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Original Article

Characterization of chia seed oils obtained by pressing and solvent extraction

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ABSTRACT

The oil yield, fatty acid composition and the physicochemical and quality characteristics of chia crude seed oils obtained by pressing and solvent extraction were determined. The extraction methods assayed influenced significantly the oil yield, obtaining about 30% more oil by solvent than by pressing. The main fatty acids ranked in the following order of abundance: α-linolenic acid (α Ln) > linoleic acid (L) > oleic acid (O) ≈ palmitic acid (P) > stearic acid (S) for both extraction systems. The *n*-3/*n*-6 FA ratio of chia oils ranged from 3.18 to 4.18, being markedly higher than that reported for other vegetable oils. The main triacylglycerols were: αLnαLnαLn > αLnαLnL > αLnLL > αLnαLnP > αLnLO ~ αLnLP, which represent about 87–95% of the total content of these compounds. The quality and composition of some minor constituents of chia seed oils were influenced by the extraction process. Oils presented a moderate content of bioactive components, such as tocopherols, polyphenols, carotenoids and phospholipids; the high unsaturation level determined their low oxidative stability.

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1. Introduction

Vegetable oils are utilized for many food and industrial purposes. Despite the vast range of vegetable oils sources, world consumption is dominated by palm, soybean, rapeseed, and sunflower oils with 38.1, 35.7, 17.8, and 18.2 million tons consumed per year, respectively (American Soybean Association, 2007). In recent years there has been a parallel development of underexploited promising plant species as a source of dietary or specialty oils. Many of them contain significant quantities of oils and/or a high proportion of nutritionally, medicinally or industrially desirable fatty acids.

The composition of vegetable oils is important from the nutrimental point of view. Thus, *n*-3 fatty acids (FAs) play a very essential role in physiology, especially during fetal and infant growth (Bowen and Clandinin, 2005). It is also important for the prevention of cardiovascular diseases, being antithrombotic, anti-inflammatory, antiarrythmic and favoring plaque stabilization (Galli and Marangoni, 2006). Thus, health authorities of many countries, such as AFSSA, the French Agency fir Food Safety, as well as British and German authorities have independently promoted the intake of foods containing high amounts of *n*-3 FAs and a favorable *n*-3/*n*-6 fatty acid (FA) ratio. With this recent concern about specific FA, the food industry has looked for particular fats and oils containing these compounds, to optimize the "fat profile" of the final products (Dubois et al., 2007). Although use of oils with high levels of *n*-3 has traditionally been restricted owing to their instability and flavor reversion, availability of stabilized products has allowed manufacturers to offer a variety of products (Shahidi, 2008).

Salvia hispanica L. and other *Salvia* members of the family *Labiatae* have long been used as a food ingredient and are especially well-known by American Indians and rural Mexicans. It has been cultivated under environments ranging from tropical to subtropical conditions. Plants are not very frost tolerant but can be grown as summer annuals in greenhouses in some parts of Europe (Huxley, 1992). Today, chia is grown commercially in Mexico,

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Bolivia, Argentina, Ecuador, and Guatemala. Chia (*S. hispanica* L.) seeds have about 25–38% oil by weight which contains the highest proportion of α-linolenic acid (~60%) of any known vegetable source (Ayerza, 1995; Palma et al., 1947). Dubois et al. (2007) have clustered oils according to their FA profiles, and have included chia seed oil in PUFA class (α-linolenic + linolenic acid subclass). These authors highlight that chia seed oil is a very interesting source with regard to providing a good equilibrium between the two essential FA. Currently, chia seed oil is not widely used commercially even though it has characteristics that are well-suited for industrial applications, and they can contribute to healthy human diets.

Commercial production of vegetable oils is based on pressing and solvent extraction. Since chia seed oil is a natural product, it has a variable chemical composition depending on several factors such as the cultivation environment (Ayerza, 1995; Coates and Ayerza, 1998) and the extraction system (Daukšas et al., 2002).

To date, solvent extraction of chia seeds and some scanty data on their composition have been reported (Álvarez-Chávez et al., 2008; AOCS, 1998; Ayerza, 1995; Bushway et al., 1981; Coates and Ayerza, 1998), but there is little information about chia seed oil and the influence of the extraction system on its fatty acid profile and physicochemical characteristics (Velasco Vargas et al., 2004). Moreover, no information was found about the triacylglycerol composition, presence of the antioxidants, metals, wax esters and pigments in chia seed oil, and about its oxidative stability. Further knowledge on chia oil composition may lead to seek different uses in the food industry, such as the development of functional foods, or medicinal, pharmaceutical and other non-food industrial applications.

The objectives of this work were to study the oil yield, fatty acid composition and the physicochemical and quality characteristics of chia seed oils obtained by pressing and solvent extraction.

2. Materials and methods

2.1. Seeds

Commercial chia seeds used in this study were purchased from two different sources: Functional Product S.A., Argentina (set 1) and Apsaxela, Guatemala (set 2) (20 kg each). They were manually cleaned, homogenized and packed in hermetic plastic vessels and stored at 5 °C until further use. Randomized samples of each independent set of commercial chia seeds (approximately 7–8% d.b. moisture content) picked by a sample splitter (CPASA, Centro Proveedor Agropecuario, Buenos Aires, Argentina) were used to obtain oils by pressing or solvent extraction. Each set of oils obtained by both extraction systems were analyzed in duplicate.

2.2. Oil extraction

2.2.1. Solvent extraction

Seed samples (40 g) were grinded using a coffee mill (Braun, Type 4041, Mexico) for 60 s. These materials were extracted with *n*-hexane in a Soxhlet apparatus by thermal cycles at 80 °C for 8 h, following the IUPAC Standard Method (IUPAC, 1992). The solvent was removed using a rotary vacuum evaporator at 40 °C (Büchi, Flawil, Switzerland), under nitrogen stream. The oil content was gravimetrically determined and expressed as weight percent on dry basis (%, d.b.).

2.2.2. Pressing

The seed moisture content at the time of pressing was adjusted to enhance oil recovery and to avoid choking problems during press operation. The pressing capacity, calculated as the weight of product oil collected in a known period of time, was the highest when the seed moisture content was around 10% (w/w). Moistening increased seed plasticity and contributed to press feeding owing to its effect as barrel lubricant. As soon as the desired moisture content was attained, seeds were immediately expressed at 25–30 °C using a Komet screw press (Model CA 59 G, IBG Monforts, Mönchengladbach, Germany), with a 5-mm restriction dye and a screw speed of 20 rpm. The screw press was first run for 15 min without seed material but with heating *via* an electrical resistance-heating ring attached around the press barrel, to raise the screw-press barrel temperature to the desired temperature. Running temperature was checked with a digital thermometer inserted into the restriction dye. Oil obtained was stored at 4 °C, without further treatment. The oil content was gravimetrically determined and expressed as weight percentage on dry basis (%, d.b.).

Oils obtained by both extraction systems were stored in dark vessels with a nitrogen atmosphere at 4 °C until their use.

2.3. Oil analytical determinations

2.3.1. Fatty acid composition

The fatty acid composition was determined as methyl esters: 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 boron triflouride–methanol-complex (20% solution in methanol) (Merck) plus 1 mL methanol for 45 min at 60 °C, and then extracted from the methanolic phase with petroleum ether. GC analysis: 1 μL hexane solution of fames was injected on column in GC (Hewlett Packard 6890) equipped with a capillary column Supelco 11090-02A Omegawax (30 m × 0.250 mm, i.d. 25 μm). The separation was carried out at 175–220 °C (3 °C/min) with helium as carrier (25.1 psi) and a FID detector at 260 °C (Christie, 2003). The results were expressed as the relative percentage of each individual fatty acid (FA) presents in the sample.

2.3.2. Analysis of positional distribution of fatty acids by ^{13}C NMR spectroscopy

The ^{13}C NMR spectra were recorded on a spectrometer Avance III 600 (Bruker, Karlsruhe. D), magnetic flux density 14.1 T equipped with a QNP cryo probe head and an automated sample changer Bruker B-ACS 120. Computer Intel Core2 Duo 2.4 GHz under MS Windows XP and TopSpin 2.1 were used for the acquisition of data; the processing was made by Bruker TopSpin 2.1 software, following a standard operation procedure SAA-GMR028-01. The samples were prepared dissolving 200 mg in 1 mL of $CDCl_3$ and 200 μL DMSO-d_6. Further, samples were dried over 200 mg anhydrous sodium sulfate. The number of scans (NS) for the ^{13}C NMR was 1024.

2.3.3. Triacylglycerol composition

Oil samples (10 mg) were dissolved in 2 mL of $CHCl_3/CH_3OH$/HCOOH (50:50:0.1) and their triacylglycerol composition were determined by HPLC/APCI-MS.

The chromatographic apparatus consisted of a gradient Rheos 2000 pump (Flux Instruments, Basel, CH), a degassing unit on-line-Degasser ERC-3415 α (ERC GmbH, Riemerling, G), a 6000LP diode array UV detector (Thermo Finnigan, San José, CA), a HTC PAL System autosampler (CTC Analytics AG, Zwingen, CH), and a thermostated column compartment (column oven L-5025, Merck, Darmstadt, G). The HPLC conditions were: chromatographic column Nucleosil 120 C_{18} (125 m × 4 mm × 5 μm), mm), a flow rate of 0.4 mL/min, an injection volume of 10 μL, column temperature of 40 °C and a mobile phase (A: acetonitrile, B: 0.1% ammonium acetate in 2-propanol w/v). A gradient

was applied as follows: % solvent A decreased from 60 to 35, from 0 to 31 min, after that increased from 35 to 60 until 35 min and then was maintained constant up to 45 min. The injector needle was washed with the mobile phase before each injection. The UV detection at 200 nm and positive-ion APCI-MS were coupled in series. The Finnigan LTQ mass spectrometer (Thermo Electron Corp., San José, CA) in the mass range m/z 500–1000 was used with the following setting of tuning parameters: capillary temperature 250 °C, sheath gas flow 20 psi, the drying gas flow rate of 20 L/min, temperature of the APCI vaporizer was 450 °C. The reconstructed ion current chromatograms in the region m/z 800–1000 were used for the peak integration. To analyze the data a computer PIV 2.8 GHz under Windows XP and Software Xcalibur 2.0 were used.

2.3.4. Physical and chemical parameters

Iodine and saponified values, refractive index, unsaponifiable matter and free fatty acid contents were determined according to AOCS recommended practices Cd 1c-85, Cd 3a-94, Cc7-25, Ca6a-40 and Ca 5a-40, respectively (AOCS, 1998). Total phosphorus content was measured by the method IRAM 5597 (IRAM, 1970).

2.3.5. Color

Minolta colorimeter (CR-400, Konica Minolta Sensing Inc., Japan) was used to detect L^*, a^*, and b^* values of oil samples. L^* value indicates lightness; a^* value indicates redness; and b^* value indicates yellowness. Each value was the mean of 10 determinations (Choo et al., 2007).

2.3.6. Carotenoid and chlorophyll determinations

Extraction and quantification of carotenoids was performed according to Messina et al. (2009). Oil samples (0.5 g aliquots) were filled to a total volume of 3 mL with of 0.9% NaCl, and mixed volume to volume with 1% of pyrogallol in ethanol. Saponification was performed for 30 min at 70 °C with 0.9 mL 12 N KOH. Then, samples were extracted twice with *n*-hexane (3 volumes per extraction) after the addition of distillate water, evaporated under nitrogen flow, resuspended in *n*-hexane, diluted in absolute ethanol and filtered through a 0.45-micropore nylon membrane before injection.

HPLC conditions: The HPLC pump (model P4000) with a membrane vacuum degasser and a 20 μL loop injector were purchased from TSP (Thermo Separation Products Inc., USA), and connected to an Alltima C18 column (250 mm × 4.6 mm), 5 μm particle size. The electrochemical detector (Decade, Antec Leyden, The Netherlands) was equipped with a flowcell with Ag/AgCl and glassy carbon reference and working electrodes respectively.

The mobile phase used for electrochemical detection was modified from the technique described by de Rijke et al. (1997). The flow-rate was 1 mL/min and the reference cell was set at +700 mV. Recovery of β-carotene was 98%. Calibration curves were performed with β-carotene standard (Sigma–Aldrich, St. Louis, USA) diluted in absolute ethanol.

Chlorophyll content was determined by spectrophotometric analyses at 670 nm, in cyclohexane using the specific extinction values (Minguez-Mosquera et al., 1991).

2.3.7. Wax ester content

Wax composition was determined by separation with a silica gel chromatographic column and analysis by GC. A Varian 3700 GLC with FID detector and on-column injection (Varian Associates Inc.), an HP5, 11 m. 0.32 mm (0.52 μm) capillary column (Hewlett-Packard, Palo Alto, CA), and a Millenium 2010 data processor (Millipore Corporation, Milford, MA) were used (Carelli et al., 2002).

2.3.8. Metals

Fe and Cu contents in chia seed oils were measured by flame atomic absorption spectrometry using a GBC 902 AA spectrometer. Oil samples were mineralized (550 °C, 16 h) to obtain carbon free white ashes. Ashes were dissolved in chlorhidric acid, filtered and analyzed by triplicate. Results were expressed as mg/kg oil.

2.3.9. Tocopherol analysis

Oil tocopherol content was determined by normal phase HPLC using a Hewlett Packard chromatography system (HPLC Hewlett Packard 1050 Series, Waldbronn, Germany) following the procedures described in IUPAC 2.432 (IUPAC, 1992) and AOCS Ce8-89 (AOCS, 1998). Approximately 0.25 g oil in 5 mL hexane was placed in an ultrasonic bath for 2 min and protected from light. A 20 μL aliquot of this solution was 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, Phillpsburg, USA) as mobile phase at a flow rate of 1.5 mL/min. Tocopherols were detected using a fluorescence detector (Agilent, 1100 Series) with the excitation/emission wavelength set at 290/330 nm, and quantified using a six-point external standard curve.

2.3.10. Polyphenol analysis

Oil samples (2 g) were dissolved with 5 mL of hexane. Polyphenolic compounds were extracted with 2 mL each time of a mixture of acetonitrile and 10% acetic acid in the proportion (60:40), (50:50) and (40:60) and stirring by vortex. The samples were centrifuged and the polar phase was collected. The pooled extracts were evaporated to dryness by using a speed-vac evaporator Heto VR-1. The residue was dissolved in acetonitrile:10% acetic acid (50:50) and analyzed by HPLC/APCI-MS.

These analyses were carried out with a Surveyor Plus Chromatograph (quaternary pump and autosampler) coupled to a LTQ XL Linear Ion Trap (Thermo Fisher Scientific).

The chromatographic separations were performed with a C_{18} 150 mm × 2.1 mm 335 μm XTerra (Waters) and guard column C_{18} 4 mm × 2 mm (Phenomenex), and the mobile phase consisted of 0.1% acetic acid in acetonitrile:water (95:5) (solvent A) and 0.1% acetic acid in water (solvent B), at 300 μL/min and 20 °C. A gradient was applied as follows: % solvent A increased from 10 to 50, from 0 to 5 min and was maintained until 12 min and decreased to 10 at 13 min and maintained at 10 for the last 4 min until next injection. Injection volume was 10 μL. All the assays were carried out by duplicate.

Mass detection was carried out using APCI source operating in positive ion mode. Polyphenols formed $[M+H]^+$ and were detected as follows: m/z 181 for caffeic acid, m/z 287 for kaempferol, m/z 303 for quercetin, m/z 319 for myrcetin, and m/z 355 for chlorogenic acid. Myrcetin was used to create the tuning file for the instrumental method. Quantitation of samples was performed by external standard method in the range of 100–3 μM for the polyphenols (de Rijke et al., 2006; Taga et al., 1984).

2.3.11. Oxidative stability analysis

Oil oxidative stability was evaluated by the Rancimat (Mod 679, Metrohm) method, using 5 g oil sample warmed at 98 °C with air flow of 20 L/h. Stability was expressed as the induction time (h), according to Gutiérrez (1989).

2.4. Statistical analysis

Each separate set of seed oil was analyzed in triplicate. Data were processed using the Statgraphics Plus statistical package (Version 4.0 for Window, Manugistics Inc., USA). Normal distribution of the variables was checked by the Kolmogorov–Smirnov test ($p \leq 0.05$) and variance check was done by Cochran's test. To test

the significance of the differences among the extraction systems, for each variable analyzed, data were processed by one-way ANOVA. When it was necessary, data were transformed to do not violate the assumptions underlying the ANOVA test. Means were separated according to Tukey's multiple comparison tests.

3. Results and discussion

The oil yield and the FA composition of chia seeds extracted by solvent and pressing are presented in Table 1. The oil yield ranged from 20.3 to 33.6%, which was lower than data published by Ayerza (1995), but in the same range than that reported by Velasco Vargas et al. (2004) by solvent extraction. On average, the fatty acids ranked in the following order of abundance: α-linolenic acid (C18:3) > linoleic acid (C18:2) > oleic acid (C18:1) ≈ palmitic acid (C16:0) > stearic acid (C18:0) > vaccenic vaccenic acid (18:1). In agreement with findings reported in earlier studies (Ayerza, 1995; AOCS, 1998), the main FA was α-linolenic acid (64.5–69.3%). The *n*-3/*n*-6 FA ratio of chia oils ranged from 3.18 to 4.18, being these values markedly higher than that of most vegetable oils, e.g. canola oil (0.45), olive oil (0.13), soybean oil (0.15), and walnut oil (0.20) (Belitz and Grosch, 1999). Therefore, the incorporation of chia seed oil into the diet would be very beneficial due to the vegetable oils with a high content of PUFAs have been well documented to provide numerous health benefits (Bowen and Clandinin, 2005). The comparison between both extraction systems studied show that there were significant differences ($p \leq 0.05$) for oil yield, but not for FA composition ($p > 0.05$). The highest yield was obtained by solvent extraction which resulted, on average, about 30% higher than the total oil yield obtained by pressing. This fact may be attributed to a greater ability of the organic solvent (compared to pressing) to extract most of the lipid components. The results obtained for FA composition were in agreement with those recorded by Brevedan et al. (2000) and Concha et al. (2006) for sunflower and rosehip oil, respectively. A significant negative correlation ($r = -0.91$; $p = 0.0015$) was found between PUFAs and SFAs, which is according to the information reported about the synthesis of both types of FAs (Belitz and Grosch, 1999).

The ^{13}C NMR is an analytical technique that allows obtaining information about the glyceride and fatty acid composition of oils. The carbonyl signal region is sensitive for position analysis of the different fatty acid types; by this way it is possible to make the distinction of *sn*1/3 and *sn*2. (Diehl, 2008). The olefinic region, which has a larger dispersion of chemical shifts, can be utilized to quantify those acyl groups which have superimposed carbonyl peaks. In this way, a combination of both the olefinic and carbonyl spectral regions can provide the necessary information to quantify the full acyl positional distribution of chia seed oil.

A typical ^{13}C NMR spectrum of the chia oils analyzed is reported in Fig. 1. This spectrum shows resonances grouped in four sets of signals: carbonyls (from 172 to 174 ppm), unsaturated carbons (from 125 to 135 ppm), glycerol backbone carbons (from 60 to 80 ppm), and aliphatic carbons (from 15 to 35 ppm). The spectral region of the ^{13}C spectrum of chia oil in the range where the carbonyl carbon resonate (172.8–173.3 ppm) is amplified in Fig. 1a. In fact, within this spectral region, the low field (high frequency) and high field (low frequency) carbonyl group resonances are due to chains esterified in the *sn*1,3 positions and the *sn*2 position, respectively. These two sets of carbonyl resonances are separated by a shift of about 0.4 ppm. This shift was explained by noting that C=O groups of 2-position chains experiment two vicinal carbonyls interaction while just only one for carbonyls of *sn*1,3-chains (Mannina et al., 1999). The 1,3- and 2-linoleyl, linolenyl resonances overlap in the carbonyl region. In order to obtain 1,3-acyl and 2-acyl composition of these two fatty acids, the olefinic spectrum must be analyzed. Fig. 1b shows an expanded view of the ^{13}C NMR olefinic spectrum of chia seed oil. Multiple olefinic carbon peaks of an unsaturated acyl group are the single most important aspect of the olefinic region. Unresolved unsaturated acyl groups in the carbonyl region, such as linoleyl and linolenyl, have four and six olefinic carbons, respectively, to choose from. A differentiation of linoleyl and linolenyl is possible as ω-3 vs. ω-6 as a sum from carbon atoms at the fatty acid chain ends (methyl and *n*-2) as well as from the olefinic region including the regioselective position. This fact allows for many possible combinations to quantify the linoleyl and linolenyl content, as well as the positional distribution when peak regions are congested. In Table 2, the percentage of fatty acid chains on the glycerol moiety positions is reported. It is clear that saturated fatty acids (palmitic and stearic acids) and Δ-11 are highly distributed over the *sn*1,3 positions. The *sn*1,3:2 acyl ratio of the oleic acid was about 60:40. Regarding the polyunsaturated acids, 8.9–12.3% of linoleic and 33.5–41.7% of α-linolenic acid are distributed in position 1,3, which represent about 55% and 64%, of the total linoleic and α-linolenic acids present in chia oils, respectively.

Furthermore, it is possible to note that the extraction system did not affect the distribution of the FA on the glycerol positions. Results obtained by ^{13}C NMR are in good agreement with the fatty acid composition of chia seed oil obtained by GC (Table 1).

The triacylglycerol composition determined by HPLC/APCI-MS of chia oils is listed in Table 3. This analysis was made for chia oils

Table 1
Oil yield (%, d.b.) and fatty acid composition (% of total fatty acid) determined by GC of two independent sets of *Salvia hispanica* L. seed oils extracted by solvent and pressing.

	Set 1		Set 2	
	Solvent extraction	Pressing	Solvent extraction	Pressing
Oil yield	33.6 ± 0.4[b]	24.8 ± 0.5[a]	26.7 ± 1.9[b]	20.3 ± 0.5[a]
Fatty acids				
Palmitic acid (16:0)	6.2 ± 0.4[a]	6.6 ± 0.3[a]	5.5 ± 0.1[a]	5.9 ± 0.1[a]
Stearic acid (18:0)	3.0 ± 0.7[a]	3.1 ± 0.1[a]	2.7 ± 0.2[a]	4.4 ± 0.9[a]
Oleic acid (18:1)	5.3 ± 1.1[a]	5.4 ± 0.4[a]	5.8 ± 0.3[a]	5.5 ± 0.4[a]
Vaccenic acid (18:1)	0.5 ± 0.01[a]	0.5 ± 0.02[a]	0.4 ± 0.02[a]	0.5 ± 0.02[a]
Linoleic acid (18:2)	19.7 ± 0.0[a]	20.3 ± 0.2[a]	16.6 ± 1.2[a]	17.5 ± 0.2[a]
α-Linolenic (18:3)	65.6 ± 0.8[a]	64.5 ± 0.2[a]	69.3 ± 1.0[a]	66.7 ± 0.4[a]
SFA	9.3 ± 0.3[a]	9.8 ± 0.4[a]	8.3 ± 0.1[a]	10.3 ± 1.1[a]
PUFA	85.4 ± 0.8[a]	84.9 ± 0.0[a]	85.9 ± 0.2[a]	84.1 ± 0.6[a]
PUFA/SFA	8.7 ± 0.2[a]	9.2 ± 0.4[a]	10.4 ± 0.1[a]	8.2 ± 0.9[a]
n-3/*n*-6 FA ratio	3.32 ± 0.03[a]	3.18 ± 0.03[a]	4.18 ± 0.4[a]	3.81 ± 0.0[a]

Mean values ± standard deviation ($n = 3$) followed by different letters differ at $p \leq 0.05$, according to Tukey (HSD) test.
SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; *n*6/*n*3 FA ratio (linoleic/α-linolenic acid).
Set 1: Argentinean seeds.
Set 2: Guatemalan seeds.

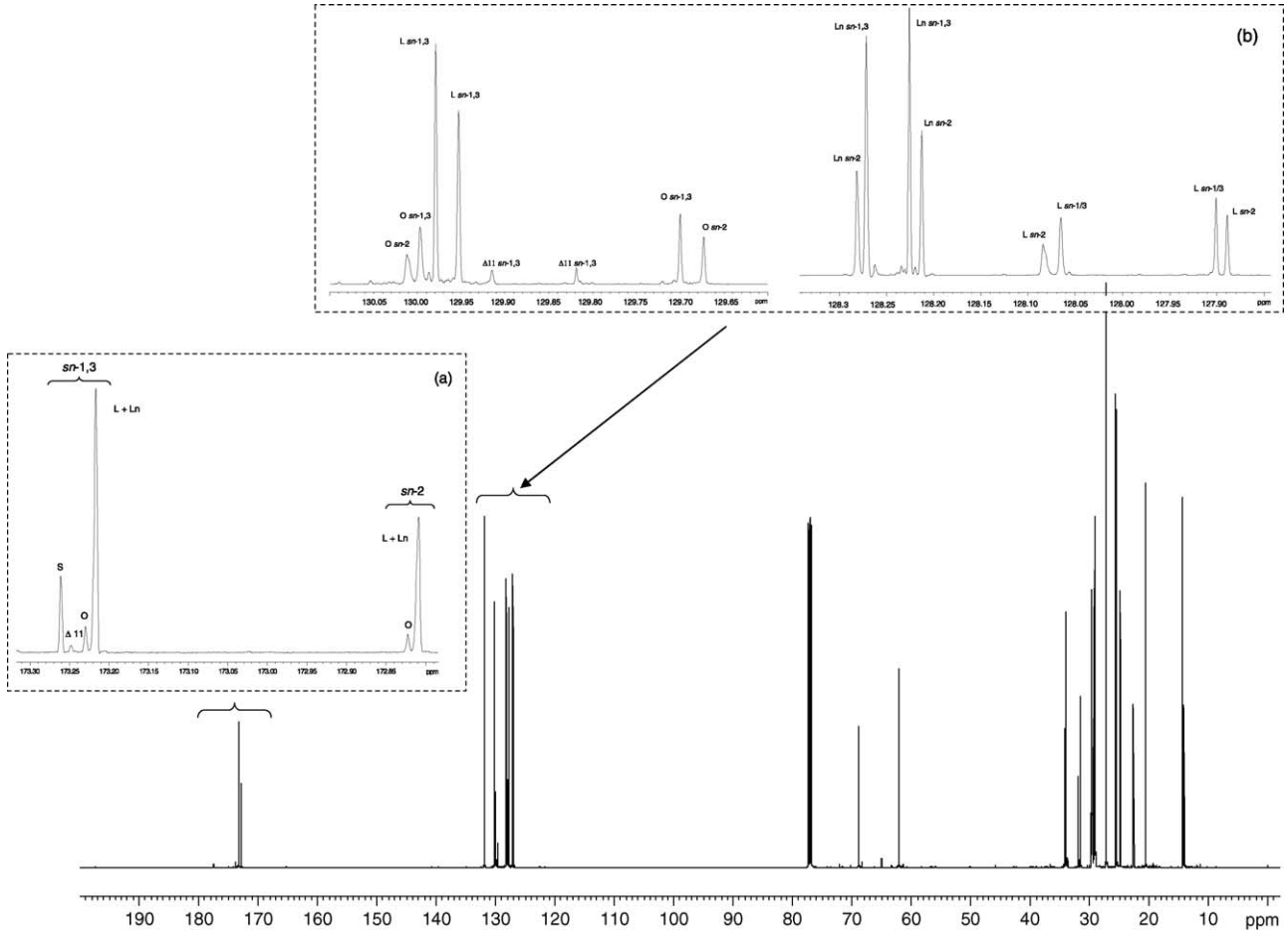

Fig. 1. ^{13}C NMR spectrum of *Salvia hispanica* oil. Insets (a) and (b) are expanded views of the carbonyl and olefinic region, respectively. Reported letters refer to different types of fatty acid chains according to: S = saturated; O = oleic; Δ11 = vaccenic; L = linoleic; Ln = α-linolenic.

Table 2
Acyl positional (*sn*1,3 vs. *sn*2) distribution of saturated and unsaturated fatty acids in *Salvia hispanica* L. seed oils extracted by solvent and pressing, as determined by ^{13}C NMR.

	Set 1		Set 2	
	Solvent extraction	Pressing	Solvent extraction	Pressing
*sn*1,3 fatty chains, %				
Saturated	12.3	12.5	9.8	9.8
Vaccenic acid	1.1	1.1	0.8	1.0
Oleic	6.1	6.1	4.4	4.4
Linoleic	12.3	12.1	8.9	9.0
α-linolenic	33.5	33.8	41.7	41.4
*sn*2 fatty chain, %				
Oleic	4.4	4.5	2.8	2.9
Linoleic	9.9	9.9	7.4	7.7
α-linolenic	18.7	19.0	23.8	23.4
Total (*sn*1,3+ *sn*2)				
Saturated (1,3:2)	12.3 (100:0)	12.5 (100:0)	9.8 (100:0)	9.8 (100:0)
Vaccenic acid (1,3:2)	1.1 (100:0)	1.1 (100:0)	0.8 (100:0)	1.0 (100:0)
Oleic (1,3:2)	10.5 (58:42)	10.6 (58:42)	7.2 (61:39)	7.3 (60:40)
Linoleic (1,3:2)	22.2 (55:45)	22.0 (55:45)	16.3 (55:45)	16.7 (54:46)
α-linolenic (1,3:2)	52.2 (64:36)	52.8 (64:36)	65.5 (64:36)	64.8 (64:36)

Set 1: Argentinean seed oils.
Set 2: Guatemalan seed oils.

obtained by solvent extraction, with low (set 1) and high (set 2) level of α-linolenic acid due to there were not significant differences in the FAs composition of oils obtained by different processes, as shown previously. Identified triacylglycerols (TGs) were composed of the five FAs reported by the GC analysis. Twelve different TGs were found in both oils, representing αLnαLnαLn, αLnαLnL, αLnLL, αLnαLnP, αLnLO, and αLnLP about 87–95% of the total TGs. It is interesting to note that the α-linolenic acid is present in all of the most TGs found and the main differences in the oils with low and high content of this fatty acid are in the αLnαLnαLn triacylglycerol content, with a higher level in set 2 than in 1 (Table 3).

Table 3
Retention times (t_R) and relative peak area (%) of triacylglycerols identified in set 1 and set 2 of *Salvia hispanica* L. oils obtained by solvent extraction, selected according to their low or high α-linolenic acid content, respectively.

Triacylglycerol	Set 1		Set 2	
	t_R (min)	%	t_R (min)	%
αLn αLn αLn	5.55	32.8	5.56	47.0
αLn αLn L	6.23	20.3	6.21	20.8
αLnLL	7.11	13.8	7.08	11.7
αLnαLn P	7.35	7.7	7.35	6.7
αLnLO	8.05	7.0	8.07	3.9
αLnLP	8.35	5.3	8.40	4.7
αLnOO + αLnOP	9.46	8.3	9.40	4.1
αLnPP	9.93	0.8	9.94	0.2
LLS	10.71	1.1	10.75	0.2
αLnOS	11.10	2.1	11.04	0.6
αLnSP	11.39	1.0	11.37	0.1

αLn = alpha linolenin; L = linolein; O = olein; P = palmitin; S = stearin.
Set 1: Argentinean seed oil.
Set 2: Guatemalan seed oil.

Table 4 shows the physicochemical and quality characteristics of the two independent sets of chia seed oils obtained by solvent and pressing.

The average FFA values ranged between 0.70 and 2.05 mg KOH/g oil. These values were lower than those accepted for other vegetable oils obtained by pressing, for example, olive oil (Codex Alimentarius Commission, 1999). Chia oils presented significantly higher ($p \leq 0.05$) FFA values in solvent than in pressure system. These results are similar to those reported by other authors, who found that sunflower oils obtained by hexane extraction presented a higher acidity than oils obtained by pressing (Brevedan et al., 2000).

Iodine values (208.5–215.0) were somewhat higher than those published elsewhere, whereas saponified values (193.0–193.1) were similar to those previously reported (AOCS, 1998; Velasco Vargas et al., 2004). Unsaponifiable matter content was 0.68–1.27%, including this range the data published (1.2%) by AOCS (1998). No significant differences ($p > 0.05$) were found between both extraction systems for these physicochemical indices.

Refractive index ranged from 1.4763 to 1.4798, recording significant differences ($p \leq 0.05$) between the oils obtained by both extraction systems (Table 4).

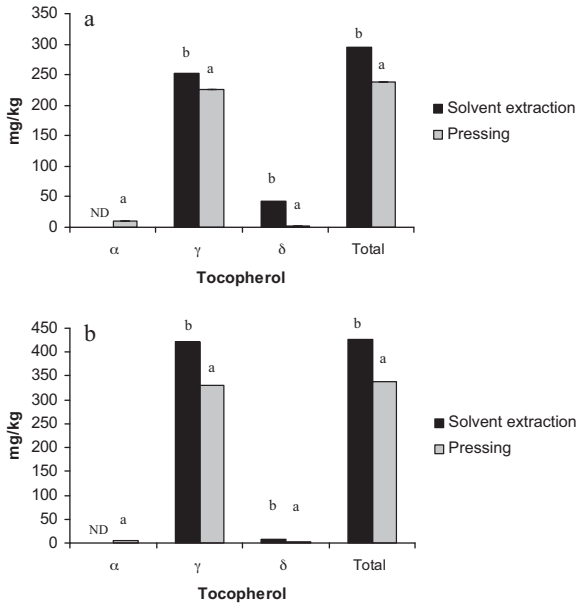

Fig. 2. Tocopherol profile of two independent sets of chia (*Salvia hispanica* L.) seed oils obtained by solvent and pressing extraction: (i) set 1; (ii) set 2. Bars with different letters are significantly different between extraction systems at $p \leq 0.05$, according to Tukey (HSD) test. ND: not detected.

Regarding CIELAB color, significant differences ($p \leq 0.05$) were found between the L^* and a^* values of the oils. There are no color standards for chia seed oils and the L^* a^* b^* measurements could be used for color classification. The lowest L^* values, reflecting brightness, were found in oils obtained by pressing which appeared consequently darker than the solvent extracted oils. In all cases, parameter a^* assumed a negative but low value, which is in the same range than that reported by Meléndez-Martínez et al. (2006) for β-carotene. A significant negative correlation ($r = -0.92$; $p = 0.0014$) was found between L^* and a^* parameter. All chia seed oil samples showed positive values of parameter b^*, characteristic of yellow colors, with no significant differences ($p > 0.05$) between both extraction systems.

The color of vegetable oils is associated with the total pigment content; presence of carotenoids but not chlorophyll pigments

Table 4
Physicochemical characteristics of two independent sets of *Salvia hispanica* L. seed oils obtained by solvent and pressing.

Physicochemical characteristic	Set 1		Set 2	
	Solvent extraction	Pressing	Solvent extraction	Pressing
Acid value (mg KOH/g oil)	2.05 ± 0.02^b	0.91 ± 0.03^a	1.64 ± 0.06^b	0.70 ± 0.01^a
Iodine value (g I_2/100 g oil)	210.5 ± 1.1^a	208.5 ± 0.6^a	215.0 ± 0.9^a	209.4 ± 1.2^a
Saponified value (mg KOH/g oil)	193.09 ± 0.07^a	193.12 ± 0.04^a	193.01 ± 0.03^a	192.99 ± 0.01^a
Unsaponifiable matter (%)	1.27 ± 0.49^a	0.85 ± 0.20^a	1.00 ± 0.38^a	0.68 ± 0.03^a
Refractive index (25 °C)	1.4768 ± 0.0007^a	1.4794 ± 0.0001^b	1.4763 ± 0.0007^a	1.4798 ± 0.0000^b
Color (CIELAB)				
L^* value	43.177 ± 0.081^b	42.855 ± 0.031^a	43.032 ± 0.003^b	39.720 ± 1.371^a
a^* value	-4.545 ± 0.191^b	-3.757 ± 0.060^a	-4.850 ± 0.056^b	-2.087 ± 0.067^a
b^* value	28.385 ± 1.895^a	25.900 ± 0.440^a	21.467 ± 1.576^a	23.865 ± 1.318^a
β-Carotene (mg/kg)	0.58 ± 0.09^a	1.21 ± 0.32^a	0.53 ± 0.04^a	0.58 ± 0.13^a
Chlorophylls (mg/kg)	nd	nd	nd	nd
Metal content (mg/kg)				
Cu	0.2 ± 0.0^a	0.1 ± 0.0^a	0.3 ± 0.0^a	0.3 ± 0.1^a
Fe	1.8 ± 0.1^b	0.3 ± 0.0^a	3.9 ± 0.2^b	3.4 ± 0.2^a
Phosphorus content (mg/kg)	46 ± 22^a	225 ± 1^b	100 ± 20^a	128 ± 21^b
Total waxes (mg/kg)	142 ± 1.4^b	108 ± 2.8^a	180 ± 2.8^b	92 ± 1.4^a
Induction time (h)	2.4 ± 0.1^a	2.8 ± 0.1^a	2.4 ± 0.1^a	2.4 ± 0.1^a

Mean values ± standard deviation ($n = 3$) followed by different letters differ at $p \leq 0.05$, according to Tukey (HSD) test.
nd: not detected.
Set 1: Argentinean seed oils.
Set 2: Guatemalan seed oils.

was detected in chia seed oils. Total carotenoid content varied from 0.53 mg/kg to 1.21 mg/kg; most of the measured amount corresponds to β-carotene which is predominant in seed oils; α-carotene was not detected. These values are higher than those reported in soybean (0.3 mg/kg) and sunflower (0.1 mg/kg), similar to flaxseed (0.7 mg/kg) and maize (0.9 mg/kg) but lower than rapeseed (1.7 mg/kg) (Tuberoso et al., 2007). No significant differences ($p > 0.05$) were found between the oils extracted.

According to the oxidant–antioxidant balance hypothesis, recognized in biological systems, there is a continuous competition between reactive oxygen species and antioxidants that are classified as protective or repair systems and depend on the oxidative susceptibility of tissue lipids and other biological substrates (Frankel, 2005).

The oxidative stability of foods is dependent on the composition, concentrations and activity of reaction substrates, prooxidants and antioxidants. In order to minimize the use of food additives, the oxidative stability can be potentially improved by preserving or enhancing the endogenous oxidation control systems of foods (Decker, 1998).

Trace of metals, particularly copper and iron ions, are known to be effective prooxidants in lipid oxidation, so they are undesirable in oils. Both metal contents in chia seed oils (Table 4) were lower than the maximum level accepted for virgin vegetable oils (Codex Alimentarius Commission, 1999), and than those reported in literature for crude and degummed sunflower oils (Brevedan et al., 2000). Regarding the extraction system, solvent extracted chia oils showed similar concentrations of copper and higher concentrations of iron than pressed extracted oils. This fact is similar to that reported for sunflower oils (Brevedan et al., 2000).

Total phosphorus content ranged from 46 to 225 ppm. These values resulted lower than those reported in the literature for sunflower (441–932 ppm) (Brevedan et al., 2000) and soybean crude oils (600–800 ppm) (List et al., 1978). Extractability of these compounds is known to be dependent on the extraction method (Kamal-Eldin and Appelqvist, 1995). Total phosphorus

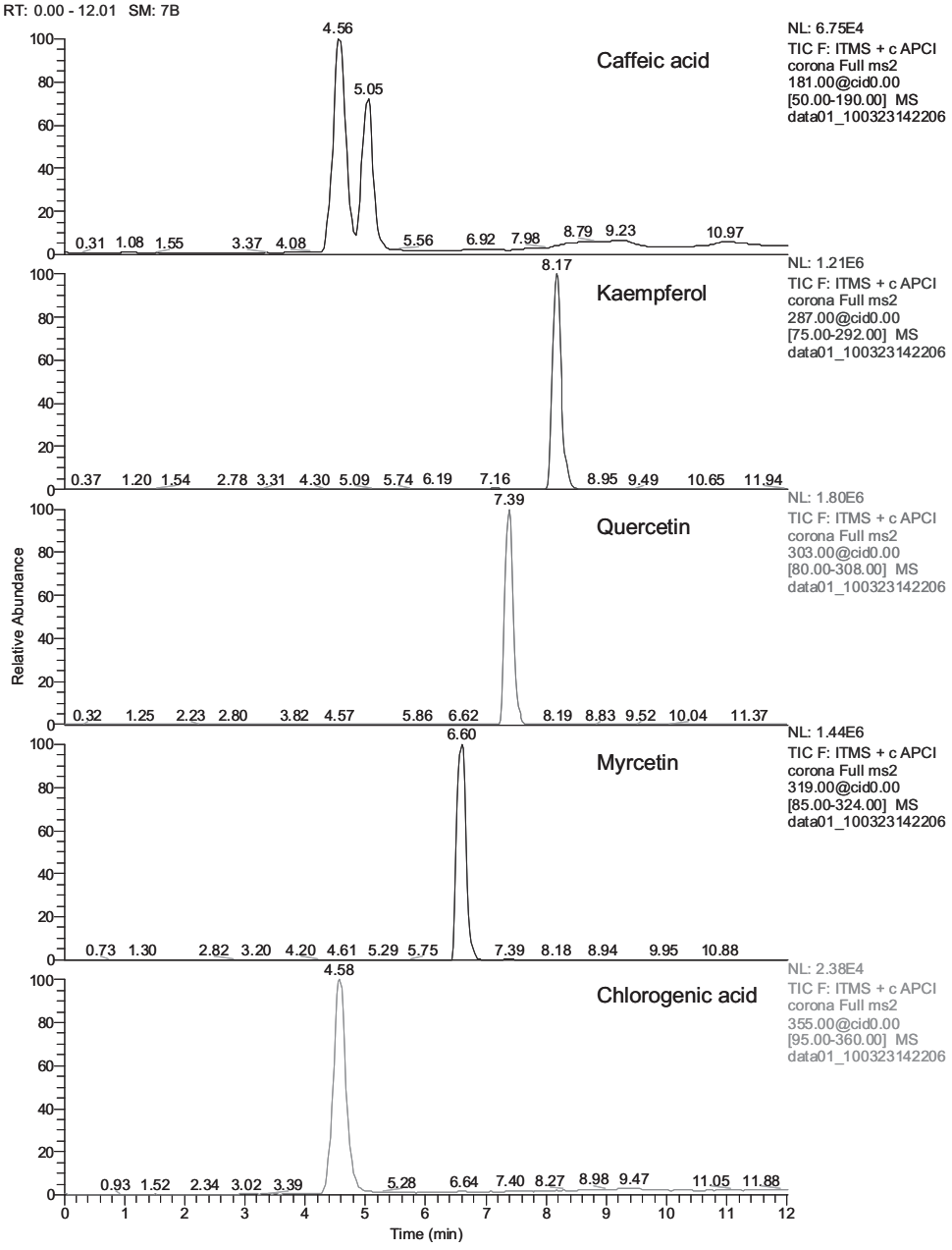

Fig. 3. Typical chromatogram of a standard solution of 100 μM of each polyphenolic compound.

content was significantly higher ($p \leq 0.05$) in oils obtained by pressure system than those extracted by solvent, which could be attributed to the low solubility of phospholipid in hexane.

Wax esters content presented in chia seed oils varied between 92 and 180 mg/kg, which is lower than that reported for cultivated (400–1100 mg/kg) and wild (678–1128 mg/kg) sunflower oils (Pérez et al., 2004). The main wax components were esters between 34 and 48 carbon atoms, with a high concentration in the C48 fraction (set 1: 18–46%; set 2: 36–41%), followed by C38 (set 1: 17–24%; set 2: 9–15%) and C36 fractions (set 1: 13–18%; set 2: 6–14%). Extracted oils showed higher concentrations of waxes than those obtained by pressing due to its high solubility in hot hexane. This different between extraction methodologies were also found by Carelli et al. (2002) in hybrid sunflower oils.

Figs. 2 and 4 show the profiles of tocopherol and polyphenolic compounds of the two independent sets of chia seed oils obtained by solvent and pressing extraction. Chia seed oils contained about 238–427 mg/kg of tocopherols, mainly γ-tocopherol (>85%) and δ-tocopherol; α-tocopherol was found in variable concentrations (0.4–9.9 mg/kg) while β-tocopherol was not detected. Total amount of tocopherols in chia oils was similar to data reported for peanut oil (398.6 mg/kg), but lower than those recorded in flaxseed (588.5 mg/kg), sunflower (634.4 mg/kg) and soybean (1797.6 mg/kg) oils (Tuberoso et al., 2007). Tocopherol contents were significantly higher ($p \leq 0.05$) in oils obtained by solvent extraction than by pressing. Usually high amounts of tocopherols are associated with high PUFA content (Tuberoso et al., 2007); the results obtained showed a significant positive correlation between α-linolenic acid with each of both total tocopherols ($r = 0.95$; $p = 0.0034$) and γ-tocopherol ($r = 0.94$; $p = 0.0055$).

Fig. 3 shows a typical chromatogram for a standard solution of 100 μM of each polyphenol. The retention times for all the sterols are between 4 and 9 min. The top signal corresponds to m/z 181,

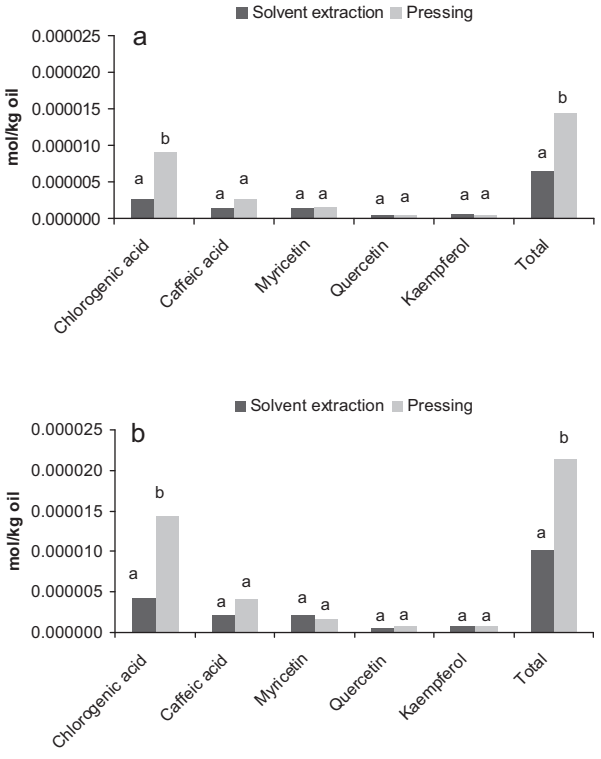

Fig. 4. Polyphenolic compounds profile of two independent sets of chia (*Salvia hispanica* L.) seed oils obtained by solvent and pressing extraction: (i) set 1; (ii) set 2. Bars with different letters are significantly different between extraction systems at $p \leq 0.05$, according to Tukey (HSD) test.

this precursor ion is observed in both, caffeic acid (RT: 5.02 min) and chlorogenic acid (4.58 min). Fortunately it can be observed that the potential interference is minimized chromatographically. The total polyphenolic content in chia seed oils ranged from 6×10^{-6} to 2.1×10^{-5} mol/kg. The major phenolic compounds were chlorogenic and caffeic acids, followed by myricetin, quercetin and kaempferol (Fig. 4). These compounds are the same substances detected in chia whole seeds (Reyes-Caudillo et al., 2008; Taga et al., 1984). Chlorogenic acid and total polyphenolic compounds were significantly ($p \leq 0.05$) higher in pressure than in solvent system. It is interesting to note that most of the polyphenolic compounds found in chia seed oil are not present in other oilseeds (Tuberoso et al., 2007).

Regarding the oxidative stability test using Rancimat, no statistically differences ($p > 0.05$) were found among both extraction methods (Table 4). The correlation analyses revealed only a significant correlation ($r = 0.76$; $p = 0.0268$) between total phosphorus content and induction time. Some authors reported that phospholipids may increase the oxidative stability of vegetable oils owing to their antioxidant properties and synergism with tocopherols (Gordon and Rahman, 1991). However, this accelerated stability test showed that chia oils have a low oxidative stability. In spite of the presence of antioxidant compounds, the high content of PUFAs makes chia seed oil very instable. In this way, innovative technologies to protect *n*-3 polyunsaturated fatty acids using antioxidants, adequate preparation, refining and/or packaging of the oil, are needed.

4. Conclusion

Noting the increasing popularity of chia oil as a very important source of *n*-3 and *n*-6 fatty acids, this study presents a view of the characteristics of chia seed oil obtained by pressing and solvent extraction and could serve as a starting point to define quality standards since there are little specifications available for this non-traditional vegetable oil. The obtained data suggest the potential value-added use of these seed oils as dietary sources of essential fatty acids, for optimal human health. Thus, this oil may be used to make suitable oil blends with others which contain low levels of these compounds.

The experimental results show that the oil yield was much lower in pressing than in solvent extraction. The composition of some minor constituents and quality of chia seed oils were influenced by the extraction process. Nevertheless, the fatty acid composition was similar in oils obtained by both extraction systems. Twelve triacylglycerol molecular species were identified, most of them with at least one α-linolenic acid esterifiying the glycerol; trilinolenin (αLnαLnαLn) was the major compound. The high level of PUFAs would be the main cause for the low oxidative stability prevailing over the antioxidant effects associated with bioactive components (tocopherols, polyphenols, carotenoids, phospholipids) present in chia seed oils. In further studies their oxidative stability may be improved by the addition of natural antioxidants, such as phenolic compounds.

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