
C18:0	2.78	2.63	2.50	2.43
C18:1 <i>cis</i> 9	5.74	5.58	5.04	4.95
C18:2 (<i>cis</i> 9, 12)	18.40	17.71	16.83	16.67
C18:3 (<i>cis</i> 9, 12, 15)	64.22	65.69	68.26	68.84
SFA	10.81	10.21	9.17	8.89
MUFA	6.57	6.39	5.74	5.59
PUFA	82.63	83.39	85.09	85.52
UFA	89.20	89.78	90.83	91.11
n-6/n-3	0.29	0.27	0.25	0.24
UFA/SFA	8.26	8.79	9.90	10.25

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Table 2. Fatty acid composition (% of total fatty acids) of oils extracted by SFE from LOCS (25-45 MPa, 40°C- 60°C) and HOCS (45 MPa and 40°C). Extraction time: 240 min; CO₂ flow rate: 40 g/min.

Fatty acid (%)	LOCS				HOCS
	25 MPa		45 MPa		45 MPa
	40°C	60°C	40°C	60°C	40°C
C16:0	6.34	8.60	6.87	8.41	7.57
C18:0	2.64	3.30	2.50	3.36	3.37
C18:1 <i>cis</i> 9	5.29	7.22	5.46	7.21	6.49
C18:2 (<i>cis</i> 9, 12)	17.21	23.47	17.63	23.43	21.30
C18:3 (<i>cis</i> 9, 12, 15)	67.45	55.36	66.25	55.58	59.17
SFA	9.32	12.74	9.81	12.59	11.94
MUFA	6.05	8.13	6.31	8.13	7.43
PUFA	84.63	79.13	83.89	79.28	81.06
UFA	90.68	87.26	90.19	87.41	88.49
n-6/n-3	0.26	0.42	0.27	0.42	0.36
UFA/SFA	9.73	6.85	9.20	6.94	8.38

Table 3. TAG molecular species composition (%) of oil extracted from LOCS and HOCS by PLE (60°C) and SFE (CO₂ flow rate: 40 g/min; extraction time: 240 min). CN (Carbon number). D:M (dichloromethane:methanol 2:1, v/v). EtOH (ethanol).

	Extraction	Operational conditions	TAG molecular species (%)			
			<CN50	CN50	CN52	CN54
LOCS	PLE	D:M	7.9	6.7	37.0	48.4
		EtOH	7.6	6.3	37.8	48.3
	SFE	25 MPa 40°C	3.8	6.0	38.3	51.8
		25 MPa 60°C	3.7	6.6	37.5	52.2
		45 MPa 40°C	5.2	5.9	35.2	53.7
		45 MPa 60°C	3.9	6.4	35.7	54.0
HOCS	PLE	D:M	5.0	5.2	35.1	54.7
		EtOH	6.0	4.9	34.5	54.6
	SFE	45 MPa 40°C	5.0	3.9	28.3	62.9

