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Article

Subcritical Extraction of *Salvia hispanica* L. Oil with *N*-Propane: Composition, Purity and Oxidation Stability as Compared to the Oils Obtained by Conventional Solvent Extraction Methods

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This study evaluated the Chia (*Salvia hispanica* L.) oil composition in terms of fatty acids (FA), sterols, acylglycerols and oxidative stability obtained via subcritical *n*-propane fluid extraction (SubFE-propane), in different temperatures and pressure conditions, as compared to Bligh & Dyer (BD), Soxhlet (SE) and Folch (FLS) extractions. Total lipid varied from 23.25 to 30.21% and the best yield was obtained by both SubFE-propane extraction at 45 °C and 12 MPa (A). α-Linolenic acid (18:3n-3) was the most abundant FA and SubFE-propane extraction provided the best results for the sum of n-3 and PUFA. All oil samples were similar in regard to triacylglycerols (TAG) profiles as measured via direct electrospray ionization mass spectrometry (ESI-MS) analysis. The total amounts of stigmasterol, β-tocopherol and tocopherol total were highest in the Chia oil obtained by BD, campesterol and sitosterol by SE and γ-tocopherol by SubFE-propane extraction. The SubFE-propane oil also presented the best (2 to 5 times) oxidation stability. SubFE-propane was the most efficient extraction method for Chia oil, providing the highest extraction yields, purity, oxidation stability and diverse profile of sterols.

Keywords: lipids extraction, fatty acids, subcritical extraction, principal components analysis, phytosterols

Introduction

Chia, *Salvia hispanica* L. is an oilseed crop with potential use as human food.[1,2] This seed is composed of 30% of oil[3,4] rich in α-linolenic acid (50 to 60%)[2-5] and is gluten-free, thereby handling it appropriate to be consumed by celiac.[5] As for other oilseeds, chia also presents several bioactive components, such as phytosterols and tocopherols,[6,7] and free phytosterols serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes. Most phytosterols contain 28 or 29 carbons and one or two carbon-carbon double bonds, typically one in the sterol nucleus and sometimes a second double bond in the alkyl side chain. Phytostanols are a fully-saturated subgroup of phytosterols which act on cellular functions, preventing inflammation[8] and acting in several other diseases.[6,9,10]

The α-linolenic acid (18:3n-3) is known to act on the prevention of cardiovascular diseases, decreasing the risk of heart and other chronic diseases such as type 2 diabetes and cancer, and protecting against Alzheimer's disease.[10-12] The conventional methods of oil extraction used in food industry can eventually remove or degrade components such as α-linolenic acid, phytosterols and tocopherols. Supercritical fluid extraction (SFE) is an alternative method for lipid extraction,[13-16] presenting several advantages, such as the use of a solvent with low density, viscosity, surface tension, mild conditions of temperature and pressure, which cause no degradation of the bioactive components.[17,18]

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Carbon dioxide (CO_2) is the solvent most commonly used for SFE, due to beneficial properties such as low temperature (31 °C) and critical pressure (7.29 MPa).[19-22] Many authors have applied the subcritical fluid extraction SFE method for oilseeds, using CO_2 and *n*-propane as solvent, because it can preserve oil quality characteristics and extract free lipids from toxic residues.[19–23] However, *n*-propane (critical temperature of 97 °C and critical pressure of 4.19 MPa) seems to be a better alternative than CO_2 for SFE oil extraction, due to this solvent highest solubility in lipids.

This work evaluated *Salvia hispanica* L. oils extracted by subcritical *n*-propane fluid (SubFE-propane), under different pressure and temperature conditions, and compared to different conventional lipid extraction methods. The composition of the oil obtained by subcritical *n*-propane fluid extraction (SubFE-propane) was evaluated via gas chromatography with flame ionization detector (GC-FID), gas chromatography coupled to mass spectrometry (GC-MS) and electrospray ionization mass spectrometry (ESI-MS).

Experimental

Samples

Four packs of 500 g of 4 different lots of Chia (*Salvia hispanica* L.) samples were provided by Dubai Trade and Industry Food Production, Catuípe-RS, Brazil. The grains were crushed, homogenized and stored in vacuum packaging at room temperature, and protected from light.

Total lipids (TL)

Total lipids (TL) was extracted according to Folch *et al.*[24] (FLS), Bligh *et al.*[25] (BD), and AOAC Official Method 991.36 - Soxhlet[26] (SE) and were expressed in dry basis percentage (% DB^{-1}).

Subcritical *n*-propane extraction method

For lipid extraction with pressurized *n*-propane, 30.0 g of dried sample was filled into the extractor, on a laboratory scale for lipid extraction with subcritical *n*-propane, that was pressurized via a pump-type syringe with a temperature-controlled thermostatic bath at 10 °C, as described by de Souza *et al.*[27]

Different temperature and pressure conditions were used as the two main factors for the 2^2 factorial design (Table 1), with three replications of the central point. The answer was the final oil quantity (extraction yield).

The extraction was carried out with 1 $cm^3\ min^{-1}$ of propane flow, controlled by an expansion valve (Autoclave Engineers) maintained at 80 °C using a thermoregulator (Tholz, model CTM-04E). Lipids were collected in weighed glass vials and lipid content was determined gravimetrically in 5 periods of 5 to 60 min on an analytical balance (Marte, model AM 220) and were expressed in dry basis percentage (% DB^{-1}).

Fatty acid quantification

Fatty acid methyl esters (FAME) were prepared by the methylation of TL[28] and analyses were performed in triplicate. Methyl esters were separated by gas chromatography (Trace Ultra 3300 model - Thermo Scientific) equipped with a flame ionization detector and a cyanopropyl capillary column (100 m × 0.25 i.d., 0.25 μm film thickness, CP7420 Varian, EUA). The injector and detector temperatures were 240 °C. The gas flow rates used were 1.2 $cm^3\ min^{-1}$ carrier gas (H_2), 30 $cm^3\ min^{-1}$ make-up gas (N_2), 35 and 300 $cm^3\ min^{-1}$ flame gases (H_2 and synthetic air, respectively). The sample splitting rate was 1:80 and the samples (2 μL) were injected in duplicate. The main operational parameters were as follows: the column temperature was held at 185 °C for 7.5 min, programmed to increase at 4 °C min^{-1} to 235 °C and maintained at this temperature for 1.5 min; the total run time was 25 min. The peak areas were determined by the ChromQuest 5.0 software. For fatty acid identification, retention times were compared with those of standard methyl esters.

Quantification (in mg FA g^{-1} of TL) was performed against tricosanoic acid methyl ester as an internal standard (23:0).[29] Theoretical FID correction factor values[30] were used to obtain concentration values. FA content was calculated in mg g^{-1} of total lipids using equation 1:

$$FA = \frac{A_x \cdot M_{IS} \cdot CF_X}{A_{IS} \cdot W_X \cdot CF_{AE}} \quad (1)$$

Table 1. Factors and levels for the 2^2 factorial design

Factors	Symbol	Unit	Type	Levels		
				−1	0	+1
Temperature	T	ºC	Numeric	30	45	60
Pressure	P	MPa	Numeric	8	10	12

where FA is expressed as mg g^{-1} total lipids, A_X is the peak area, A_{IS} is the peak area of the internal standard (IS) methyl ester of tricosanoic acid (23:0), W_{IS} is the IS weight (mg) added to the sample (mg), W_X is the sample weight (mg), CF_X is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of FA rather than as methyl esters. The results were converted from FA mg g^{-1} of oil.

Phytosterols and tocopherols quantification

Phytosterols and tocopherols were simultaneously evaluated by gas chromatography coupled to mass spectrometry.[31] The extracted oils were previously derivatized[32] and the analysis was performed in a gas chromatograph (Thermo–Finnigan, model Thermo Focus GC) equipped with a capillary column DB-5 (5% phenyl, 95% methylpolysiloxane) fused silica, 30 m, 0.25 mm i.d. and 0.25 mm thick film stationary phase (J & W Scientific) coupled to a mass spectrometer (Thermo–Finnigan, model DSQ II) equipped with an electron ionization source (EI). The system of data acquisition was performed by Xcalibur software accompanying database of spectra contained in the NIST MS Search spectral library version 2.0. Flow rate of gas was 1.0 cm^3 min^{-1} for the carrier gas (He - 5.0). The injections were performed in triplicate; the injection volume was 2 μL and the sample splitting rate was 1:10. The temperature of the injector and detector was 280 °C. The initial temperature of the column was 200 °C for 8 min, programmed to increase to 235 by 3 °C min^{-1}, and then to 280 by 15 °C min^{-1}; the column remained at this temperature for 15 min. The temperature of the transfer line between GC and MS was 250 °C.

Quantitation were carried out in relation to the internal standard 5 α-cholestane (Sigma, Brazil), according to Li *et al*.[33]

ESI(+)-MS analysis

For ESI(+)-MS analysis, the oils were dissolved in 1.0 cm^3 of HPLC-grade methanol and injected into the ESI source of the mass spectrometer (BRUKER, model HCT ultra ETD II) with an auxiliary syringe pump by a flow of 400 μL h^{-1}. Spectra were acquired under the following conditions: capillary and skimmer of −3000 and 40 V, respectively, source temperature of 300 °C, and in the *m/z* 100-1200 range.

Differential scanning calorimeter (DSC)

The oxidative stability of the oils extracted by different extraction methods was evaluated by the midpoint.[34] An amount of 12.0 ± 0.5 mg of oil was placed in platinum capsules and introduced into the differential scanning calorimeter (DSC) (Netzsch, model STA 6000 PerkinElmer) to be analyzed at four different temperatures: 110, 120, and 140 °C. While the temperature was being increased, the sample was kept in contact with an inert atmosphere (N_2) with a flow of 50 cm^3 min^{-1}, contacting with a flow of 50 cm^3 min^{-1} of oxygen 4.5 in the set temperature.

Statistical and principal components analysis (PCA)

Proximate composition, phytosterols and tocopherols analyses were performed in triplicate and fatty acid analysis was done in quadruplicate. Means and standard deviations of the analytical error propagation were calculated. The results were submitted to variance analysis (ANOVA) and mean values were compared by Tukey's test, using the Statistica software,[35] version 8.0. The Principal component analyses (PCA) were performed with the Statistica software, version 8.0.

Results and Discussion

Total lipids (TL)

Table 2 shows the percent of TL extracted by SubFE-propane (A-E) and conventional solvent extraction methods (BD, SE e FLS). SubFE-propane A and B condition, BD and SE extraction methods presented significant differences in total lipid extraction.

Table 2. Percentage of TL extracted by different methods in dry basis

Extraction Methods	Temperature / ºC	Pressure / MPa	TL / %
A	45	10	$28.16^{a,a,AB}$
B	30	8	$23.61^{b,b,AB}$
C	30	12	$24.43^{b,ab,AB}$
D	60	8	$25.77^{b,ab,AB}$
E	60	12	$27.24^{b,ab,AB}$
BD	25	0.1	23.25^{B}
SE	65	0.1	30.21^{A}
FLS	25	0.1	27.92^{AB}

TL: Total lipids; BD: Bligh and Dyer; SE: Soxhlet; FLS: Folch, Less & Stanley. A, B, C, D and E: letters representing the testing of extraction with subcritical fluid. Averages of triplicates ± standard deviation absolute. [a]Average of triplicates of the center point. Means followed by different italic lowercase letters in the same column demonstrated significant difference by Tukey test ($p < 0.05$) to different conditions of subcritical extraction chia oil with *n*-propane. Means followed by different uppercase letters in the same column are significantly different by Tukey test at 5% probability to different methods of chia oil extraction; [b]Parameters used in subcritical fluid extraction.

The TL values for BD, SE and FLS were 23.25, 30.21 and 27.92% (Table 2), respectively. BD value is similar to 21.69% (in wet basis) obtained by Sargi *et al.*,[36] which also studied Brazilian Chia oil by BD. SE percent (30.21%) is similar to about 32.5% obtained by Olivos-Lugo *et al.*[37] and Monroy-Torres *et al.*,[38] which studied the Mexican Chia oil extracted by SE. Ixtaina *et al.*[39] obtained by supercritical fluid extraction with CO_2 solvent, in 4 h, the same TL amount obtained in Table 2 with *n*-propane solvent in 1 h.

Fatty acid quantification

Table 3 shows the results for FA composition. The main FA found in the extracted oils were: palmitic acid (16:0), stearic (18:0), oleic (18:1 n-9), linoleic (18:2 n-6) and alpha-linolenic acid (18:3 n-3), and this composition agree with those found previously.[3–5,36]

Ixtaina *et al.*[39] also obtained LNA values ranged from 44.4 to 63.4 mg g^{-1} LT (Table 2), in Mexican Chia oil. The FA present in the highest amount was alpha-linolenic acid, with approximately 600 mg of AG g^{-1} of total lipids, which is equivalent to 60% of the total mass of oil, as also reported previously,[3–5] when studying different cultivars of chia. The quantify of 18:3 n-3 obtained was the highest in C (subcritical fluid conditions).The sum of total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total n-6 fatty acids (n-6) and n-6/n-3 ratio showed no significant differences in extraction yields.

Phytosterols and tocopherols quantitation

Table 4 shows the composition of phytosterols and tocopherols obtained for Chia Oil, in mg of the compound by 100 g^{-1} oil.

For campesterol and sitosterol, the highest values were found for the oil obtained by Soxhlet extraction. Ciftci *et al.*[40] in chia oil sterols extracted by Folch *et al.*[24] and observed that the sum of sitosterol, stigmasterol and campesterol were 205.7, 124.8 and 47.2 mg 100 g^{-1}, respectively. The values in Table 4 were different likely because other variety of chia was evaluated. Similar results were obtained by Ixtaina *et al.*[39] for mechanical and solvent lipid extraction method of tocopherol total amount, from 24 to 46 mg 100 g^{-1} of Chia Oil.

ESI(+)-MS analysis

All chia oils obtained by different techniques and conditions were directly analyzed by ESI(+)-MS and Figure 1 shows representative lipid profiles. Cabral *et al.*[41] has showed that ESI(+)-MS offers a rapid and efficient technique for vegetable oil typification via triacylglycerols (TAG) profile.[41] These spectra can also reveal the level of oil oxidation.[42]

Note that both glycerolipids (diacylglicerol (DAG) and triacylglycerol (TAG)) and glycerophospholipids (glycerophosphoinositol (PI), glycerophosphocholine (PC), glycerophosphoserine (PS), glycerophosphates (PA),

Table 3. Quantification of fatty acids (mg g^{-1} of oil), summations and n-6/n-3 ratio of Chia oil extracted by the method of Bligh & Dyer, Soxhlet, Folch, Less & Stanley and subcritical fluid using *n*-propane

FA	0,0(A)	−1,−1(B)	−1,+1(C)	+1,−1(D)	+1,+1(E)	SE	FLS	BD
16:0	$66.01^{a} \pm 2.16$	$67.59^{a} \pm 2.86$	$61.57^{a} \pm 1.77$	$65.60^{a} \pm 6.11$	$61.93^{a} \pm 0.13$	$63.08^{a} \pm 0.87$	$67.76^{a} \pm 8.20$	$63.57^{a} \pm 0.16$
16:1n-9	$1.42^{ab} \pm 0.04$	$1.35^{abc} \pm 0.06$	$1.37^{abc} \pm 0.05$	$1.55^{a} \pm 0.22$	$1.32^{abc} \pm 0.02$	$1.30^{bc} \pm 0.03$	$1.16^{c} \pm 0.12$	$1.37^{abc} \pm 0.01$
18:0	$27.75^{a} \pm 4.04$	$25.94^{a} \pm 2.90$	$23.00^{a} \pm 0.30$	$24.89^{a} \pm 2.55$	$23.21^{a} \pm 0.09$	$23.60^{a} \pm 0.41$	$26.04^{a} \pm 4.04$	$23.27^{a} \pm 0.04$
18:1n-9 c	$56.12^{a} \pm 4.92$	$60.87^{a} \pm 11.72$	$50.52^{a} \pm 0.86$	$53.88^{a} \pm 4.32$	$50.52^{a} \pm 0.33$	$51.56^{a} \pm 1.10$	$67.35^{a} \pm 18.91$	$53.79^{a} \pm 0.15$
18:1n-7	$6.97^{a} \pm 0.10$	$7.24^{a} \pm 0.67$	$6.78^{a} \pm 0.14$	$7.21^{a} \pm 0.45$	$6.93^{a} \pm 0.02$	$6.84^{a} \pm 0.07$	$7.09^{a} \pm 0.64$	$6.91^{a} \pm 0.02$
18:2n-6	$199.79^{a} \pm 1.11$	$210.64^{a} \pm 12.10$	$200.61^{a} \pm 1.34$	$204.69^{a} \pm 5.86$	$199.47^{a} \pm 0.16$	$196.57^{a} \pm 1.28$	$216.66^{a} \pm 20.95$	$204.47^{a} \pm 0.19$
18:3n-3	$611.13^{ab} \pm 11.89$	$601.75^{ab} \pm 32.04$	$628.66^{a} \pm 0.71$	$613.56^{ab} \pm 18.30$	$626.03^{ab} \pm 1.29$	$605.35^{ab} \pm 1.53$	$568.34^{b} \pm 52.58$	$598.37^{ab} \pm 0.16$
24:0	$0.56^{bc} \pm 0.03$	$0.58^{b} \pm 0.02$	$0.53^{bc} \pm 0.02$	$0.54^{bc} \pm 0.01$	$0.58^{b} \pm 0.02$	$0.68^{a} \pm 0.08$	$0.48^{c} \pm 0.04$	$0.72^{a} \pm 0.01$
SFA	$94.32^{a} \pm 4.58$	$94.11^{a} \pm 4.08$	$85.10^{a} \pm 1.80$	$91.04^{a} \pm 6.52$	$85.71^{a} \pm 0.16$	$87.36^{a} \pm 0.96$	$94.28^{a} \pm 9.14$	$87.56^{a} \pm 0.17$
MUFA	$64.51^{a} \pm 4.93$	$69.46^{a} \pm 11.74$	$58.67^{a} \pm 0.88$	$62.64^{a} \pm 4.35$	$58.77^{a} \pm 0.33$	$59.70^{a} \pm 1.10$	$75.60^{a} \pm 18.92$	$62.06^{a} \pm 0.15$
PUFA	$810.91^{a} \pm 11.94$	$812.39^{a} \pm 34.25$	$829.27^{a} \pm 1.52$	$818.26^{a} \pm 19.22$	$825.50^{a} \pm 1.30$	$801.92^{a} \pm 2.00$	$785.00^{a} \pm 56.60$	$802.85^{a} \pm 0.25$
n-3	$611.13^{ab} \pm 11.89$	$601.75^{ab} \pm 32.04$	$628.66^{a} \pm 0.71$	$613.56^{ab} \pm 18.30$	$626.03^{ab} \pm 1.29$	$605.35^{ab} \pm 1.53$	$568.34^{b} \pm 52.58$	$598.37^{ab} \pm 0.16$
n-6	$199.79^{a} \pm 1.11$	$210.64^{a} \pm 12.10$	$200.61^{a} \pm 1.34$	$204.69^{a} \pm 5.86$	$199.47^{a} \pm 0.16$	$196.57^{a} \pm 1.28$	$216.66^{a} \pm 20.95$	$204.47^{a} \pm 0.19$
n-6/n-3	$0.33^{a} \pm 0.02$	$0.35^{a} \pm 0.08$	$0.32^{a} \pm 0.01$	$0.33^{a} \pm 0.04$	$0.32^{a} \pm 0.01$	$0.32^{a} \pm 0.01$	$0.38^{a} \pm 0.13$	$0.34^{a} \pm 0.01$

Mean values ± standard deviation; Means followed by different letters in the same row demonstrated significant difference by Tukey test ($p < 0.05$). A, B, C, D and E: letters representing the testing of extraction with subcritical fluid. MUFA = total monounsaturated fatty acids; PUFA = total polyunsaturated fatty acids; n-6 = total n-6 fatty acids; n-3 = total n-3 fatty acids; (limit of detection = 0.015 mg g^{-1}).

Table 4. Quantification of tocopherols and phytosterols (mg $100g^{-1}$) in chia oil extracted by the methods of Bligh & Dyer, Soxhlet, Folch, Less & Stanley and subcritical fluid using *n*-propane

Method	Tocopherol		Phytosterol		
	β-Tocopherol	γ-Tocopherol	Campesterol	Stigmasterol	Sitosterol
A	$7.751^{e} \pm 0.511$	$7.282^{a} \pm 0.245$	$17.724^{f} \pm 0.799$	$10.818^{f} \pm 0.888$	$132.592^{f} \pm 5.194$
B	$11.262^{d} \pm 1.521$	$6.638^{a} \pm 0.550$	$20.279^{ef} \pm 1.254$	$12.961^{ef} \pm 0.875$	$151.210^{e} \pm 5.045$
C	$20.478^{c} \pm 0.290$	$2.924^{b} \pm 0.197$	$22.269^{de} \pm 1.676$	$13.592^{def} \pm 0.702$	$154.386^{de} \pm 7.130$
D	$25.693^{a} \pm 0.598$	$1.121^{c} \pm 0.373$	$27.072^{cd} \pm 2.248$	$17.267^{bcd} \pm 0.431$	$182.863^{c} \pm 7.384$
E	$23.305^{b} \pm 0.604$	$2.711^{b} \pm 0.863$	$24.705^{de} \pm 1.389$	$16.485^{cde} \pm 1.965$	$170.147^{c} \pm 3.713$
SE	$12.460^{d} \pm 0.749$	$2.341^{b} \pm 0.227$	$37.190^{a} \pm 1.021$	$20.125^{bc} \pm 0.681$	$272.303^{a} \pm 6.880$
FLS	$22.821^{b} \pm 0.790$	n.d.	$23.323^{cde} \pm 1.241$	$20.602^{b} \pm 3.045$	$168.503^{cd} \pm 0.836$
BD	$27.304^{a} \pm 0.446$	n.d.	$30.191^{b} \pm 1.112$	$25.064^{a} \pm 0.688$	$218.996^{b} \pm 1.927$

Mean values ± standard deviation; means followed by different letters in the same row demonstrated significant difference by Tukey test ($p < 0.05$); BD: Bligh and Dyer; SE: Soxhlet; FLS: Folch and Less & Stanley; A, B, C, D and E: letters representing the testing of extraction with subcritical fluid.

glycerophosphoglycerol (PG)) were detected in protonated $[M + H]^+$, sodiated $[M + Na]^+$ and potassiated $[M + K]^+$ forms. TAG ions were detected in the *m/z* 850-1000 range, and DAG and glycerophospholipids ions were detected in the *m/z* 400–800 range.

The FA composition revealed by ESI(+)-MS for the chia oils shows major cluster of ions around *m/z* 873, a second minor cluster around *m/z* 851 and two less abundant clusters of ions around *m/z* 891 and 595. The results obtained by ESI(+)-MS in this study were compared with literature data established via ambient desorption/ionization by easy ambient sonic-spray ionization mass spectrometry (EASI(+)-MS) because according to Cabral *et al.,*[41] the ESI(+)-MS and EASI(+)-MS has been shown to provide similar profiles for the oils.

This ESI(+)-MS profile is quite different from other common vegetable oils such as soybean and corn as reported by Simas *et al*.,[42] using a direct ambient ionization technique; that is, EASI(+)-MS. Marineli *et al*.,[43] also characterized the Chia oil using EASI(+)-MS also finding a quite typical profile with predominance of α-linolenic acid. The main FA present in the TAG molecules for the Chia oil were palmitic (P, 16:0), stearic (S, 18:0), oleic (O, 18:1n-9), linoleic (L, 18:2n-6) and particularly α-linolenic (Ln, 18:3n-3).

The ions in the ESI(+)-MS were assigned as follow: *m/z* 573.5 ($[PI + H]^+$, P), *m/z* 595.5 ($[DAG + H]^+$, OP), *m/z* 613.5 ($[DAG + H]^+$, LnLn; $[DAG + Na]^+$,PLn), *m/z* 851.7 ($[TAG + Na]^+$, PPLn) , *m/z* 853.7 ($[TAG + Na]^+$, PPL; $[TAG + H]^+$, PLLn), *m/z* 855.7 ($[TAG + Na]^+$, PPO), *m/z* 873.6 ($[TAG + K]^+$, PPS; $[TAG + Na]^+$, LnLnP), *m/z* 875.7 ($[TAG + Na]^+$, PLLn), *m/z* 877.7 ($[TAG + Na]^+$, PLL or LnOP), *m/z* 879.7 ($[TAG + Na]^+$, POL; $[TAG + H]^+$, LLL), *m/z* 891.6 ($[TAG + K]^+$, PLLn), *m/z* 893.6 ($[TAG + K]^+$, PLL; $[TAG + K]^+$, POLn or LLP;), *m/z* 895.6 ($[TAG + K]^+$, POL; $[TAG + Na]^+$, LnLnLn) and *m/z* 897.6 ($[TAG + K]^+$, POO or LPS; $[TAG + Na]^+$, LLnLn).

Oils obtained by SubFE-propane and by Bligh & Dyer extractions showed nearly identical spectra (Figure 1 - A) which demonstrate very similar and characteristic TAG compositions, and no significant oxidation.[42] Chia oils obtained by Soxhlet and Folch methods slightly differ by showing additional ions particularly in the *m/z* 380-800 range. The ESI(+)-MS of the chia oil obtained by FLS seems to display a characteristic ion of *m/z* 780.5 ($[PC + Na]^+$, PL; $[PS + Na]^+$, LnP) (Figure 1 - FLS) .

The Soxhlet extraction oil (Figure 1 - SE) displayed a diverse set of unique ions mainly of *m/z* 397.4 ($[MAG + K]^+$, S), *m/z* 441.4, *m/z* 485.4 ($[PG + H]^+$, P), *m/z* 529.4 were detected in the m/z 850–1000, *m/z* 551.4 ($PG + K]^+$, S), *m/z* 617.4 ($[DAG + H]^+$, LnLn; $[DAG + H]^+$, OLn; ($[DAG + Na]^+$, PO; $[PI + Na]^+$, Ln), *m/z* 639.4 ($[PI + K]^+$, S; $[DAG + Na]^+$, LL or OLn), *m/z* 661.4 ($[DAG + K]^+$, SO), *m/z* 705.4 ($[PA+ H]^+$, SS) and *m/z* 749.4 ($PG + H]^+$, PO).

The differences in the ESI(+)-MS profiles of Figure 1 - A as compared to the different extraction methods (Figure 1 - FLS, BD, SE) show that the SubFE-propane Chia oil displays fortunately similar TAG profiles than that of all SE methods but SubFE-propane was able to extract considerably higher quantities of glycerophospholipids. The hydrolysis products DAG were also higher for the SE methods, likely due to the higher temperatures and longer extraction times (Figure 1 - SE).

Differential scanning calorimetry (DSC)

Table 5 shows induction temperatures obtained from differential scanning calorimetry, which determine the point where the oils begin to be oxidized. The values for the SubFE-propane oil indicate higher resistance to oxidation at

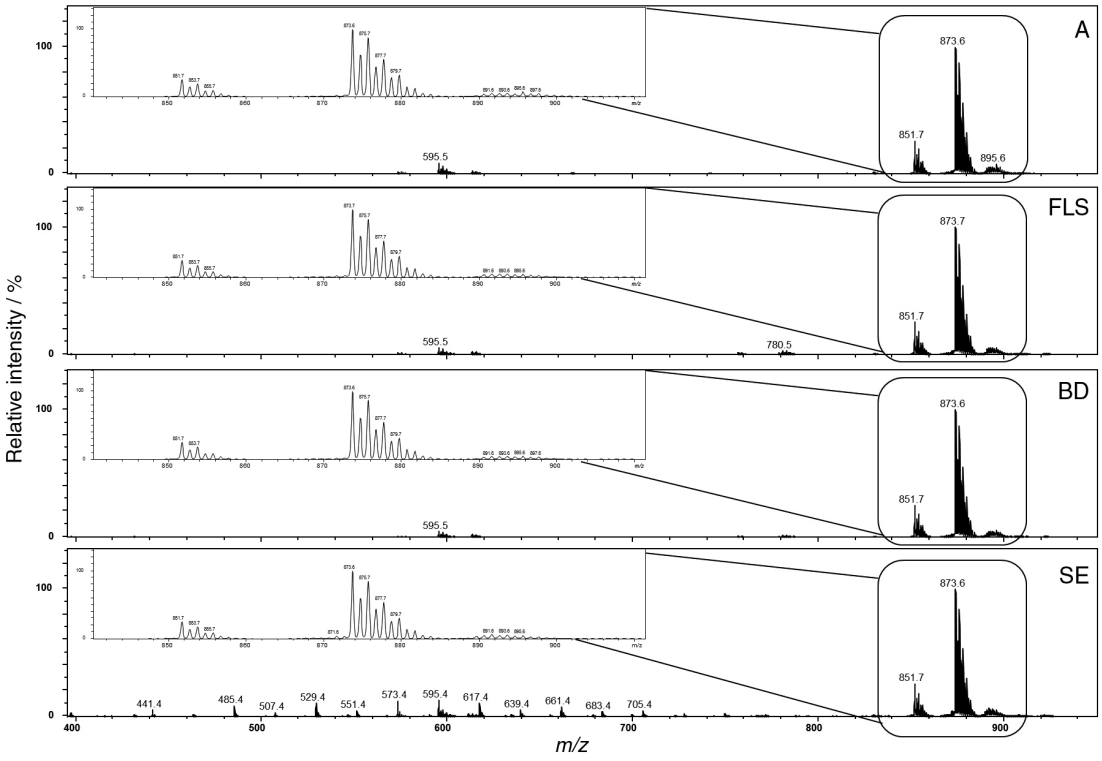

Figure 1. ESI(+)–MS spectra for Chia oils in methanol solutions obtained by different extraction methods, as indicated.

Table 5. Time of oxidative induction obtained by differential scanning calorimetry (DSC) and its logarithmic regression equation among T_0 and the temperatures of the isotherms for chia oils extracted by the methods of Bligh & Dyer, Soxhlet, Folch, Less & Stanley and subcritical fluid using *n*-propane

Extraction method	Temperature / ºC			Regression equation	R^2
	110	120	140		
	DSC T_0 / min				
A	49.4	25.5	11.3	$T = 461.82 - 47.19 \log_{10} T_0$	0.9661
FLS	27.0	10.8	2.3	$T = 423.53 - 28.46 \log_{10} T_0$	0.9953
BD	31.3	16.4	4.5	$T = 432.74 - 33.18 \log_{10} T_0$	0.9612
SE	38.3	17.7	5.0	$T = 436.18 - 33.88 \log_{10} T_0$	0.9951

A: subcritical *n*-propane extraction at 45 °C and 10 MPa; BD: Bligh & Dyer; SE: Soxhlet; FLS: Folch, Less & Stanley.

all temperatures compared to all other SE oils, showing the effectiveness of the SubFE-propane method. The values for A were from two to five times longer than those measured for the other oils. Similar results were also observed for other oils from oleaginous obtained via SFE-CO_2 and SubFE-propane *versus* conventional SE extractions with hexane.[21,22,44]

Principal Components Analysis

PCA was performed to try to find correlations for the amount of key components (SFA, MUFA, PUFA, n-3 and n-6) in relation to the different conditions of subcritical *n*-propane extraction method (Figure 2). The variance explained was 71.19 and 26.16% for PC1 and PC2,

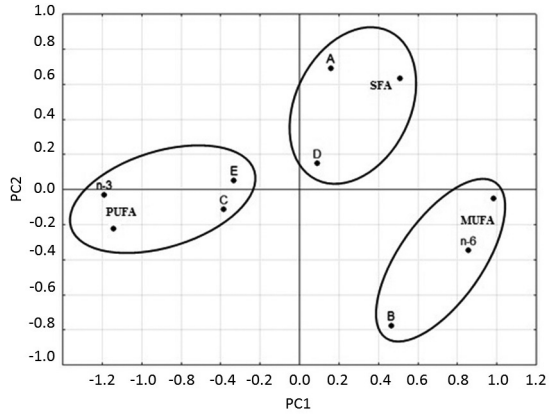

Figure 2. PCA of sums de FA for Chia Oil A, B, C, D and E; letters representing the testing of extraction with subcritical fluid. MUFA = total monounsaturated fatty acids; PUFA = total polyunsaturated fatty acids; n-3 = total n-3 fatty acids.

respectively, reducing the five variables for two and totaling 97.35%. Conditions C and E were more efficient in relation to the sum of n-3 PUFA and AGPI extraction.

Conclusions

SubFE-propane has been found to represent indeed a promising alternative for oil extraction with superior results as compared with conventional SE methods. The oil obtained by SubFE-propane is less oxidized compared to oils obtained by SE, BD and FLS and has higher purity. SubFE-propane at 45 °C and 10 MPa when applied to Chia oil extraction showed to be fast providing the highest oil yield, purity, and best oxidation stability with comparable levels of biologically active components.

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