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Effect of soxhlet and cold press extractions on the physico-chemical characteristics of roasted and non-roasted chia seed oils

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Abstract

While peroxide values of roasted and non-roasted chia seed oils obtained by cold press changed between 3.65 (non-roasted) and 14.12 meqO₂/kg (roasted), peroxide values of chia seed oils extracted by Soxhlet extraction system were determined between 2.17 (non-roasted) and 8.53 meqO₂/kg (roasted). Total wax contents of chia seed oils ranged between 56.74 mg/kg (roasted seed oil obtained by cold press system) to 138.87 mg/kg (non-roasted seed oil extracted by Soxhlet extraction). The linolenic acid contents of roasted and non-roasted chia oils obtained by cold press and Soxhlet extraction systems varied between 66.24 and 67.84% to 64.98 and 66.75%, respectively. $\beta + \gamma$ -Tocopherols contents of roasted and non-roasted chia seed oils from cold press and Soxhlet extraction systems were determined between 901.6 and 917.3 mg/kg and 795.6 to 857.1 mg/kg, respectively. The rosmarinic acid contents of non-roasted and roasted chia seed oils obtained by cold press system decreased from 2.17 to 1.28 mg/g (p < 0.05), while oil extracted from Soxhlet method showed slight increase from 2.67 to 2.92 mg/g in non-roasted and roasted seeds respectively. This study revealed that roasting and extraction methods had significant effects on the micro constituents of oil from chia seeds. Due to these properties cold presses and non-roasted can be recommended.

Keywords Chia seed oil \cdot Cold press \cdot Soxhlet extraction \cdot Antioxidant activity \cdot Fatty acid \cdot Tocopherols \cdot Phenolic compounds

Introduction

The seeds of *Salvia hispanica* L. known as "Chia" and "Chia sage" were an important staple food. Chia, a member of Labiatae family, is one of the newly identified food crop that may has broad applicationpotentials in food systems due to its bioactive and nutraceuticals properties [1–3], and it is an herbaceous plant grown semi-annually and is a member of Lamiaceae [4]. Presently, it is cultivated on small scale and grown in both tropical and subtropical regions of the world [5]. The use of chia as a food date back to 3500 BC and its

² Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia popularity as a potential food crop in central Mexico date back to 1500 BC. Borneo et al. [6] and Valenzuela et al. [7] described chia seeds as an excellent source of dietary fat, protein, fiber, minerals and polyphenols. Bioactive compounds including quercetin, myrcetin, kaempferol and chlorogenic acids have also been identified in chia seeds and oil [2, 8–11]. However, the seeds of chia are still counted within underexploited plant materials and its potential for use as a good source of specialty oil has just been established. Apart from being used in oil production, chia seeds could also be consumed when roasted or milled as gruel. In addition to that, chia is also consumed as fruit juice when soaked in water [12]. The oil content of chia seeds varied between 25-35% and majority of the fatty acids present in chia oil are polyunsaturated fatty acids [8], with α -linolenic acid accounting for about 68% of the total polyunsaturated fatty acids [5, 6]. Chia seeds contain a significant amount of lipids, with omega-3-fatty acids accounting for about 60% of the total lipids [13]. Chia seeds have potential antioxidant activity such as myrcetin, quercetin, kaempferol and caffeic acid [13–16]. Chia seeds oil is distinctive and this

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is attributed to the presence of high levels of omega-3fatty acid at levels above any other natural oil [4, 14–17]. Omega-3 fatty acids have many health promoting properties when consumed and is also an important raw material in cosmetic industry [15]. Pressing is slow and time-consuming and oil recovery depends on many factors including cracking, de-hulling, heat conditioning of seeds prior to extraction [18]. Solvent extraction, on the other hand, is a cost-effective and fast method of oil extraction that permits high recovery of available oil. However, high inflammability of the solvent used in solvent extraction and high cost of extraction systems, with possible presence of solvent remnants in the oil are the major problems associated with solvent extraction [19]. Oil recovery, shelf life and phytochemical composition of oil depend on the extraction method used. Information about the effect of extraction methods on the phytochemical composition of chia seed oil is not available in literature. Therefore, the aim of this study was to determine the effect of cold press and Soxhlet extraction methods on the physicochemical and quality characteristics of roasted and non-roasted chia seed oil.

Materials and methods

Materials

Chia seeds were purchased from the local market in Konya, Turkey. The seeds were cleaned, sorted, dried and sieved to remove all types of impurities. The seeds were then packed in air tight polypropylene bags and stored in refrigerator till further used.

Oven roasting

Prior to oil extraction, chia seeds were divided into two portions (100 g each). One portion was used for oil extraction without roasting, while the other was roasted in a tray oven (Nüve FNO55, Ankara, Turkey) at 120 °C for 30 min. The heating was carefully done and adequately monitored to prevent over-heating of the samples. Thereafter, both roasted and unroasted samples were subjected to oil extraction using cold press and Soxhlet methods. The purpose of chia seeds roasting before extraction is to obtain more oil.

Cold press

Oil was extracted from the chia seeds using cold press method. After extraction, the oil was allowed to stand for sedimentation period of 1 week in order to remove solid impurities. After that, the oil was filtered and kept in sealed dark coloured bottles under nitrogen at 4 $^{\circ}$ C.

Soxhlet extraction

Chia seeds were ground and extracted for 6 h using petroleum ether (50 °C) in a Soxhlet extractor, followed by evaporation of solvent under reduced pressure. Extracted oil was kept in sealed glass bottles at -18 °C until further analysis.

Both cold press and soxhlet extraction methods are preferred because they are the most widely used systems in oil extraction.

Physicochemical properties

Standard AOAC [20] methods were used to determine the acidy, peroxide value, density, iodine value, refractive index, saponifiable and unsaponifiable values for chia seed oil samples. Wax precipitation was carried out using a procedure as proposed by Burger et al. [21].

Extraction of chiasamples

In order to study the phenolic contents and antioxidant activity, extraction method as reported by Talhaoui et al. [22] was followed after some modifications. Chia seed oils were mixed with 20 mL of methanol followed by 15 min sonication and 10 min centrifugation at $5000 \times g$. The extraction was carried out in two cycles and centrifuged, and the supernatants were separated and concentrated at 37 °C in a rotary evaporator under vacuum. The extracts volume was made up to 25 mL using methanol and kept at 4 °C until used for analysis.

Total phenol

The totalphenolic content of the chia seed oils were extracted using methanol and quantified with Spectrophotometer (absorbance at 765 nm) using Folin-Ciocalteu reagent (FCR) as described by Madaan et al. [23]. Gallic acid standard curve was constructed and used to evaluate the total phenolic, which was expressed as Gallic acid equivalent. For preparation of the standard solution, 10 mg of Gallic acid and 100 mL of 50% methanol were mixed, followed by dilution to concentrations of 12.5, 25, 50 and 100 µg/mL. An aliquot of 0.076 mL from each of these dilutions was kept in a glass tube and diluted further to 0.76 mL using distilled water. About 0.12 mL of Folin-Ciocalteu solution (1 N) was added to the oil samples, held at 37 °C for 5 min, incubated at the same temperature and thereafter 0.32 mL of Na₂CO₃ (20% w/w) was added and total volume in test tubes made 2 mL each using distilled water. The samples and standard mixtures prepared in the same manner were vortexed and allowed to stand at room temperature for 30 min, after which the absorbance was measured at 765 nm using UV/VIS Spectrophotometer (Shimadzu, Japan) and distilled water was used as a blank. Dilute methanolic extract (0.76 mL) was used for the estimation of the plant samples. The same approach was used for the standard.

Total flavonoid

The method described by Dewanto et al. [24] was used to determine the total flavonoids ofoil extracts. In the procedure, distilled water was thoroughly mixed with methanol extracts, followed by addition of NaNO₂ solution. Then, AlCl₃ solution was added after 5 min. The samples were kept for 6 min and thereafter 1 M NaOH was added. Total volume of the mixture was made to 5 mL with distilled water and the tubes were vigorously stirred. The resulting solution was pink-colored and its absorbance was determined by Spectrophotometer at 510 nm against the blank. Catechol was used to construct the calibration curve and the flavonoids in the sample were expressed as mg Catechol equivalents per gram of dry weight (mg CE/g DW).

Antioxidant activity

Antioxidant activity of chia seed oil was determined using the method described by Lee et al. [25]. DPPH solution in methanol was used for the assessment of antioxidant activity of chia seed oil extracts. Briefly, 1 mL extract was added to 2 mL DPPH methanolic solution followed by vigorous shaking. The mixture was then incubated for 30 min at 37 °C and absorbance was measured by Spectrophotometer at 517 nm.

Determination of phenolic compounds

The analysis of individual phenolic compounds in the extracts from chia seed oil was carried out using Shimadzu-HPLC equipped with PDA detector and Inertsil ODS-3 (5 μ m; 4.6×250 mm) column. The mobile phase consisted of 0.05% acetic acid in water (A) and acetonitrile (B) and flow rate was set at 1 mL/min and 20 μ L of the samples were injected at 30 °C. The peaks were recorded at 330 nm and sample was run for 60 min.

Fatty acid composition

Chia seed oil samples were first esterified as described in ISO-5509 [26] procedure followed by the identification of fatty acid methyl esters through comparison of retention time for those from the samples with fatty acid standards. The samples were injected into a gas chromatography system (Shimadzu GC-2010) equipped with a capillary column (Tecnocroma TR-CN100, 60 m \times 0.25 mm, film thickness: 0.20 µm) and flame-ionization detector (FID). The injection

block and detector temperature was set at 260 °C and nitrogen was used as mobile phases ata flow rate of 1.51 mL/min. The total flow rate was 80 mL/min whereas the split rate was 1/40. Column temperature was set as 120 °C for 5 min followed by an increment of 4 °C/min until reached 240 °C where it was held for 25 min.

Tocopherol content

The contents of tocopherols in either cold pressed or Soxhlet extracted oil samples were determined following the method of Spika et al. [27] using a HPLC systemconsisted of Shimadzu-HPLC equipped with PDA detector and Li Chro CART Silica 60 (4.6×250 mm, 5 μ ; Merck, Darmstadt, Germany) column. Briefly, 20 μ L aliquots of oil samples was instantaneously injected into the Diol phase HPLC column 25 cm $\times 4.6$ mm ID (Merck, Darmstadt, Germany) at a flow rate of 1.3 mL/min. Standard solutions of tocopherols (α , β , γ and δ -tocopherol) were used at 0–100 mg/L concentrations for comparison and quantification.

Statistical analysis

Complete randomized split block design was used for carrying out the experiment. All analytical measurements were carried out in triplicate. The obtained data were analyzed using analysis of variance (JMP version 9.0, SAS Inst. Inc., Cary, N.C., U.S.A). The results were expressed as means \pm standard deviation of independent chia seed oil samples [28].

Results and discussion

The physico-chemical and quality characteristics of chia seed oil extracted from roasted and non-roasted chia seeds using two different extraction methods are presented in Table 1. Varied effects of roasting and extraction method on the physicochemical properties of chia seeds oil were observed. Regardless of the extraction method used, roasting of chia seeds prior to oil extraction significantly (p < 0.05) increased the acidity and peroxide and iodine values as higher values of these parameters were observed in oil extracted from roasted seeds by both methods compared to the values of that extracted from non-roasted seeds. This could be attributed to the fact that heating processes causes oxidation of oils and consequently increase their acid, peroxide and iodine values. In addition, roasting also could cause releasing of the free fatty acids thereby increasing the acid values of the extracted oils. Similar observation has been reported during microwave heating of soybean oil [29]. In addition, increased peroxide value of chia oil extracted from thermally pretreated seeds has also been reported [30]. Roasting negatively

Tabl	e 1	Physico	-chemical	properties of	f roasted	and	non-roasten	chia seed	l oils	extracted	by t	two (different	extraction	meth	iods
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Properties	Cold press		Soxhlet extraction			
	Roasted	Non-roasted	Roasted	Non-roasted		
Acidity (mgKOH/g)	$3.87 \pm 0.76a^*$	$1.79 \pm 0.44b$	8.53±1.18a	$2.17 \pm 0.65b$		
Peroxide value (meqO ₂ /kg)	14.12±1.27a**	$3.65 \pm 0.29b$	$17.92 \pm 1.13a$	$5.84 \pm 1.56b$		
Iodine value $(gI_2/100 \text{ g oil})$	193.21 ± 1.17a	$192.86 \pm 2.38b$	$198.51 \pm 2.34a$	$197.61 \pm 3.48b$		
Refractive Index (n_D^{20})	1.4765 ± 0.0013	1.4758 ± 0.0017	1.4769 ± 0.0015	1.4783 ± 0.0021		
Density (g/mL)	$0.9239 \pm 0.0015 d$	$0.9247 \pm 0.0011c$	$0.9261 \pm 0.0012b$	$0.9286 \pm 0.0014a$		
Saponification value (mg KOH/g)	$217.36 \pm 3.28b$	$220.54 \pm 2.77a$	$191.44 \pm 3.44b$	194.18±4.39a		
Unsaponifiable matter (%)	$0.687 \pm 0.017 b$	$0.756 \pm 0.038a$	$0.973 \pm 0.0015b$	$1.341 \pm 0.024a$		
Total phenol (mgGAE/g)	$0.33 \pm 0.11b$	$0.73 \pm 0.17a$	$0.39 \pm 0.05b$	$0.87 \pm 0.19a$		
Antioxidant activity (%)	$0.27 \pm 0.07 b$	$0.54 \pm 0.09a$	$0.21 \pm 0.09b$	$0.47 \pm 0.13a$		
Total flavonoid (mgCE/g)	$0.15 \pm 0.03c$	0.14 ± 0.07 c	$0.19 \pm 0.03b$	$0.28 \pm 0.07a$		
Total wax (mg/kg)	$56.74 \pm 2.67b$	$109.21 \pm 3.38a$	$97.63 \pm 2.46b$	$138.87 \pm 5.36a$		

*Mean ± standard deviation

**Values in each row with different letters are significantly different (p<0.05)

(p < 0.05) affected the saponification value, unsaponifiable matter, total phenolics, antioxidant activity and total wax of chia seed oils as these attributes were significantly lower in oil extracted from roasted chia seeds by both extraction methods compared to that of non-roasted seeds. This could be attributed to degradation or polymerization of these constituents during thermal treatments. Total flavonoids were significantly higher in oil extracted from non-roasted seeds than that of roasted seeds under Soxhlet extraction method. However, neither extraction method nor roasting affected the refractive index and density. Regardless of treatment (roasting and non-roasting) of chia seed samples before oil extraction, with few exceptions, most of the physicochemical properties of chia seeds oil extracted by Soxhlet method were higher than those extracted by cold press. This could due to the extraction of more amounts of these constituents with the oil by the combined effects of organic solvent and heating. Chia oil presented significantly higher acidity values in Soxhlet extraction system then in pressing system. Ayerza and Emir [31] reported that peroxide values of Tzotzol and Iztac chia oils obtained in Soxhlet system were determined as 0.61 meqO₂/kg and 0.82 meqO₂/kg, respectively. Iodine value, peroxide value, refractive index and density values of black chia seed oil were determined as 197.68 gI₂/100 g oil, 2.67 meqO₂/kg, 1.46 n²⁰_D and 0.89 g/mL, respectively [32]. The highest saponifiable value of roasted and nonroasted chia oils obtained by cold pressed were determined as (217.36 mg KOH/g) and (220.54 mg KOH/g), respectively. Ixtaina et al. [13] reported that acid value, iodine value, saponifiable value, unsaponifiable matter, refractive index, and total wax of chia oils extracted by both solvent extraction and pressing methods were determined as 2.05 and 0.91 mg KOH/g, 210.5 and 208.5 gI₂/100 g, 193.09 and 193.12 mgKOH/g, 1.27 and 0.85%, 1.4768 and 1.4794 (25 °C) and 142 and 108 mg/kg, respectively. The values of antioxidant activity for chia seeds sold in the market ranged from 1.474 to 2.602 mmol TEAC/g for 2 and 40 min, respectively [33]. The contents of total phenol and flavonoid contents of Chia seed extracts were determined as 2.639 mg GAE/kg and 0.162 g equivalent/kg, respectively [34]. Chia seed extracts contained 0.88 to 0.94 mg GAE/g total phenol [9, 35]. The IC_{50} found was of 3.841 mg/mL and 45.004 mmol trolox equivalent/kg of chia seed extract [34]. In the DPPH treat, the highest antioxidant activity was found for cold pressed oil. Sargi et al. [33] reported that antioxidant capacity of Chia seed was 1.72%. Total wax content determined in Chia seed oils changed between 56.74 mg/kg (oil obtained by pressing after roasting) and 138.87 mg/kg (non-roasted seed oil extracted by Soxhlet extraction). The result obtained in this present study is less than the values reported for cultivated chia (400-1100 mg/kg) and sunflower oils (678-1128 mg/kg) [36]. Chia seed contain 5-6% mucilage, which can be used as a good source of dietary fiber [9, 17]. It was observed statistically differences among the physico-chemical and quality characteristics of roasted and non-roasted chia seeds oils obtained by cold press and Soxhlet extraction systems (p < 0.05).

Fatty acid composition of chia oil extracted fromroasted and non-roasted seeds by cold press and Soxhlet extraction methods are presented in Table 2. Roasting and extraction methods significantly (p < 0.05) affected the fatty acids composition in chia seed oil. With exceptions of palmitic and oleic acids, fatty acids of chia oil extracted from non-roasted seeds by both extraction methods were significantly higher than those of roasted seeds, indicating that roasting of the seeds prior to oil extraction could negatively affect most of the fatty acids of chia seeds oil. In addition, extraction method also affected the fatty acids profile of chia seeds
 Table 2
 Fatty acid compositions

 of roasted and non-roasted
 chia seed oils extracted by two

 different extraction methods
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Fatty acids	Cold press		Soxhlet extraction			
	Roasted	Non-roasted	Roasted	Non-roasted		
Myristic	$0.04 \pm 0.01b^*$	$0.05 \pm 0.01a$	0.03 ± 0.01 ab	$0.04 \pm 0.01a$		
Palmitic	7.19±1.17ab**	$7.28 \pm 0.69a$	6.58 ± 1.23 ab	6.67 <u>+</u> 1.18a		
Palmitoleic	$0.05 \pm 0.01 \text{b}$	$0.09 \pm 0.03a$	$0.03 \pm 0.01 \text{b}$	$0.06 \pm 0.03a$		
Stearic	$2.17 \pm 0.78b$	$2.33 \pm 0.91a$	$1.97 \pm 0.56b$	$2.07 \pm 0.37a$		
Oleic	9.06 ± 1.23 ab	$9.48 \pm 1.17a$	8.12 ± 0.78 ab	$8.51 \pm 0.51a$		
Linoleic	18.34±1.56b	19.61 ± 1.34a	$17.25 \pm 2.23b$	$18.97 \pm 1.45a$		
Linolenic	$66.24 \pm 2.51b$	$67.84 \pm 2.67a$	64.98±1.81b	$66.75 \pm 2.39a$		
Arachidic	$0.15 \pm 0.03b$	$0.17 \pm 0.01a$	$0.11 \pm 0.05 b$	$0.14 \pm 0.03a$		

*Mean ± standard deviation

**Values in each row with different letters are significantly different (p < 0.05)

oil. In this sense, values of fatty acids of chia oil extracted from roasted and non-roasted seeds by cold press method were higher than those extracted by Soxhlet methods suggesting the negative effects of Soxhlet extraction method on fatty acids of chia seed oil. Roasting and extraction methods significantly (p < 0.05) affected the fatty acids composition in chia seed oil. While palmitic acid contents of roasted and non-roasted chia oils obtained by pressing and Soxhlet system range between 7.19-7.28%, palmitic acid contents of the treated chia oils varied between 6.58 and 6.67%, respectively. The most abundant fatty acid of chia oils obtained by both cold press and Soxhlet extraction was linolenic acid. Extraction methods did not affect the levels of palmitic, palmitoleic and arachidic acid contents of chia oil samples. Linolenic acid composition of roasted and non-roasted chia oils obtained by pressing ranged between 66.24 and 67.84%, linolenic acid present in roasted and non-roasted chia oils extracted by Soxhlet extraction system varied between 64.98 and 66.75%. In addition, linoleic acid contents of roasted and non-roasted chia oils obtained by pressing and Soxhlet varied between 18.34 and 19.61% to 17.25 and 18.97%, respectively. Also, the highest oleic acid contents were found in roasted (9.06%) and non-roasted chia oils (9.48%) obtained by cold press. Information about fatty acid composition of chia seeds have been reported by different researchers. Statistically significant differences were observed among the fatty acid compositions of roastedand non-roasted chia seeds oils obtained by cold press and Soxhlet extraction systems (p < 0.05). Sargi et al. [33] reported that chia seed oil contained 5.8% palmitic, 2.4% stearic, 6.1% oleic, 17.4% linoleic and 54.4% linolenic (omega-3) acids. According to Coelho and de las Mercedes Salas-Mellado [37], chia oilcontains 34.4% lipids out of which 62% are omega-3-fatty acid, 17.4% omega-6 and 10.5% omega-9-fatty acid. In a study on chia seed by Da Silva Marineli et al. [38], alphalinolenic acid was the most prominent (62.80 g/100 g), followed by linoleic (18.23 g/100 g), palmitic (7.07 g/100 g), oleic (7.04 g/100 g) and stearic acid (3.36 g/100 g). Barreto et al. [39] reported that majority of lipids in chia seed were polyunsaturated fatty acids, with linolenic being the most abundant (64.4%), followed by linoleic acid (19.5%). Similar values (63.8-64.8% linolenic and 18.9-22.5% linoleic acids) were reported for chia oil by Ayerza and Coates [17]. Ayerza and Emir [31] obtained 6.5 and 6.2% palmitic, 3.65 and 4.1% stearic, 6.65 and 6.8& oleic, 17.5 and 18.4% linoleic and 64.5 and 63.3% linolenic acids in Tzotzol and Iztac chia oils, respectively. While palmitic, stearic, oleic, linoleic and alpha-linolenic acids of chia oil extracted by solvent extraction were recorded as 6.2%, 3.0%, 5.3%, 19.7% and 65.6%, respectively, the same fatty acids of chia oil obtained by pressing were determined as 6.6%, 3.1%, 5.4%, 20.3% and 64.5%, respectively [13]. In addition, the results obtained for fatty acid composition in this present study are similar to the findings of many researchers [5, 17, 31]. Polyunsaturated linoleic and linolenic fatty acids are essential fatty acids required by humans and must be consumed through food intake [40]. Although fish sea foods such as salmon, herring, sardines and mackerel are rich in polyunsaturated fatty acids, chia seed oil is also recognized as an excellent source of omega-3 fatty acid since they are the only plant containing high levels of the essential fatty acid [41]. Differences observed in fatty acid composition of chia oil may be attributed to climatic factors and differences in cultivation locations. The linolenic/linoleic acid ratio of chia oil is higher than that reported for other vegetable oils [4, 5].

Table 3 shows the effect of extraction methods (Soxhlet and cold press) on tocopherols and tocotrienol contents of roasted and non-roasted chia seed oils. Noticeably, α -tocopherol, $\beta + \gamma$ -tocopherol and δ -tocopherols were the most abundant tocopherols in roasted and non-roasted chia oils obtained by cold press and Soxhlet extraction methods. Higher values of tocopherols and tocotrienol were seen in chia oil extracted from non-roasted seeds by both methods compared to those extracted from roasted seeds demonstrating the destruction impacts of roasting (heat) on these essential compounds. In same line with that, tocopherols

 Table 3
 Tocopherol contents

 of roasted and non-roasted
 chia seed oils extracted by two

 different extraction methods
 (mg/kg)

Tocopherols	Cold press		Soxhlet extraction			
	Roasted	Non-roasted	Roasted	Non-roasted		
α-Tocopherol	131.3±3.67*b	138.4 ± 1.89a	127.4±3.58b	$135.6 \pm 2.74a$		
$\beta + \gamma$ -Tocopherol	901.6±5.49b**	$917.3 \pm 5.54a$	$795.6 \pm 4.86b$	857.1 <u>+</u> 6.69a		
δ-Tocopherol	33.8±2.34b	$38.6 \pm 3.67a$	$24.7 \pm 1.29b$	$35.8 \pm 3.92a$		
β-Tocotrienol	0.11±0.03b	$0.18 \pm 0.03a$	0.05 ± 0.01 b	$0.09 \pm 0.03a$		
γ-Tocotrienol	$0.06 \pm 0.01 \text{b}$	$0.11 \pm 0.03a$	$0.03 \pm 0.01 \text{b}$	0.05 ± 0.03 a		

*Mean ± standard deviation

**Values in each row with different letters are significantly different (p < 0.05)

and tocotrienol of oil extracted from roasted and non-roasted chia seeds by cold press approach were higher compared to those extracted with Soxhlet method. These findings suggested that heating (roasting and Soxhlet extraction) reduced the tocopherols and tocotrienol contents of chia seed oil. As seen in Table 3, α -tocopherol, $\beta + \gamma$ -tocopherol and δ -tocopherols were the most abundant tocopherols in roasted and non-roasted chia oils obtained by cold press and Soxhlet extraction methods. While α -tocopherol contents of roasted and non-roasted chia oils extracted by cold press and Soxhlet systems ranged between 131.3 and 138.4 mg/ kg, α-tocopherol contents of chia oils extracted by Soxhlet system varied between 127.4 and 135.6 mg/kg, respectively. In addition, $\beta + \gamma$ -tocopherols contents of roasted and nonroasted chia oils extracted by cold press and Soxhlet systems ranged between 901.6 and 917.3 mg/kg to 795.6 and 857.1 mg/kg, respectively. Also, the highest δ -tocopherol content was found in non-roasted chia oils obtained by cold press (38.6 mg/kg), followed by non-roasted Soxhlet oil (35.8 mg/kg) and roasted cold pressed oil (33.8 mg/kg). The highest levels of β - and γ -tocotrienol contents was obtained in oil extracted from non-roasted chia seeds by cold press (0.83 and 0.67 mg/kg, respectively). It was observed statistically differences among the tocopherol contents of roasted and non-roasted chia seeds oils obtained by cold press and Soxhlet extraction systems (p < 0.05). Dobrowski et al. [18] reported that chia oil contains low levels of carotenoids, while high amounts of tocopherols were observed. Amato et al. [2] found carotenes in oil, as well as tocopherols, with y-tocopherol as the main component, followed by α -and δ-tocopherol. According to Ixtaina et al. [13], tocopherol content of chia seed oils ranged between 238 and 427 mg/ kg, with γ -tocopherol (>85%) and δ -tocopherol accounting for majority, while varying levels (0.4–9.9 mg/kg) of α tocopherol were obtained. Tocopherols values obtained for chia oils in this present study compared favourably with the values reported for peanut oil (398.6 mg/kg), but lower than the values reported for flaxseed (588.5 mg/kg), sunflower (634.4 mg/kg) and soybean (1797.6 mg/kg) oils [42]. The result showed that tocopherol composition of solvent extracted oil was significantly (p < 0.05) higher than the 653

cold press extracted oil. High levels of tocopherols in oil are related to the PUFA content [42].

Phenolic compounds of roasted and non-roasted chia oils as affected by extraction methods are shown in Table 4. Both system oils are rich in rosmarinic, chlorogenic, caffeic acids. The most abundant compound in chia seed oil among all compounds and throughout all treatments is rosmarinic. With exception to rosmarinic of chia oil extracted by Soxhlet method, values of all phenolic compounds were higher in oil extracted from non-roasted seeds by both methods compared to those of roasted seeds demonstrating negative impacts of roasting of chia seeds prior to oil extraction on the phenolic composition of the chia seed oil. Regardless of roasting treatments, the values of phenolic compounds in oil extracted with Soxhlet method were higher than that of oil extracted by cold press suggesting the enhancing potentials of Soxhlet method on the phenolic profile of chia seeds oil. These findings tend to suggest that combing non-roasting of chia seeds and oil extraction with Soxhlet method could improve the phenolic compounds in extracted chia seed oils. While the rosmarinic acid contents of roasted and non-roasted chia oils obtained by cold press vary between 1.28 and 2.17 mg/g, rosmarinic acid contents of roasted and non-roasted chia oils obtained by Soxhlet system changed between 2.92 and 2.67 mg/g, respectively. In addition, roasted and non-roasted chia oils obtained by Soxhlet extraction system contained 0.21 and 0.28 mg/g caffeic, 0.07 and 0.09 mg/g cinnamic, 0.20 and 0.23 mg/g chlorogenic acid, 0.14 and 0.17 mg/g quercetin, 0.17 and 0.21 mg/g kaempferol, 0.11 and 0.14 mg/g rutin, 0.96 and 1.08 mg/g genistein, 2.92 and 2.67 mg/g rosmarinic and 0.41 and 0.84 mg/g myrcetin. Generally, chia seed oils from Soxhlet extraction had higher phenolic components than cold press oils. Also, phenolic compounds of non-roasted chia oils obtained by both extraction methods were found higher when compared to roasted chia oils. Coelho and de las Mercedes Salas-Mellado [37] reported that chia seed extract contain 4.68 µg/g cinnamic acid, 30.89 µg/g caffeic, 0.17 µg/g quercetin. Chia seed extracts contained 0.0307-0.509 mg/ mL kaempferol and 0.379-0.881 mg/mL caffeic acids, 0.125-0.268 mg/mL quercetin, 0.301-0.509 mg/mL kaempferol and 0.379–0.881 mg/mL total phenols [9]. The results Table 4Phenolic compoundsof roasted and non-roastedchia seed oils extracted by twodifferent extraction methods(mg/g)

Phenolics	Cold press		Soxhlet extraction			
	Roasted	Non-roasted	Roasted	Non-roasted $0.07 \pm 0.03a$		
Gallic acid	0.02 ± 0.01 ab*	$0.03 \pm 0.01a$	0.05 ± 0.03 b			
(+)-Catechin	$0.11 \pm 0.05b^{**}$	$0.15 \pm 0.03a$	$0.13 \pm 0.05 b$	$0.21 \pm 0.03a$		
Syringic	$0.04 \pm 0.01 b$	$0.07 \pm 0.03a$	$0.11 \pm 0.01b$	$0.18 \pm 0.07a$		
Cinnamic acid	0.03 ± 0.01 ab	$0.04 \pm 0.01a$	0.07 ± 0.01 ab	$0.09 \pm 0.03a$		
Caffeic acid	$0.03 \pm 0.01 \text{b}$	$0.05 \pm 0.01a$	0.09 ± 0.03	0.11 ± 0.05		
Quercetin	$0.05 \pm 0.01 \text{b}$	$0.09 \pm 0.03a$	$0.14 \pm 0.03b$	$0.17 \pm 0.07a$		
Kaempherol	$0.09 \pm 0.03b$	0.13±0.01a	$0.17 \pm 0.05 b$	$0.21 \pm 0.09a$		
Rutin	0.03 ± 0.01 ab	$0.04 \pm 0.01a$	0.06 ± 0.03 ab	$0.08 \pm 0.05a$		
Genistein	$0.71 \pm 0.13b$	$0.88 \pm 0.09a$	$0.96 \pm 0.15b$	$1.08 \pm 0.17a$		
Rosmarinic	$1.28 \pm 0.67a$	2.17 ± 0.54 ab	$2.92 \pm 0.28b$	$2.67 \pm 0.32a$		
Myrcetin	$0.23 \pm 0.07b$	$0.35 \pm 0.09a$	$0.41 \pm 0.11b$	$0.84 \pm 0.09a$		
Isorhamnetin	$0.14 \pm 0.03b$	$0.17 \pm 0.05a$	$0.23 \pm 0.07b$	$0.31 \pm 0.05a$		

*Mean ± standard deviation

**Values in each row with different letters are significantly different (p < 0.05)

also showed that chia oils contained myrcetin, quercetin, caffeic and chlorogenic acid in oil from both extraction methods. Chlorogenic and caffeic acids were the most abundant phenolic compounds in chia oil, while myrcetin, quercetin and kaempherol are found in minor fractions. It was observed statistically differences among the phenolic compounds of roasted and non-roasted chia seed oils obtained by cold press and Soxhlet extraction systems (p < 0.05). Chia seed oil comprises of many antioxidants such as tocopherol, phytosterol and carotene [16] and phenolic compounds such as chlorogenic and caffeic acid [7, 14], which have many health-promoting properties and protect people from various diseases [43, 44]. Ayerza and Emir [31] reported that Tzotzol and Iztac Chia genotypes contained 0.115 and 0.121 mg/g myrcetin, 0.007 and 0.006 mg/g quercetin, 0.025 and 0.024 mg/g kaempherol, 0.226 and 0.218 mg/g chlorogenic acid and 0.139 and 0.149 mg/g caffeic acid, respectively. The result obtained in this present study differs from those in literature. The physicochemical properties, fatty acid compositions, tocopherol contents and phenolic compounds of roasted chia seed oils were high due to the heat treatment applied to the unroasted seed oils. In addition, the physico-chemical properties, fatty acid compositions, tocopherol contents and phenolic compounds of both roasted and non-roasted oils obtained by the Soxhlet extraction system were lower than the cold press. This increase may be due to heat treatment and solvent applied during the exfoliation.

Conclusion

This study showed the effect of roasting and extraction systems for all chemical properties, antioxidant activity, total phenol, fatty acids, tocopherol contents and phenolic compounds. The peroxide value shows that natural antioxidants that have been identified such as γ -tocopherol, protects the oxidation of omega-3 fatty acids. The shelf life, oil content and phytochemicals present in chia oil depend on the extraction method used. The presence of Omega-3 fatty acid has relevance because of the numerous benefits associated with its consumption. Among tocopherols, α -tocopherol, $\beta + \gamma$ -tocopherol and δ -tocopherols were the most abundant tocopherols in roasted and non-roasted chia oils extracted by cold press and Soxhlet system. Tocopherol contents of roasted and non-roasted chia oils obtained by cold press were found higher as compared to chia oils extracted by Soxhlet system. Roasting and extraction methods significantly affected the micro constituents and quality of the oil. The result obtained from this present study demonstrate that chia oil could promote good health and protect human body against many non-infectious disesases due to the presence of many health-promoting phytochemicals.

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