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Supercritical carbon dioxide extraction and conventional extraction of chia seed oils: chemical composition and lipid oxidation

Supercritical carbon dioxide extraction of chia oils

[1]Renata Adriana Labanca ; [2]Cecilia Svelander ; [3]Lovisa Eliasson ; [1]Raquel Linhares Bello de Araújo ; [2-3]Lilia Ahrné ; [2]Marie Alminger

(1) Department of Food Science, Federal University of Minas Gerais, Brazil, 31270-010 Belo Horizonte, MG, Brazil,

(2) Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, Gothenburg, Sweden

(3) SP Technical Research Institute of Sweden, Gothenburg, Sweden

email addresses: renata@bromatologiaufmg.com.br

Abstract

Chia seed (Salvia Hispanica L.) oil was obtained by supercritical CO_2 (SC-CO_2) extraction and conventional solvent extraction (CSE). The seeds were from Brazil (seeds and stabilized flour), Sweden and the Netherlands. The processing parameters related to chia oil extraction using different seeds countries have not been studied yet. The chia oils were characterized with regard to fatty acid (FA) composition, antioxidant properties (ABTS, DPPH and FRAP), Peroxide Index (PI), TBARS (thiobarbituric acid reactive substances) and tocopherol. The yield of the extracted oil was 25.7-32.2% with CSE and 27.8-31.8% by SC-CO_2. The oils mainly contained unsaturated fatty acids (84.6-88.1%), the total concentration was similar between the different extraction methods. The ratio of omega-6/omega-3 fatty acid was 0.40, being markedly better than that reported for other vegetable oils. The SC-CO_2 extracted oil showed greater antioxidant capacity compared with CSE extracted oil, whereas no significant differences were observed for levels of TBARS and PI. The total tocopherol content ranged from 141 to 601 mg/Kg and consisted mainly of gamma-tocopherol (88%). This study reveals that chia seed oil could be an interesting functional food ingredient. We also propose that SC-CO_2 extraction is a better option than CSE for extraction of chia seed oil as it does not require use of organic solvents and is more environmentally friendly.

Introduction

Epidemiological studies have shown that a balanced diet rich in fruit, vegetables and grains may play a crucial role in preventing chronic diseases, such as heart disease, cancer, diabetes and Alzheimer's disease. According to some studies, the consumption of foods rich in vitamin E (tocopherols and tocotrienols) and omega-3 fatty acids may have a positive association with an increased gene expression of hepatic antioxidant enzymes, which results in an improvement of the antioxidant capacity as a whole. In a prospective analysis of more than 45,000 men in the Health Professionals Follow-up Study, for example, the consumption of Omega-3 fatty acids, both of marine and vegetal origin, was linked to a reduced cardiovascular risk, with little influence by the omega-6 intake (1).

It can be stated that while the consumption of

food rich in vitamin E and omega-3 has been touted as a protective factor for diseases, the consumption of supplements alone still faces problems regarding dosing and effectiveness. This is the reason why there has been a growing interest in eating food rich in natural antioxidants. The main explanation for the difference between these sources is the synergistic effect between antioxidants and other bioactive compounds present in food. There may also be other factors which have not yet been elucidated that give power to the antioxidant action of foods (2, 3, 4).

Whole grains are rich sources of bioactive compounds and in recent years there has been an increase of research to identify new grains to add to our diet. Chia seeds are considered due to their high content of protein, lipids, antioxidants and dietary fiber. Chia seeds have a high content of unsaturated fatty acids, of which almost 60% is omega-3 fatty acids. Furthermore, they contain natural antioxidants, such as phenolic compounds, like chlorogenic acid, caffeic acid, quercetin and kaempferol and carotenoids, e.g. lycopene, beta-carotene, alfa-carotene, lutein, that may protect the polyunsaturated fatty acids from suffering fast autoxidation (5).

The consumption of chia seeds leads to an increase in the proportion of n-3 fatty acids in the diet, which in turn can lead to health benefits for the general population. A recent study has reported that patients that consumed chia seeds showed a significant reduction in weight, body mass index and waist circumference compared with patients who did not consume chia seeds. Although chia is still not a well-known food, its global production has increased in recent years due to its health properties and increasing popularity. However, detailed studies to further characterize the oil and investigate effects of different extraction techniques and conditions on antioxidant activity are still limited. Compared with conventional solvent extraction of lipids, supercritical fluid extraction (SC) is an attractive method for lipid extraction, 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 (2). Carbon dioxide (CO_2) is the solvent most commonly used for supercritical fluid extraction (SC), due to beneficial properties such as low temperature (31°C) and critical pressure (7.29 MPa).

In this context, the objective was to compare the chemical and nutritional characterization of chia oil produced by supercritical CO_2 and conventional solvent extraction methods respectively, and to assess the potential for chia oil as a functional food. The extractions were performed on commercially available chia seeds bought in Brazil, Sweden and the Netherlands. The resulting oils were analyzed with regard to fatty acid composition by GC-MS (gas chromatography-mass spectrometry), tocopherol by HPLC (high performance liquid chromatography), antioxidant properties (DPPH, ABTS and FRAP), total phenolic content (Folin-Ciocalteu assay), Peroxide Index and TBARS.

Materials and methods

Chemicals

Methanol, chloroform, hexane, toluene, acetyl chloride, petroleum ether, and Standards (alpha, delta and gamma) tocopherol were purchased from Sigma (United Kingdom). 2,2,4-trimetylpenthane were supplied by Fisher Chemical (Loughborough, UK).

The reagents used for antioxidant properties, PI and TBARS were 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethyl chroman-2- carboxylic acid (Trolox), cellulose (spruce powder), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), cumene hydroperoxide (CPO), methyl tricosanoate

(23:0), BHT (butylated hydroxytoluene), ammolnium thiocyanate, barium chloride, ferrous sulphate, trichloroacetic acid, thiobarbituric acid, 1,1,3,3-tetraethoxypropane (TEP), potassium persulfate and ferrous sulphate from Sigma Chemical. Sodium carbonate was also used.

Samples

The experiments were carried out using chia samples obtained at local supermarkets in Brazil (seeds and stabilized flour), Sweden and the Netherlands. After the purchase, seeds and flour were kept at -20 ± 2 ° C until the time of analysis.

Batch grinding was carried out on 50 g of chia seeds for 30 s using a coffee mill. After grinding, the particle size of each sample was determined using sieves of standard screen mesh (1.60; 1.25; 0.73 and 0.50 mm) stacked on each other with the smallest mesh screen at the bottom and the largest at the top. The sample was placed on the top screen and the stack was shaken mechanically for 1 min using a motorized Fritsch vertical vibratory sieve shaker. The screen which retained particles were removed, weighed, and the mass of the individual screen increments were converted to mass fractions of the total sample, with particle size <0.73 mm.

Analysis

Moisture determination

Moisture content of the chia seeds was determined in triplicate using a moisture balance encompassing the mass balance and dryer. A temperature of 80°C was used and initial sample weight was 0.5 ± 0.05 g.

Extraction of Lipids

Samples were subjected to a hexane extraction. Five grams of milled sample was mixed with 50 mL hexane for 2 h on orbital rotary plate (150 rpm) in room temperature (20°C). The extraction procedure was repeated twice and the combined fractions dried under N_2. The extracted oil was weighted for yield determination, and the tubes with extracts were stored dark and cool (-20°C) until analysis.

The SC-CO_2 oil extraction was carried out in a system from Waters (SFE-500M1-2-C50, Waters, Milford, Massachusetts, USA). The system consisted of a stainless steel extraction vessel of 500 ml with heating jacket set at 60°C, a CO_2 pump set at 40 g/min, a back pressure regulator to control the pressure in the system to 350 bar and a 500 ml cyclonic separator set at 25°C and 10 bar. The extraction basket was loaded with approximately 50 g of milled chia sample and glass wool was used to fill up the remaining space of the basket. The extraction condition was selected based on previous work by Ixtania et al. (6), but with an increased extraction time from 60 to 80 min. The CO_2 was of purity 99.9% (AGA, Sweden) and the oil was collected in Falcon tubes from the cyclone after 80 min extraction. The extracted oil was weighted for yield determination, and the tubes with extracts were stored dark and frozen (-20°C) until analysis.

GC-MS analysis of Fatty acids

Fatty acids were analyzed by adding 5 mL toulen to 25 mg oil together with 100 µl internal standard (fatty acid 17:0, 5 mg/mL) and vortexing of the tubes for 30 s, according to the method described by Cavonius et al., (7). After adding 1.0 mL 10 % (w/v) acetyl chloride solution, the tubes were vortexed 15 s and left to stand at 70°C (heat block) for 2 h. After addition of 1 mL mQ water and 2 mL petroleum ether, the tubes were vortexed 15 s and centrifuged (5,000×g for 5 min). The organic phase was transferred to a new tube, evaporated under N_2 at 40°C, and the methylated fatty acids resuspended in 1 mL of 2,2,4-trimethylpentane. The extract was injected into an Agilent 7890 A GC system equipped with a J&W DB-wax column (30

m×0.250 mm×0.25 µm) and interfaced with a Agilent 5975 C triple-axis mass spectrometric (MS) detector in electron impact mode. Injection volume was 1 µL with a 15:1 split at an inlet temperature of 275°C. The carrier gas was helium, with a fixed flow of 1 mL/min throughout the temperature program, which was as follows—100°C for 0 min, ramp at 4°C/min to 205 °C, thereafter ramp at 1 °C/min to 230°C, hold 5 min. Fatty acids were quantified against the internal standard, summed, and expressed as the relative percentage of each individual FA present in the sample.

Tocopherol analysis by HPLC

The analyses were performed using a method previously described by Larsson et al. (8). An aliquot of 50 µL chia seed oil was diluted to 1 mL with 95% methanol and analyzed by HPLC (HPLC, PU-2089 Plus HPLC pump, As-2057 Plus, injection, Jasco, Tokyo, Japan) on a Kromasil C18 column (150 mm × 2.1 mm, 5 µm) (Eka Chemicals, Bohus, Sweden). The mobile phase consisted of 95% methanol in water, and the flow rate was 0.4 mL/min. Peaks were detected with a Jasco FP-920 spectrofluorometric detector (Tokyo, Japan) using an excitation wavelength of 295 nm and emission wavelength of 330 nm. Data acquisition and processing were carried out using the Chrompass Jasco software (Tokyo, Japan).
The samples were quantified against standard curves that ranged from 30 to 120 mg/L (r2 > 0.98). The theoretical limits of quantification and detection of the method for alpha, gamma and delta was 16.0; 13.2; 8.6 and 6.2; 3.0; 2.1 mg/L, respectively. Values less than these were considered not detectable (nd).

Antioxidant activity and Lipid autoxidation products

Extraction was carried out according to Rufino et al. (9). Briefly, the procedure was as follows: freshly produced oil samples were weighed (5.00g) in centrifuge tubes and subsequently extracted. After addition of 40 ml of methanol/water (50:50, v/v) the samples were incubated (with agitation at 150 rpm) at room temperature for 1 h. The tubes were centrifuged at 25,400xg for 15 min and the supernatant was recovered. An acetone/water mixture (70:30, v/v) was added (40 ml) to the residue, and extraction was performed at room temperature for 60 min followed by centrifugation. Methanol and acetone extracts were combined, made up to 100 ml with distilled water and used to determine antioxidant capacity. A spectrophotometric method was employed to determine the antioxidant activity using a spectrophotometer Tecan Saphire-2 microplate reader (Salzburg, Austria).

The ABTS assay was based on the method by Rufino et al. (9) with modifications. ABTS radical cations were produced by reacting 7 mM ABTS stock solution with 145 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Ethanolic solutions of known Trolox concentrations were used for calibration and the results were expressed Mµ Trolox equivalents (TE)/g oil.

The antioxidant capacity was determined by the modified DPPH method AOAC Official Method 2012.04 (10) which is based on the quantification of free radical-scavenging. A methanol solution containing 0.06 mM DPPH was prepared. After adjusting the blank with methanol, an aliquot of 100 µl oil extract was added to 3.9 ml of the DPPH solution. The decrease in absorbance at 515 nm was measured at 1 min intervals for the first 10 min, and then at 5 min intervals until stabilization. Using the Trolox calibration curve the results were expressed Mµ Trolox equivalents (TE)/g oil.

The antioxidant capacity of each sample was estimated by FRAP assay, following the procedure described in the literature (9) with modifications. Briefly, 2.7 ml of freshly prepared FRAP reagent (TPTZ, $FeCl_3$ and acetate buffer) at 37°C was mixed with 90 µl of oil extract and 270 µl of distilled water. Using a blank containing FRAP reagent as reference, absorbance was measured at 595 nm after 30 min. Antioxidant activity of a sample is expressed as the percentage of absorption inhibition and in terms of Mmol Fe_2SO_4/g oil.

TBARS were determined in the methanol/water phase obtained after the chloroform/methanol extraction as described by Schmedes & Hølmer et al. (11). 1,1,3,3-Tetraethoxypropane (TEP) was used to prepare a standard curve for quantification. TBARS values for each sample and the results were expressed as milligrams per kilogram of malonaldehyde sample (mgMDA/Kg).

To determine the amount of lipid hydroperoxides in the chloroform extract obtained after the chloroform/methanol extraction, the method was used as described by Larsson et al. (8). Quantification was done using a standard curve made from cumene hydroperoxide. Results were expressed as micromoles of peroxide per kilogram of lipid.

Statistical analysis

Statistical evaluation of the samples was made with one-way ANOVA, followed by Tukey's post-hoc test when initial testing revealed significant ($p < 0.05$) differences between the samples. Tests were performed with the PAST software (statistical version 2.7 for Windows). In addition, the two extraction methods, CSE and SC-CO_2 extraction, were compared with a paired samples t-test by the SPSS statistics software (Version 19, IBM, USA).

Results and discussions

Moisture content and oil yield

The moisture content was 5.13-6.02 g/100 g for the different chia seeds and 9.10 ±0.04 g/100 g for stabilized flour (Table 1), which is in agreement with previously reported values of 10.41, 6.75 and 6.52% respectively (12, 13, 14). Moisture content may vary due to growing, harvesting and storing conditions, as well as geographical locations such as altitude, temperature, relative humidity and soil characteristics, which can explain the differences observed in Table 1.

The average oil yield from the chia seeds was 31.3 ±0.8 g/100 g for the SC-CO_2 extraction and 31.1 ±1.2 g/100 g for the CSE, and the oil yield for stabilized flour was somewhat lower, 27.8 ±0.1 g/100 g with SC-CO_2 and 25.7 ±0.1 g/100 g with CSE (Table 1). The assayed extraction methods did not significantly influence the oil yield for seeds, but for flour about 8% more oil was obtained with SC-CO_2 extraction compared with CSE. SC-CO_2 extraction conditions were based on the study by Ixtaina et al. (6), where a yield of 12.4% was obtained for the same pressure and temperature, but with an extraction time of 60 min. The greatly increased yield in our study justifies the increased extraction time to 80 min.

Fatty acids

Fatty acids were quantified against the internal standard, summed, and the results presented as % of total fatty acids (Table 2). The main constituent in the oil was polyunsaturated 3-linolenic fatty (18:3) acid (omega-3), ranging from 52.50% to 55.85% of the total content. When linolenic fatty acid content was measured as g/kg of seed and compared among extraction, no significant ($P < 0.05$) differences were detected. The content of palmitic acid (16:0) differed significantly ($P < 0.05$) depending on extraction method and the origin of chia seeds. The lowest yield, 6.37%, was obtained by SC-CO_2 extraction of seeds obtained in Sweden and the highest yield, 7.89%, by SC-CO_2 extraction of chia

bought in The Netherlands. For the other main quantified fatty acids no significant ($P < 0.05$) differences were observed. These results are in line with those of Ixtaina et al. (3), who tested different conditions of SC-CO_2 extraction and concluded that the fatty acid composition of chia seed oils was not affected by the extraction process.

The omega-6/omega-3 dietary ratio has been reported to have a great influence on health and proposed omega-6/omega-3 ratios in several countries shows a coverage range from 4 to 5:1. For a healthy diet, the recommentations are today to reduce the omega-6/omega-3 ratio. Thus, Chia oil, which is a rich source of alpha-linolenic acid can therefore contribute to a lowering of the ratio. The present study showed that regardless of the extraction method used, the omega-6/omega-3 ratio was between 0.35 and 0.40, which is significantly lower compared with other vegetable oils. Similarly, the PUFA:SAT ratio (Polyunsaturated fatty acid/Saturated fatty acid) showed mean values of 6.50, which is higher compared to other vegetable oils (4).

Tocopherols

The composition of tocopherols in chia oil is shown in Table 3. The total tocopherol content ranged from 141 to 601 mg/Kg and consisted mainly of gamma-tocopherol, which ranged between 85 and 91% of the total tocopherol content (Table 3). Similar results were obtained by Ixtaina et al. (4) using a mechanical and solvent lipid extraction method for extraction of tocopherol, with a total amount from 240 to 460 mg/Kg Chia Oil. Zanqui et al. (2), observed slightly higher gamma and delta tocopherol levels of 664 and 257 mg/Kg, respectively in chia oil extracted by SC-CO_2, which may be due to the differences in origin of the seeds. Likewise, there was a significant difference in the tocopherol content for the four samples used in the current study. Higher tocopherol levels were only found after supercritical extraction for the chia seeds sample bought in Brazil. For Chia oil from the other three samples, the highest gamma tocopherol values were obtained by CSE extraction.

Antioxidant activity and Lipid autoxidation products

The results from the different antioxidant activity assays with chia samples showed values between 251 to 320 with DPPH, 124 to 169 with ABTS and between 139 to 166 with the FRAP assay (µmole Trolox equivalents (TE)/g sample, Table 4). It is important to note that the antioxidant activity of a bioactive compound from a natural source is influenced by several factors, such as the country or region in which the plant has grown and the form in which the sample is prepared for analysis; whether in powder, extract or as an isolated fraction. A factor that limits the comparison of studies is the extraction procedure used since it is a critical process for some matrices, particularly when there may be insoluble components with antioxidant capacity, which lead to underestimated Trolox equivalent antioxidant capacity values in some cases.

In vitro antioxidant activity of chia have been reported previously, but mainly for chia seeds and not chia oil. Reyes-Caudillo et al. (5) evaluated the antioxidant activity by ABTS of chia seed extracts from two Mexico states, Jalisco and Sinaloa, and concluded that the Jalisco extract had greater radical scavenging activity than the Sinaloa extract, but both comparable to Trolox, which is used as reference. Vásquez-Ovando et al. (15) evaluated the antioxidant activity of a fiber rich chia seed fraction by the ABTS method and found an activity corresponding to 488.8 Mµ Trolox equivalent (TE) / g, which is around three times higher compared with that found for the Chia oils in the current study. Similarly, the antioxidant activity of the chia seed oils in the current study was lower than those reported in a recent study by Marineli et al. (16), where the antioxidant activity of chia seeds was

436.61 ± 9.67 µmol Trolox/g with the DPPH method, 405.71 ± 6.55 µmol Trolox/g samples for the FRAP method and 517.30 ± 09.09 µmol Trolox/g samples by the ORAC method. Sargi et al. (17) also reported a higher antioxidative activity for chia seeds with the ABTS, DPPH and FRAP methods; 2:56 ± 0:03, 1.72 ± 0:09, and 2.86 ± 0:10 in Trolox equivalent antioxidant capacity (TEAC) / g chia seeds, respectively.

The high antioxidant activity in chia has been linked to the presence of phenolic compounds such as caffeic and chlorogenic acid, as well as quercetin, one of the most powerful and stable pure compounds in which antioxidant activity has been evaluated (4, 14). Phenolic compounds are generally hydrophilic and this may explain the lower antioxidative capacity for the chia seed oils compared with chia seeds. Indeed, the total polyphenolic content in chia seed oils ranged from 6 x 10^{-6} to 2.1 x 10^{-5} mol/kg, Ixtaina (4) and for chia seeds between 0.88 and 0.92 mg GAE/g (6). However, the antioxidant potential of a product is not related only to their total phenolic content, but also the composition of phenolic compounds and lipophilic antioxidants such as vitamin E. It should be noted that the antioxidant activity of chia oil was similar to other oils such as olive oil (152 Mµ Trolox/g oil - FRAP) and high if compared with sunflower oil (65.3 Mµ Trolox/g oil – FRAP and 11.6 Mµ Trolox/g oil - ABTS), suggesting that chia oil may have more beneficial health effects than some other vegetable oils.

The peroxide index value of chia oil (2.06 mEq peroxide/kg) was similar to those reported for chia oil by Ayerza and Coates (18) in South America, by Marinele (19) in Chile and by Ixtaina, Nolasco, and Tomás (19) in Argentina, values 3.25, 2.56 and 1.00, respectively. An important finding was to observe that none of the samples exceeded the upper limit of peroxide index value (10.0 mEq active oxygen/kg oil) established by the Codex Alimentarius (20). Chia oil contains bioactive components that are probably responsible for maintaining low peroxide values and therefore present a good oxidative stability.

Regarding the secondary lipid autoxidation product, MDA, the value of 4.06 mg MDA/kg was similar for all treatments and oils, with lower results than those reported for Chia oil by Marineli et al. (16), 17.46 mg MDA/kg. The TBARS analysis is also related to consumer health as the MDA and other reactive substances has demonstrated carcinogenic properties. Although the TBA test is one of the most commonly used chemical assays to determine secondary oxidation products, this method has limitations, such as lack of specificity and sensitivity. It is therefore important to report that low levels were found independent of the extraction method used.

Conclusions

In summary, under the conditions studied the supercritical extraction at 60°C; 350 bar; 80 min did not differ from convectional extraction (two cycles of 1g product/10 ml hexane/150 rpm: 2 hours) for the fatty acid profile, all samples had a high content of ω-3 fatty acids but did not significantly differ for any of the evaluated parameters, as yield, fatty acid profile, tocopherol content, antioxidative capacity and lipid autoxidation products.

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Tables

Table 1. Moisture content (g/100 g samples) and oil yield (g/100 g samples) of Chia seeds (*Salvia Hispanica L.*) from Brazil (seeds and stabilized flour), Sweden and the Netherlands. The oils were obtained by supercritical CO_2 (SC-CO_2) and conventional solvent extraction (CSE). Values are reported as Mean±SD of three measurement replicates

Samples	Moisture	Yield SC-CO_2	Yield CSE
Brazil seeds	5.13 ± 0.03 ^{a}	31.7 ^{A}	31.3 ± 0.1 ^{bcA}
Brazil flour	9.10 ± 0.04^{d}	27.8 ^{B}	25.7 ± 0.1 ^{aC}
Sweden	6.02 ± 0.05 ^{c}	31.8 ^{D}	32.2 ± 0.1 ^{cD}
Netherlands	5.48 ± 0.06 ^{b}	30.4 ^{E}	29.8 ± 0.3 ^{bE}
Average	*5.69 ± 3.09*	*30.4 ± 1.9*	*29.7 ± 2.9*

Means followed by the same lower case letter in columns and uppercase letter in line do not differ according to Tukey's test at 5% significance.

Table 2. Mean values of fatty acid composition (% of total fatty acids) of chia (*Salvia Hispanica L.*) seed oil from Brazil (seeds and stabilized flour), Sweden and the Netherlands obtained by supercritical CO_2 (SC-CO_2) and conventional solvent extraction (CSE). Values are reported as Mean±SD of three measurement replicates

Samples	16:0	18:0	18:1	18:2-ω6	18:3-ω3	SAT	PUFA	ω6/ ω3	PUFA/ SAT
Brazil seeds SC-CO_2	6.89 ± 0.01^{b}	3.81 ± 0.41^{a}	7.28 ± 0.01^{a}	20.79 ± 0.01^{a}	54.60 ± 0.01^{a}	13.40± 0.04^{b}	79.30± 0.04^{b}	0.38^{b}	5.92 ^{b}
Brazil flour SC-CO_2	6.96 ± 0.03^{b}	3.96 ± 0.03 ^{a}	7.49 ± 0.04^{a}	20.85 ± 0.01^{a}	54.45 ± 0.01^{a}	13.55 ± 0.03^{b}	78.85± 0.01^{b}	0.38^{b}	5.82 ^{b}
Sweden SC-CO_2	6.37 ± 0.20^{a}	4.37 ± 0.50^{a}	7.94 ± 0.70^{a}	19.75 ± 0.01^{a}	55.85 ± 0.14^{a}	13.10 ± 0.01^{b}	79.15± 0.01^{b}	0.35^{a}	6.04 ^{b}
Netherlands SC-CO_2	7.89 ± 0.05^{c}	4.19 ± 0.05 ^{a}	7.74 ± 0.01^{a}	20.85 ± 0.01^{a}	52.50 ± 0.01^{a}	15.10± 0.09^{b}	77.10± 0.01^{a}	0.40^{b}	5.11^{a}
Brazil seeds CSE	6.89 ± 0.01^{b}	3.67 ± 0.01 ^{a}	7.21 ± 0.01^{a}	21.69 ± 0.01^{a}	54.35 ± 0.03^{a}	13.28± 0.04^{b}	79.42± 0.04^{a}	0.40^{b}	5.98 ^{b}
Brazil flour CSE	6.72 ± 0.03^{b}	3.87 ± 0.23 ^{a}	7.45 ± 0.04^{a}	21.23 ± 0.01^{a}	54.19 ± 0.60^{a}	13.30 ± 0.13^{b}	79.20± 0.13^{b}	0.39^{b}	5.95 ^{b}
Sweden CSE	6.71 ± 0.02^{b}	4.33 ± 0.50 ^{a}	7.65 ± 0.70^{a}	20.76 ± 0.40^{a}	54.02 ± 0.03^{a}	13.40 ± 0.01^{b}	78.90± 0.01^{b}	0.38^{b}	5.89 ^{b}
Netherlands CSE	6.71 ± 0.05^{b}	4.21 ± 0.05 ^{a}	7.65 ± 0.01^{a}	20.16 ± 0.01^{a}	54.41 ± 0.03^{a}	11.95± 0.57^{a}	80.35± 0.72^{c}	0.37^{b}	6.72^{c}

Means followed by the same lower case letter in columns do not differ according to Tukey's test at 5% significance.

Table 3. Tocopherol content (mg/Kg oil) of Chia (*Salvia Hispanica L.*) seed oil from Brazil (seeds and stabilized flour), Sweden and the Netherlands obtained by supercritical CO_2 (SC-CO_2) and conventional solvent extraction (CSE). Values are reported as Mean±SD of three measurement replicates

Samples	αT (alpha)	γT (gamma)	δT (delta)	total
Brazil seeds SC-CO_2	222 ± 12^{d}	335 ± 12^{d}	44 ± 4^{b}	601 ± 12^{f}
Brazil flour SC-CO_2	nd	100 ± 9^{a}	41 ± 3^{b}	141 ± 9^{a}
Sweden SC-CO_2	nd	159 ± 7^{b}	24 ± 2^{a}	183 ± 7^{b}
Netherlands SC-CO_2	nd	158 ± 8^{b}	19 ± 2^{a}	177 ± 8^{b}
Brazil seeds CSE	115 ± 11^{c}	319 ± 15^{d}	51 ± 4^{b}	485 ± 15^{d}
Brazil flour CSE	58 ± 4^{a}	160 ± 12^{b}	24 ± 2^{a}	242 ± 12^{c}
Sweden CSE	79 ± 3^{b}	361 ± 16^{d}	63 ± 4^{c}	503 ± 16^{e}
Netherlands CSE	nd	202 ± 12^{c}	109 ± 12^{d}	314 ± 12^{d}

Means followed by the same lower case letter in columns do not differ according to Tukey's test at 5% significance. nd: not detected.

Table 4. Antioxidant Capacity (Mμ Trolox/g samples) and Oxidation Indices of Chia (*Salvia Hispanica L.*) seed oil from Brazil (seeds and stabilized flour), Sweden and the Netherlands obtained by supercritical CO_2 (SC-CO_2) and conventional solvent extraction (CSE). Values are reported as Mean±SD of three measurement replicates.

Samples	ABTS	DPPH	FRAP	PI	TBARS
	Mμ Trolox/g			mEq peroxide/kg	mg MDA/kg
Brazil seeds SC-CO_2	139 ± 27^{a}	279 ± 38^{a}	149 ± 9^{a}	2.08 ± 0.14^{a}	3.69 ± 0.21^{a}
Brazil flour SC-CO_2	126 ± 9^{a}	316 ± 3^{a}	154 ± 5^{a}	2.06 ± 0.05^{a}	4.16 ± 0.22^{a}
Sweden SC-CO_2	153 ± 18^{a}	312 ± 9^{a}	156 ± 15^{a}	2.03 ± 0.09^{a}	4.05 ± 0.10^{a}
Netherlands SC-CO_2	124 ± 15^{a}	251 ± 36^{a}	166 ± 9^{a}	2.14 ± 0.11^{a}	4.03 ± 0.05^{a}
Brazil seeds CSE	127 ± 11^{a}	320 ± 7^{a}	160 ± 6^{a}	1.97 ± 0.06^{a}	3.99 ± 0.14^{a}
Brazil flour CSE	136 ± 19^{a}	306 ± 33^{a}	139 ± 15^{a}	2.06 ± 0.15^{a}	4.38 ± 0.19^{a}
Sweden CSE	169 ± 24^{a}	315 ± 11^{a}	150 ± 14^{a}	1.95 ± 0.07^{a}	4.02 ± 0.26^{a}
Netherlands CSE	165 ± 9^{a}	277 ± 28^{a}	159 ± 5^{a}	2.15 ± 0.11^{a}	3.94 ± 0.32^{a}

Means followed by the same lower case letter in columns do not differ according to Tukey's test at 5% significance.