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Fractionation of Chia Oil for Enrichment of Omega 3 and 6 Fatty Acids and Oxidative Stability of Fractions

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Abstract Omega 3 and 6 fatty acids were enriched in the olein fraction of chia oil via dry fractionation at −30°C. Concentrations of C18:3 (linolenic acid, ω-3) and C18:2 (linoleic acid, ω-6) were 78.16 and 25.42% in the olein fraction. HPLC characterization indicated enrichment of caffeic and chlorogenic acids, quercetin, and phenolic glycosides in the olein fraction. Total antioxidant activities of chia oil and the olein and stearin fractions were 42.5, 53.8, and 34.6%, respectively. After 6 months of storage at 4°C, the concentration of ω-3 in the olein fraction decreased from 78.19 to 76.16%, with a 10% decrease in the ω-3 concentration when the olein fraction was stored at 25°C. The stearin fraction of chia oil exhibited the longest induction period, followed by chia oil and the olein fraction. Amounts of ω-3 and 6 fatty acids can be enriched in the olein fraction from 11.92 and 61.28% to 15.22 and 72.16%, respectively, with reasonable storage stability at low temperature.

Keywords: chia oil, omega fatty acid, olein, stearin, phenolic compound

Introduction

Omega 3 and 6 are essential fatty acids and ω-9 is a non-essential fatty acid (1). Soybean and canola oils containing ω-3 and 6 fatty acids are not good sources of these fatty acids. Fish oil is regarded as a good source of omega fatty acids. Health benefits of omega fatty acids for reductions in levels of harmful LDL cholesterol and increases in levels of beneficial HDL cholesterol in the blood of fish eating community members has been reported (2). Therapeutic effects of ω-3 fatty acids in chronic inflammatory bowel disease, asthma, cystic fibrosis, rheumatoid arthritis, brain development and function, depression, and bipolar disease has been established (3). United Kingdom dietary guidelines suggest that 0.2 g of ω-3 fatty acids should be included in the average diet of individuals to minimize incidents of cardiovascular disease (4).

Chia (*Salvia hispanica* L.) of family *Labiaceae*, usually identified as sage in Spain and chia or black chia in Mexico (5), originated from Mexico and northern parts of Gualtemala. The first use of chia seeds as a human food was recorded in 1500 BC and in prehistoric times chia seeds were a staple food in the region of modern Mexico (6). Chia oil is an excellent source of omega fatty acids (1). Chia seeds produce approximately 30-33% good quality essential/edible oil that is widely used for preparation of omega-3 capsules (7). The ω-3 fatty acids are comprised of the 3 indispensable α-linolenic, eicosapentaenoic, and decosahexaenoic fatty acids, while ω-6 is comprised of linoleic and arachidonic fatty acids (1). Chia is regarded as the richest source of ω-3 and 6 fatty acids among all known sources of omega fatty acids. The European Parliament has declared chia as a gluten free protein novel food with no anti-nutritional factors (6). Recently, chia seeds were used in formulation of biscuits, pasta, cereal bars, cakes, and snacks (8).

Dry fractionation of fats and oils is a widely used commercial technique that is preferable to solvent fractionation. Use of solvents for fractionation of fats presents health concerns and, at a commercial level, solvent use is not economical. Modification of fatty acid compositions of fats and oils of both vegetable and animal origin via dry fractionation/crystallization has been achieved and the fatty acid compositions of derived fractions were considerably different from the parent feedstock with the olein fraction as a good source of unsaturated fatty acids (9). In recent years, increased consumer knowledge regarding health benefits associated with intake of omega fatty acids and a focus of food manufacturers on supplementation of foods with bioactive compounds of plant origin has substantially increased demand for functional foods. Little is currently known regarding enhancement of omega fatty acids in chia oil using fractionation. This study aimed to increase omega fatty acid contents in chia seed oil via dry fractionation, to extend applications in preparation of food products, and to study the chemical characteristics

of olein and stearin fractions at 2 different storage temperatures (4 and 25°C).

Materials and Methods

Materials Black chia (*Salvia hispanica* L.) seeds were purchased from a market in Lahore, Pakistan in June of 2015, and cleaned with water and sun dried. Oil was extracted from roasted seeds using a Soxhlet distillation apparatus with *n*-hexane. Extracted oil was stored in amber glass bottles at −60°C in a freezer (Sanyo, Osaka, Japan) prior to use. Chemicals used in this study were HPLC grade and procured from Sigma-Aldrich, St. Louis, MO, USA.

Fractionation of chia seed oil Oil was heated to 63°C, in a hot air oven, maintained at this temperature for 30 min, then slowly cooled in room temperature and further cooled to −30°C in a freezer (Sanyo) for 4 h. Olein and stearin fractions were separated using a Buckner funnel, filter paper, with an attached vacuum pump (Buchi, Flawil, Switzerland) at 0.53 bar pressure. The filtrate was composed of low melting point glycerides and regarded as an olein fraction. The fraction that remained on the filter paper, mainly comprised of high melting point glycerides, was designated as a stearin fraction. The olein and stearin fractions were transferred to amber glass bottles and stored for −60°C in a freezer (Sanyo) for subsequent investigation (9).

Storage study Olein and stearin fractions were packaged in transparent PET bottles and stored at 4 and 25°C in an oven for 6 months using unmodified chia oil as a control. Estimation of the peroxide value, through titration, was performed at 0, 60, 120, and 180 days. Changes in fatty acid compositions were measured using fresh olein and stearin fraction and after 180 days (6 months) of storage.

Physico-chemical analysis The proximate composition of chia seeds was determined following previous methods (10). Free fatty acid contents of chia oil were determined based on oleic acid and iodine following the recommended method (11). The peroxide value was determined based on dissolution of 5 g sample in a solution prepared by dissolving 3 parts of glacial acetic acid with 2 parts of chloroform. After addition of a saturated potassium iodide solution, flasks were sealed with cork and placed in the dark for 5 min. Titration was performed using a 0.01 N sodium thiosulfate solution. The saponification value and the amount of unsaponifiable material were determined following previous methods (11). The refractive index was measured at 40°C using a model RFM 300 digital refractometer (11). Color was measured in 1 inch quartz cell on a Lovibond Tintometer (Tintometer Corporation, Dortmund, Germany). The total antioxidant activity of chia oil and the olein and stearin fractions was determined using phosphomolybdenum as a derivitization agent (12).

Fatty acid composition The fatty acid composition was determined based on conversion of oil into fatty acid methyl esters using methanol HCl 15% (Fluka; Sigma-Aldrich) as transesterification agent. Briefly, a 50 mg oil sample was weighed on three digital balance in a screw capped test tube, then 2 mL of methanol HCl was added, followed by mixing for 1 min and transfer to a heating block at 100°C for 1 h. Contents of tubes were occasionally shaken during the first 20 min. Tubes were cooled, to room temperature, then 2 mL of *n*-hexane and 2 mL of distilled water were added, followed by vortexing at 2,200 rpm for 2 min, and settling, in a stand on table, for 30 min. The supernatant was transferred to GC vials and 1 μL was injected into a 7898A GC-MS system (Agilent, Santa Clara, CA, USA) via an autosampler (Agilent) fitted with an MSD detector using a 100 mx0.25 mm ZB-5 fused silica capillary column (Zebron; Phenomenex, Torrance, CA, USA) (13).

Induction period The induction period was determined using a model 679 rancimat (Metrohm AG, Herisau, Switzerland). Oil samples of 2.5 g were oxidized in reaction vessels at 120°C under a steady stream of oxygen. (14). Total phenolic contents of chia oil and the olein and stearin fractions were determined using a spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan) with Folin-Ciocalteu reagent. One-half mL of extract was placed in a test tube with 0.5 mL of distilled water and 4.5 mL of a solution prepared by mixing 4% Na_2CO_3 solution, 2% $CuSO_4$, and 4% potassium sodium citrate in 100:1:1 ratio. Incubation (IN30; Memmert, Schwabach, Germany) of tubes for 15 min followed at 40°C, then 0.5 mL of a Folin-Ciocalteu reagent solution containing 1 part of Folin-Ciocalteu reagent and 2 parts of distilled water was added and tubes were again incubated, (IN30; Memmert) at 25°C for 10 min. The absorbance was then measured using a double beam spectrophotometer (UV-1800; Shimadzu) at 750 nm with 0 to 20 μg/mL gallic acid as a standard (15).

HPLC characterization of phenolic compounds Characterization of phenolic compounds present in chia oil and the olein and stearin fractions was performed using an HPLC apparatus equipped with a binary pump, a 50 μL loop injector, and a UV-VIS detector with a C18 column (250x4.6 mm, 100Å, 5 μm). The mobile phase was comprised of water/acetic acid/butanol (350:1:10, v/v). The injection volume was 10 μL and the flow rate was 1 mL/min with chlorogenic and caffeic acids, quercetin, and phenolic glycoside (99% purity) standards (Sigma-Aldrich). Results recorded were mean values of triplicate determinations for each oil and fraction sample. Concentrations of standards prepared in ethanol were 0.03 mg/mL caffeic acid, 0.15 mg/mL chlorogenic acid, 0.15 mg/mL quercetin, and 0.12 mg/mL phenolic glycoside. Concentrations of phenolic compounds were determined using a calibration curve, obtained from standards, where the value of the correlation coefficient for all calibration curves was between 0.952 and 0.978. Standard deviation was calculated based on peak areas and retention times of 3 consecutive determinations (16).

Statistical analysis Experiments were performed using a completely randomized design (CRD) where each treatment was replicated 3 times. Data were analyzed using both one way and 2 way analysis of variance (ANOVA) techniques and significant differences were defined at $p<0.05$ among treatments using LSD testing on SAS 9.1 software (17).

Results and Discussion

The proximate composition of chia seeds consisted of 6.13% moisture, 33.5% oil, 18.47% protein, 4.28% ash, 23.55% fiber, and 14.10% nitrogen, consistent with a previous report (18).

Physico-chemical characteristics of olein and stearin fractions of chia oil Physico-chemical compositions of chia oil and the olein and stearin fractions are shown in Table 1. Free fatty acid contents, saponification values, and colors of chia oil and fractions were not significantly ($p>0.05$) different. Fractionation/dry crystallization of fats and oils mainly involves crystallization of triglycerides with different melting profiles. Free fatty acid contents of *Moringa oleifera* oil and the olein fraction were reportedly not different (19). In this study, iodine values of the olein and stearin fractions were significantly ($p<0.05$) different from the parent chia oil with the olein fraction exhibiting a higher iodine value than the parent oil while the stearin fraction exhibited a lower value. Iodine values of chia oil and the olein and stearin fractions were 193.2, 228.46, and 161.77 cg/g, respectively. The correlation coefficient for the refractive index and iodine value of oil and fraction was R^2=0.999. The olein fraction can be regarded as a neutraceutical/functional food due to a high iodine value.

Unsaturated fatty acids are major contributors to the iodine value and the greater the number of unsaturated fatty acids, the higher the iodine value (20). The correlation coefficient between the iodine value and the ω-3 fatty acid content was R^2=0.909. Similarly, the correlation coefficient between the iodine value and melting point was R^2=0.999. The beneficial effects of unsaturated fatty acids on the human body have been documented (21). The amount of unsaponifiable material in the olein fraction was greater than for the parent chia oil and the increase was due to migration of tocopherols and phenolic substances into the olein fraction due to association with low melting point triglycerides. The tocopherol, phenolic, and squalene contents of the olein fraction of palm oil were reportedly higher than for the parent palm oil (22).

Fatty acid composition Fatty acid compositions of the olein and stearin fractions are shown in Table 2. Fractionation had an effect on the fatty acid composition and the olein and stearin fractions were considerably different in fatty acid compositions. Amounts of C18:1 (oleic acid, ω-9), C18:2 (linoleic acid, ω-6), and C18:3 (linolenic acid, ω-3) increased from 8.26, 11.92, and 61.28% to 10.16, 15.22, and 72.16% in the olein fraction, respectively. The olein fraction can be considered as a functional food with the highest content of omega fatty acids in any known food.

Omega fatty acids are reportedly cardio and hepatic protectors and anti-inflammatory agents (23). United Kingdom dietary guidelines suggest including omega fatty acids as an essential part of the diet of an average individual for decreasing the risk of cardiovascular disease (24). The olein fraction provides an opportunity for preparation of ω-3 and 6 capsules; however, application of chia oil is limited due to an inferior storage quality, compared with current commercial sources of vegetable oils. The stearin fraction of chia oil can serve as a source of omega fatty acids with a better storage quality than both the parent chia oil and the olein fraction.

Fractionation of fats and oils improves functional properties and broadens potential applications (25). The concentration of C18:1 in a high oleic acid fraction of *Moringa oleifera* oil was reportedly greater than for the parent oil (19) and fatty acid compositions and chemical characteristics of different fractions derived from palm oil were considerably different from native palm oil (22). The oxidative stability of a low melting point fraction of milk fat was different from native milk fat (26). This study was a preliminary investigation regarding fractionation of chia oil; however, detailed investigation is required for use as an ingredient in functional foods.

Total phenolic contents The total phenolic contents of chia oil and the olein and stearin fractions were 35.6±0.85, 47.2±1.23, and 28.4±0.65 mg of GAE/mL, respectively. The total phenolic contents were in the order of olein>chia oil>stearin. The higher total phenolic contents of chia oil could be correlated with the presence of caffeic

Table 1. Chemical characteristics of chia oil and the olein and stearin fractions

Parameter	Chia oil	Olein	Stearin
Free fatty acids (%)	0.28±0.03$^{a1)}$	0.26±0.02^{a}	0.25±0.02^{a}
Iodine value (cg/g)	193.2±1.27^{b}	228.4±3.52^{a}	161.7±2.19^{c}
Unsaponifiable matter (%)	1.26±0.09^{b}	1.42±0.03^{a}	1.17±0.08^{c}
Saponification value	192±2.55^{a}	195±1.24^{a}	189±2.74^{a}
Refractive index @ 40°C	1.471±0.01^{b}	1.479±0.01^{a}	1.462±0.01^{c}
Melting point (°C)	-13.5	-19.5	-9.8
Color (Lovibond scale)	R2.6+28Y^{a}	R3.0+30Y^{a}	R2.8+28Y^{a}

[1)]Within a row, means denoted by a different letter are different ($p<0.05$).

Table 2. Fatty acid composition of chia oil and the olein and stearin fractions

Fatty acid (g/100 g)	Chia oil before fractionation	Olein fraction after fractionation	Stearin fraction after fractionation
C14:0 (Myristic acid)	0.03±0.01[a1)]	0.01±0.00[a]	0.02±0.01[a]
C15:0 (Pentadecanoic acid)	0.06±0.02[a]	0.02±0.01[b]	0.04±0.01[a]
C15:1 (Pentadecenoic acid)	0.07±0.01[a]	0.05±0.01[a]	0.02±0.01[b]
C16:0 (Palmitic acid)	6.83±0.11[a]	1.52±0.05[c]	14.61±0.11[b]
C16:1 (Palmitoleic acid)	0.09±0.01[a]	0.08±0.02[a]	0.02±0.01[b]
C18:0 (Stearic acid)	2.71±0.05[a]	0.42±0.04[b]	22.62±0.06[a]
C18:1 (Oleic acid, ω-9)	8.26±0.14[b]	10.16±0.13[a]	7.15±0.08[c]
C18:2 (Linoleic acid, ω-6)	11.92±0.25[b]	15.22±0.36[a]	8.74±0.05[c]
C18:3 (Linolenic acid, ω-3)	61.28±1.36[b]	72.16±0.98[a]	44.24±0.14[c]
C20:2 (*cis*-Eicosadienoic acid)	0.05±0.01[a]	0.04±0.01[a]	ND[2)]
C20:3 (*cis*-Eicosatrienoic acid)	0.04±0.01[a]	0.05±0.01[a]	ND
C22:0 (Behenic acid)	0.11±0.01[a]	0.03±0.01[c]	0.08±0.02[b]
C23:0 (Tricosanoic)	0.05±0.02[a]	0.02±0.01[b]	0.04±0.01[a]
C24:0 (Lignoceric acid)	0.17±0.02[a]	0.04±0.01[c]	0.13±0.03[b]

[1)]Within a row, means denoted by a different letter are significantly different (p<0.05).
[2)]ND, Not Detected.

and chlorogenic acids, quercetin, and phenolic glycosides (27). The higher total phenolic content of the olein fraction was due to enrichment of phenolic substances in the low melting point fraction. The chlorogenic acid content of chia seeds was 102 μg/g (28). Chlorogenic and caffeic acid, myricetin, quercetin, and kaempferol have been isolated from chia (18). Health benefits associated with intake of natural antioxidants for scavenging of free radicals are well established.

HPLC characterization of phenolic compounds HPLC characterization of the phenolic compounds of chia oil and the olein and stearin fractions is shown in Fig. 1, 2, and 3. In this study, chlorogenic and caffeic acids, quercetin, and phenolic glycosides were identified in chia oil and fractions at different concentrations. The olein fraction showed a maximum concentration of phenolic compounds, followed by chia oil and the stearin fraction. Concentrations of chlorogenic and caffeic acids, quercetin, and phenolic glycosides in the olein fraction of chia oil were 4.65, 29.47, 0.15, and 614 μg/g, respectively, with 5.42, 38.92, 0.28, and 674 μg/g, respectively, in the olein fraction of chia oil. Significantly (p<0.05) lower values of chlorogenic acid (3.27 μg/g), caffeic acid (21.64 μg/g), quercetin (0.11 μg/g), and phenolic glycosides (571 μg/g) were present in the stearin fraction of chia oil. Enrichment of phenolic compounds in the olein fraction was due to association with low melting point glycerides. Migration of phenolic compounds from the parent palm oil to the low melting point fraction has been described (29). Association between antioxidant compounds and a low melting point fraction of the palm oil has also been documented (22). The increase in the phenolic compound content of the low melting point fraction was also evident in a higher unsaponifiable material content (Table 1). The phenolic compounds of chia oil have been extensively studied (16); however, little is known regarding the phenolic compounds of the olein and stearin fractions of chia oil. A strong antioxidant activity of chia oil has been reported (30). The olein fraction of chia oil can serve as a good source of phenolic compounds and beneficial omega fatty acids.

Peroxide value Peroxide values of chia oil and the olein and stearin fractions are shown in Table 3. Peroxide values of parent chia oil and fractions were enriched throughout a storage period of 180 days. Increase of the peroxide value during the storage period was reliant on the storage temperature and the fatty acid composition as both factors had an effect on generation of primary oxidation products. After 6 months of storage, peroxide values of the olein fraction stored at 4 and 25°C were 0.98 and 4.72 (meq of O_2/kg), respectively. After 6 months of storage, peroxide values of chia oil and the stearin fraction stored at 4°C were 0.82 and 0.63 (meq of O_2/kg), respectively. Chia oil is a good source of essential omega fatty acids due to a large number of polyunsaturated fatty acids; however, chai oil has poor oxidative stability. This study revealed that chia oil and fractions can be stored for 6 months at 4°C without serious deterioration. The impact of the fatty acid composition on the oxidative stability of some vegetable oils has been described (31). Storage conditions had an effect on the oxidative stability of soybean oil (32).

Transition in the fatty acid profile Transition in fatty acid compositions of chia oil and the olein and stearin fractions influenced by the storage period and storage temperature are shown in Table 4. A large amount of omega fatty acids in chia oil makes chia oil unique regarding essential/edible oils. In this study, changes in the fatty acid composition as a function of storage at 4 and 25°C were used to monitor changes in omega and other fatty acids. Many studies have been performed regarding oxidative stability of chia oil under ambient and accelerated storage conditions; however, detailed information is lacking regarding the stability of chia oil at low temperatures.

This study provided information regarding storage of chia oil for

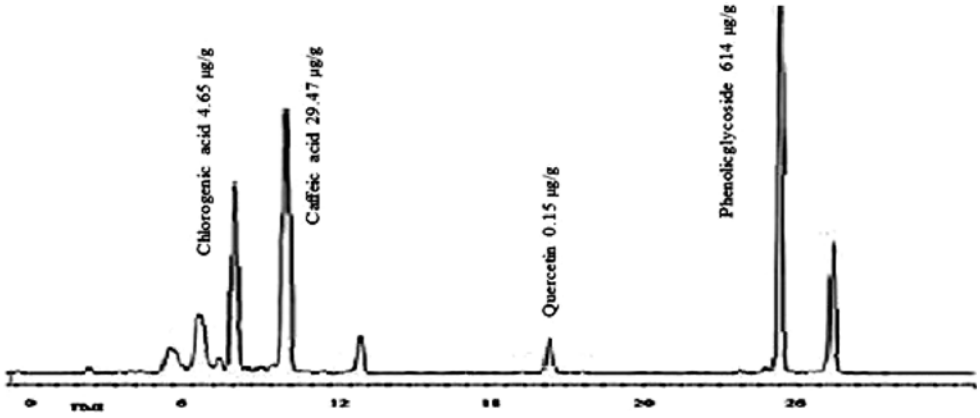

Fig. 1. HPLC characterization of phenolic compounds in chia oil.

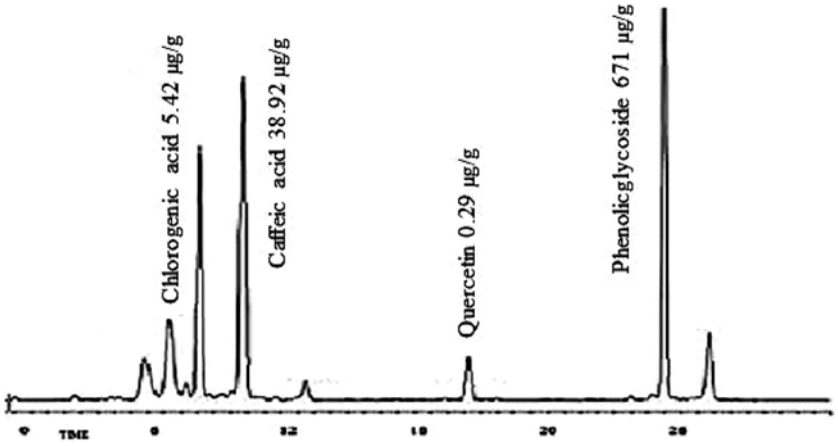

Fig. 2. HPLC characterization of phenolic compounds of the olein fraction.

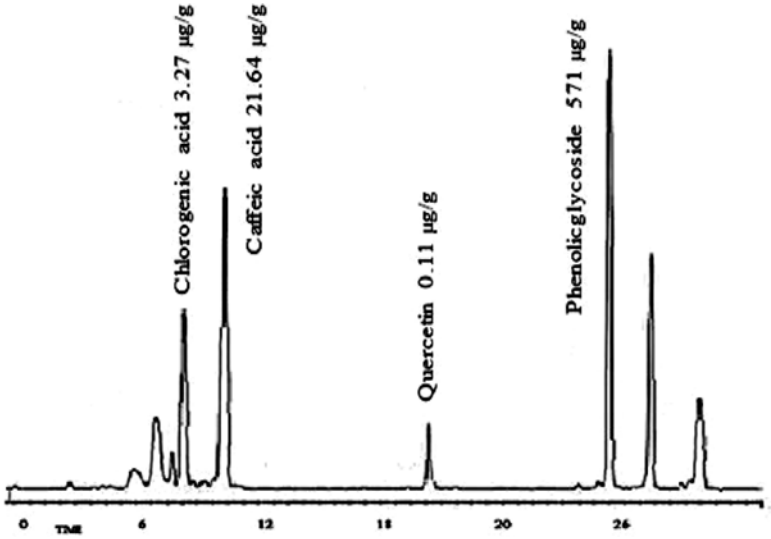

Fig. 3. HPLC characterization of phenolic compounds of the stearin fraction.

minimization of essential omega fatty acid breakdown. Stability of 3 different fatty acid profiles was examined at 2 different temperatures. Oil samples stored at 25°C suffered oxidation, whereas storage at 4°C had a lesser catalytic effect on oxidation. The catalytic effect of temperature on auto-oxidation is well established (25). Under both storage conditions at the end of 6 months of storage, the amount of unsaturated fatty acids decreased and the concentration of saturated fatty acids increased. The transition in fatty acid composition of oil

Table 3. Peroxide values (Meq of O_2/kg) of 6 month stored chia oil and the olein and stearin fractions

Oil types	Storage temperature 4°C				Storage temperature 25°C		
	0-day	60-days	120-days	180-days	60-days	120-days	180-days
Chia oil	0.25±0.03$^{I1)}$	0.32±0.02^{I}	0.82±0.06^{j}	1.47±0.12^{g}	0.79±0.07^{j}	1.73±0.14^{f}	3.19±0.18^{b}
Olein	0.27±0.02^{I}	0.35±0.04^{I}	0.98±0.04^{i}	1.92±0.08^{e}	1.15±0.05^{h}	2.58±0.13^{d}	4.72±0.21^{a}
Stearin	0.25±0.02^{I}	0.30±0.02^{I}	0.63±0.03^{k}	1.52±0.06^{g}	0.61±0.04^{k}	1.95±0.09^{e}	2.87±0.06^{c}

[1)]Within rows and columns, mean values sharing a different letter are significantly different (p<0.05).

Table 4. Transition in fatty acid composition of 6 month stored chia oil and olein and stearin fractions at 4°C and 25°C

Fatty acid (g/100 g)	Chia oil			Olein			Stearin		
	After 6 Months of Storage			After 6 Months of Storage			After 6 Months of Storage		
	Fresh	4°C	25°C	Fresh	4°C	25°C	Fresh	4°C	25°C
C14:0 (Myristic acid)	0.03±0.01^{b}	0.04±0.01^{b}	0.09±0.01^{a}	0.02±0.01^{c}	0.05±0.01^{b}	0.11±0.03^{a}	0.02±0.01^{c}	0.08±0.01^{a}	0.09±0.02^{a}
C15:0 (Pentadecanoic acid)	0.06±0.02^{c}	0.11±0.01^{b}	0.15±0.01^{a}	0.07±0.01^{d}	0.07±0.02^{c}	0.13±0.02^{a}	0.04±0.01^{c}	0.05±0.01^{c}	0.11±0.02^{b}
C15:1 (Pentadecenoic acid)	0.07±0.01^{a}	0.06±0.02^{a}	0.03±0.01^{b}	0.05±0.01^{a}	0.04±0.01^{b}	0.02±0.01^{c}	0.02±0.01^{c}	ND$^{2)}$	ND
C16:0 (Palmitic acid)	6.83±0.11^{c}	7.71±0.05^{b}	8.14±0.03^{b}	2.56±0.05^{f}	3.42±0.06^{e}	5.17±0.09^{d}	14.61±0.11^{a}	15.12±0.08^{a}	15.68±0.05^{a}
C16:1 (Palmitoleic acid)	0.09±0.01^{a}	0.08±0.02^{a}	0.05±0.01^{b}	0.08±0.02^{a}	0.07±0.01^{a}	0.05±0.01^{b}	0.03±0.01^{c}	0.02±0.01^{c}	ND
C18:0 (Stearic acid)	2.71±0.05^{c}	2.65±0.06^{c}	3.12±0.07^{b}	0.42±0.04^{e}	1.15±0.07^{d}	2.63±0.05^{c}	22.62±0.06^{a}	21.88±0.04^{a}	18.24±0.06^{a}
C18:1 (Oleic acid, ω-9)	8.26±0.14^{c}	10.89±0.012^{c}	8.51±0.16^{e}	10.16±0.13^{a}	9.38±0.12^{b}	9.24±0.32^{d}	7.15±0.08^{f}	6.82±0.02^{f}	5.75±0.13^{g}
C18:2 (Linoleic acid, ω-6)	11.92±0.25^{d}	11.24±0.10^{d}	9.62±0.08^{e}	15.22±0.36^{a}	13.75±0.09^{b}	10.44±0.22^{c}	8.74±0.05^{f}	8.19±0.03^{f}	7.21±0.07^{g}
C18:3 (Linolenic acid, ω-3)	61.28±1.36^{c}	60.38±0.88^{c}	55.17±0.72^{d}	72.16±0.98^{a}	71.19±0.93^{a}	65.15±0.43^{b}	44.24±0.14^{e}	43.11±0.04^{e}	40.41±0.03^{f}
C20:2 (*cis*-Eicosadienoic acid)	0.05±0.01^{a}	0.04±0.01^{a}	0.02±0.01^{b}	0.04±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	ND	ND	ND
C20:3 (*cis*-Eicosatrienoic acid)	0.04±0.01^{a}	0.03±0.01^{a}	ND	0.05±0.01^{a}	0.03±0.01^{a}	0.02±0.01^{b}	ND	ND	ND
C22:0 (Behenic acid)	0.11±0.01^{f}	0.15±0.02^{e}	0.26±0.04^{c}	0.03±0.01^{g}	0.12±0.02^{f}	0.31±0.05^{b}	0.09±0.02^{f}	0.21±0.02^{d}	0.37±0.06^{a}
C23:0 (Tricosanoic)	0.05±0.02^{d}	0.08±0.01^{c}	0.14±0.03^{b}	0.02±0.01^{e}	0.15±0.03^{b}	0.27±0.04^{a}	0.04±0.01^{d}	0.13±0.01^{b}	0.29±0.03^{a}
C24:0 (Lignoceric acid)	0.17±0.02^{d}	0.23±0.02^{c}	0.29±0.04^{b}	0.04±0.01^{g}	0.09±0.02^{f}	0.22±0.03^{c}	0.13±0.03^{e}	0.27±0.05^{b}	0.41±0.04^{a}

[1)]Mean values of triplicate treatments and triplicate analysis. Within a row, mean values denoted by a different letter are significantly different (p<0.05).
[2)]ND, Not Detected.

samples stored for 6 months from initial values was dependent on the fatty acid composition and the storage temperature. At 25°C after 6 months, concentrations of ω-6 and 3 in chia oil decreased from 11.92 to 9.62% and from 61.28 to 55.17%, respectively, while only 0.68% of ω-6 and 0.9% of ω-3 was lost at 4°C after 6 months. At 4°C after 6 months, concentrations of ω-6 and ω-3 fatty acids in the olein fraction decreased from 15.22 to 13.75% and from 72.16 to 71.19%, respectively. At 25°C after 6 months, 7% of ω-6 and 10% of ω-3 fatty acids were broken down into oxidation products. In the olein fraction stored at 25°C for 6 months, concentrations of ω-6 and ω-3 decreased from 15.22 to 10.44% and from 72.16 to 65.15%, respectively.

During long term storage of some vegetable oils, concentrations of saturated fatty acids increased and unsaturated fatty acids decreased due to generation of large amounts of oxidation products (33). The degree of oxidation of fats and oils greatly depends upon the fatty acid composition (34). The fatty acid composition of 6 month stored olein based butter was different from the initial value as amounts of saturated fatty acids increased and amounts of unsaturated fatty acids decreased (26).

Induction period Induction period values of chia oil and the olein and stearin fractions were 1.24±0.11, 0.82±0.05, and 1.41±0.12 h, respectively. The stearin fraction had the longest induction period, followed by chia oil and the olein fraction. Although phenolic compounds were concentrated in the olein fraction which did not exhibit a significant (p>0.05) difference in the induction period, compared with chia oil and the stearin fraction as approximately 70% of oxidation depends upon the fatty acid profile and only 30% depends upon antioxidant substances (34). The induction period recorded in this study was not different from a previously reported value where the induction period of whole chia oil was 1.0 h (35). However, little information is available regarding the induction period of the olein and stearin fractions of chia oil. The induction period indicates the anticipated shelf life of fats and oils. The shelf life of chia oil and the olein and stearin fractions can be enhanced by low temperature storage.

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