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Research Full Papers

Mineral composition, bioactive compounds and antioxidant activity of *Salvia hispanica* L as affected by thermal and non-thermal treatments

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Abstract

In this study, the effect of thermal treatments (roasting and autoclave) and non-thermal treatments (soaking and germination) on total phenolic content, total flavonoid content, antioxidant activity and bioavailability of minerals of chia seed was evaluated. Results showed thermal treatments increased the total phenolic content in samples such that the total phenolic content increased from 0.95± 0.1 mg (GAE/g) (control sample) to 1.32± 0.12 mg (GAE/g) (roasted sample) and 1.11± 0.1 mg (GAE/g) (autoclaved sample). Soaking reduced the total phenolic content in samples while germination increased the amount of total phenolic content in the samples. Using the roasting treatment had no significant impact on the total flavonoid content of samples, while using the autoclave, soaking and germination treatments reduced the total flavonoid content of samples. Roasting and autoclaving increased the antioxidant activity of samples while soaking reduces the amount of antioxidant activity among the samples and germination had no considerable effect on the antioxidant activity of samples. Moreover, germination treatment increased the macro and micro elements of minerals in samples. Thermal treatment (roasting) had no significant impact on the amount of minerals and only increased the Fe^{2+} in samples. FTIR Spectra showed thermal treatment reduced the amount of polysaccharide (1740 -1750 cm^{-1}) and protein /lipid (2800-3000 cm^{-1}) in samples.

Keywords: Chia seed; Total phenolic content; Total flavonoid content; FTIR.

Introduction

Chia seed, scientifically called *Salvia hispanica* L, is a one-year-old plant belonging to the *Lamiaceae* family which grows naturally in the Central America. Chia seed is widely used in breakfast cereal, cookies snacks, juices, cakes and yoghurt all over the world including Canada, Chile, Australia, New Zealand and Mexico(Amato *et al*. 2015). Chia seed has a lot of antioxidant compounds like chlorogenic acid, caffeic acid, myricetin, quercetin and minerals such as calcium, magnesium, potassium and iron (Barreto *et al*. 2016; Mohd Ali *et* al. 2012; Ullah *et al*. 2016). It is many years that thermal treatments such as autoclave, roasting ad microwave as well as the non-thermal treatments such as germination and soaking are used to improve the performance and nutritional properties of grains(Gómez-Favela *et al*. 2017; Yadav *et* al. 2018). Roasting is a high temperature (150– 400^0C) and short-term process which plays a critical role in creating the color, desirable taste and flavor as well as improving the nutritional value of grains together with various chemical reactions (Chandrasekara and Shahidi 2011). Jannat et al. (2010) investigated the impact of roasting conditions on the antioxidant properties and total phenol in 8 varieties of sesame. Results of this study revealed that as the roasting temperature increases, the antioxidant properties and total phenol increase in samples.

Soaking is a preliminary stage before cooking which makes the texture soft and reduces the cooking time (Xu and Chang, 2008; Yadav *et al*., 2018). While the germination is an inexpensive process starting with water absorption and ending with rooting out. During the germination process, the metabolism

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activities leads to hydrolysis of protein, carbohydrate and synthesis and aggregation of metabolites which improve the nutritional properties (Gómez-Favela *et al*., 2017). Chinma *et al.* (2015) investigated the impact of germination on the nutritional properties of rice flour. Results of this study revealed that germination for 48 (h) increases the amount of protein, magnesium, phosphorus, potassium and antioxidant properties and increases the phytic acid and total starch of rice flour (Chinma *et al.*, 2015).

According to conducted studies, there has been no research on the effects of thermal and non-thermal treatment on Chia seeds. Therefore, the present research investigated the effects of thermal treatment (autoclave and roasting) and non-thermal (germination and soaking) on the physicochemical properties of Chia seed.

Material and methods

Chia seed Argentinean was purchased from the local farmer's market in Mazandaran province of Iran. For the analytical test, chemicals were purchased from Merck, Darmstadt, Germany.

Roasting

The electric oven was used to study the impact of temperature (165°C) and time (30 min) of roasting on physicochemical properties of Chia seed.

Autoclave

Distilled water was added to chia seed in the ratio of 1:10(w/v) and autoclaved for 20 min at 121ºC and 15 psi. Then, the samples were dried by freeze- dried.

Soaking

At ambient temperature, chia seed were soaked in the water (1:4 (w/v)) for 12 h, and the excess water was removed. Then, the samples were freeze dried.

Germination

For sterilization, chia seed was immersed in a sodium hypochlorite solution (5% (w/v)) in the ratio of 1:10 (w/v) for 2 min and then, washed the chia seeds twice with distilled water. The chia seeds were germinated for 7 days at 25ºC until sprouting formed. Then, the samples were dried by freeze dried.

Before performing the test, a laboratory mill was used to mill raw chia seed and chia seed then were treated (autoclave, roasting, germination and soaking) and sieved to get average particle size of < 250 µm.

Total phenolic content

At ambient temperature, 0.2 (g) defatted sample was incorporated with 4 ml ethanol: water: HCl (80: 19: 1 ratio) for 2 h. Then, the mixture was centrifuged at 2000 (g) for 15 min. 0.2 supernatant was incorporated with of folin ciocalteous reagent and sodium carbonate (10%). At a wavelength of 760 nm, the absorption of samples was evaluated. The total phenolic content in the samples was expressed on the basis of Gallic acid (mg GAE/g) (Jogihalli *et al*., 2017).

Phenolic compounds

The HPLC (smart line, Knauer, Germany) equipped with PDA detector was used to analyze separate phenolic compounds in the chia seed. The acetic acid (5%) in water at flow rate of 1ml/ min was used as mobile phase. The injection volume was 20µl at 30ºC. The absorbance of the samples was measured at 330 nm.

DPPH Assay

At ambient temperature, 1(g) sample was incorporated with 10 ml ethanol: water: HCl (80: 19: 1 ratio) for 2 h. The mixture was placed at 40 (°C) for 4 h. For 20 min, the mixture was centrifuged at 3000×g. The DPPH was added to supernatant and the absorption of samples was evaluated at a wavelength of 515 nm (Beta *et al*., 2005; Yu and Nanguet 2013) . The percentage of scavenging radical was calculated as followed (Eq.1)

$$\text{Scavenging radical (\%)} = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \quad (1)$$

Total Flavonoid content

1.25 ml of distilled water was incorporated with 0.25 ml of sample and 75 µl of 5% (w/v) sodium nitrite ($NaNO_2$). Then, 0.15 ml of 10% (w/v) aluminum trichloride solution, 0.5 ml 1M NaOH and 0.775 ml of distilled water was incorporated to mixture. At a wavelength of 510 nm, the absorption of samples was evaluated. The total flavonoid content in the samples was expressed on the basis of quercetin (g/Kg EQ dry sample) (Jogihalli *et al*., 2017).

Mineral composition

1 (g) of samples were heated at 200ºC for complete carbonization of the material. The samples were placed in a furnace at 550ºC for 5 h. After cooling the samples, 1 ml of nitric acid was added to them. After reagent removing, the samples were placed in the furnace at 550ºC to obtain white ashes. Then, 1 ml of HCL and 2 ml of MilliQ water ware incorporated to the ashes. To help solubilization, the samples were hated at 80ºC. An optical emission spectrometer via inductively coupled plasma (ICP-OES) (Perkin Elmer, Optima 8300) was used to evaluate macro and micro minerals (Barreto *et al.*, 2016).

FTIR

FTIR (Tensor, Burker, Germany) was used to investigate the effect of thermal and non-thermal treatment on chemical changes on chia seed in the range of 400 to 4000 cm^{-1} wavenumber (López *et al.*, 2018).

Statistical analysis

Experiments were evaluated based on a completely randomized design. To compare the means and investigating the impacts of treatments, Duncan Multiple Range test was utilized. During all stages of statistical analysis, SPSS 19 was used for analysing the data. At least three repetitions were performed for each experiment.

Results and Discussions:

Total phenolic content

Impact of thermal treatment on the total phenol of chia seed is shown in table 1. Using the thermal treatments such as roasting and autoclave increases the total phenolic content in samples such that the total phenolic content increased from 0.95± 0.1 mg (GAE/g) (control sample) to 1.32± 0.12 mg (GAE/g) (roasted sample) and 1.11± 0.1 mg (GAE/g) (autoclaved sample). Applying the thermal treatment, due to breaking of the cellular matrix and better bonding of phenol compounds with pectin and cellular network and aggregation in seed shell increase the total phenol in samples (Chandrasekara and Shahidi, 2011). Table 1 shows the effect of non-thermal treatments on total phenolic content of samples. Soaking reduced the total phenol content in samples. After soaking, due to the water absorption by the seeds, phenolic compounds are transferred from seed to water due to leaching leading to the decreased total phenol content in samples. On the basis of results (Table 1), germination increased the phenol compounds in samples. During the germination process, due to the change in activity of enzymes involving in phenol compound synthesis as well as breaking of phenol compounds connections, the total phenolic content in samples is increased. The amount of change in phenol compounds depends on the cultivar type, culturing conditions, culturing time duration and extraction method (López-Amorós *et al.*, 2006; Cáceres *et al*., 2014). Chandrasekara and Shahidi (2011) reported that roasting (130^0C for 33 min.) increases the amount of total phenol compounds in peanut. Xu and Chen (2008) reported that soaking process reduces the amount of total phenol compounds in pea and lentil grains.

Total Flavonoid content

Impact of thermal and non-thermal treatments on the total flavonoid content of all samples is shown in table 1. Based on the results, using the roasting treatment has no significant impact on the total flavonoid content of all samples, while using the autoclave treatment reduced the total flavonoid content of all samples. Heating the seeds under pressure makes the cellular wall softer and more breaking leading to the leaching of more flavonoid compounds from the seed. Also, since most flavonoid compounds are in the shell and water soluble, using the treatments of soaking and germination due to leaching of flavonoid compounds reduced the total flavonoids content in samples (Suh *et al.*, 2017; Yadav *et al*., 2018).

Antioxidant activity

The effect of thermal treatment on the antioxidant activity of chia seed is shown in table 1. Using the thermal treatments such as roasting and autoclaving increases the antioxidant activity of samples. Due to the thermal activities, phenol compounds, especially Tannins, form the insoluble complex with proteins in grain as a result of which, the phenol compounds remain inside the grain. Because of water evaporation during the process, concentration of phenol compounds in seed shell increases resulting to the increased antioxidant activity of samples. On the other hand, during the roasting process, the Maillard reaction and formation of melanoidins can increase the samples' antioxidant activity more (Perrone *et al*., 2012). Based on the results (Table 1), soaking reduces the amount of antioxidant activity among the samples which is likely due to the reduction of phenol compounds by leaching in samples, while the germination did not affect significantly the antioxidant activity of samples.

Table 1. Effect of thermal and non-thermal treatment of total phenolic content, total flavonoid content and antioxidant activity

Mineral contents	Control	Roasting	Germinate
Ca	22173± 80.34^{b}	22214± 65.2^{b}	28368± 90.7^{a}
Na	395±19.2^{b}	388±20.7^{b}	5028± 85.4^{a}
Cu	23±2.4^{a}	29± 2.8^{a}	27± 1.1^{a}
P	5201± 68.7^{a}	5003± 87.1^{a}	4940± 92.8^{a}
Fe	690± 10.58^{b}	816± 20.52^{a}	721± 18.97^{b}
Zn	63±1.4^{b}	60± 2.1^{b}	76± 2.7^{a}

Means followed by the same letters in columns, are not significantly different ($p<0.05$).

Mineral content

The effect of thermal (roasting) and non-thermal (germination) treatments on bioavailability of minerals was investigated. Results (Table 2) showed that the germination treatment has increased the macro and micro elements of minerals in samples. The increase of minerals is likely due to the increase in activity of phytate enzyme resulting in the hydrolysis of phytic acid. As an anti-nutritional factor, the phytic acid plays a critical role in chelating the minerals leading to the formation of insoluble complex. While, during the germination process, phytic acid is converted into the Inositol and Orthophosphate anions due to the activity of phytate enzyme. This causes the release of minerals (Sharma *et al.*, 2017); while the thermal treatment (roasting) had no significant impact on the amount of minerals and only increased the Fe^{2+} in samples.

Table 2. Effect of thermal and non-thermal treatment on the macro and micro elements of minerals (mg/100g) of chia seed

Treatment	Total phenolic content	Total flavonoid content	Antioxidant activity (%)
Control	0.95± 0.1^{c}	0.73± 0.07^{a}	15.38± 1.1^{c}
Roasting	1.32± 0.15^{a}	0.74± 0.05^{a}	26.90± 1.7^{a}
Soaking	0.5± 0.08^{d}	0.265± 0.01^{d}	6.28± 0.9^{d}
Germinate	1.13± 0.14^{b}	0.536± 0.08^{c}	15.37± 1.5^{c}
Autoclave	1.11± 0.1^{b}	0.668± 0.04^{b}	18.67± 1.2^{b}

Means followed by the same letters in rows are not significantly different ($p<0.05$).

FTIR

The following figures show the most important peaks of the FTIR spectrum in control, germination and roasted samples. Based on obtained data, the FTIR spectrum can be classified into 6 groups: 1) 3200-3600 cm^{-1},

2) 2800-3000 cm^{-1}, 3) 1800-2200 cm^{-1}, 4) 1600-1800 cm^{-1}, 5) 1500-1600cm^{-1}, and 6) 1200-1300 cm^{-1} (Fig 1) (Tulukcu *et al.*, 2019).

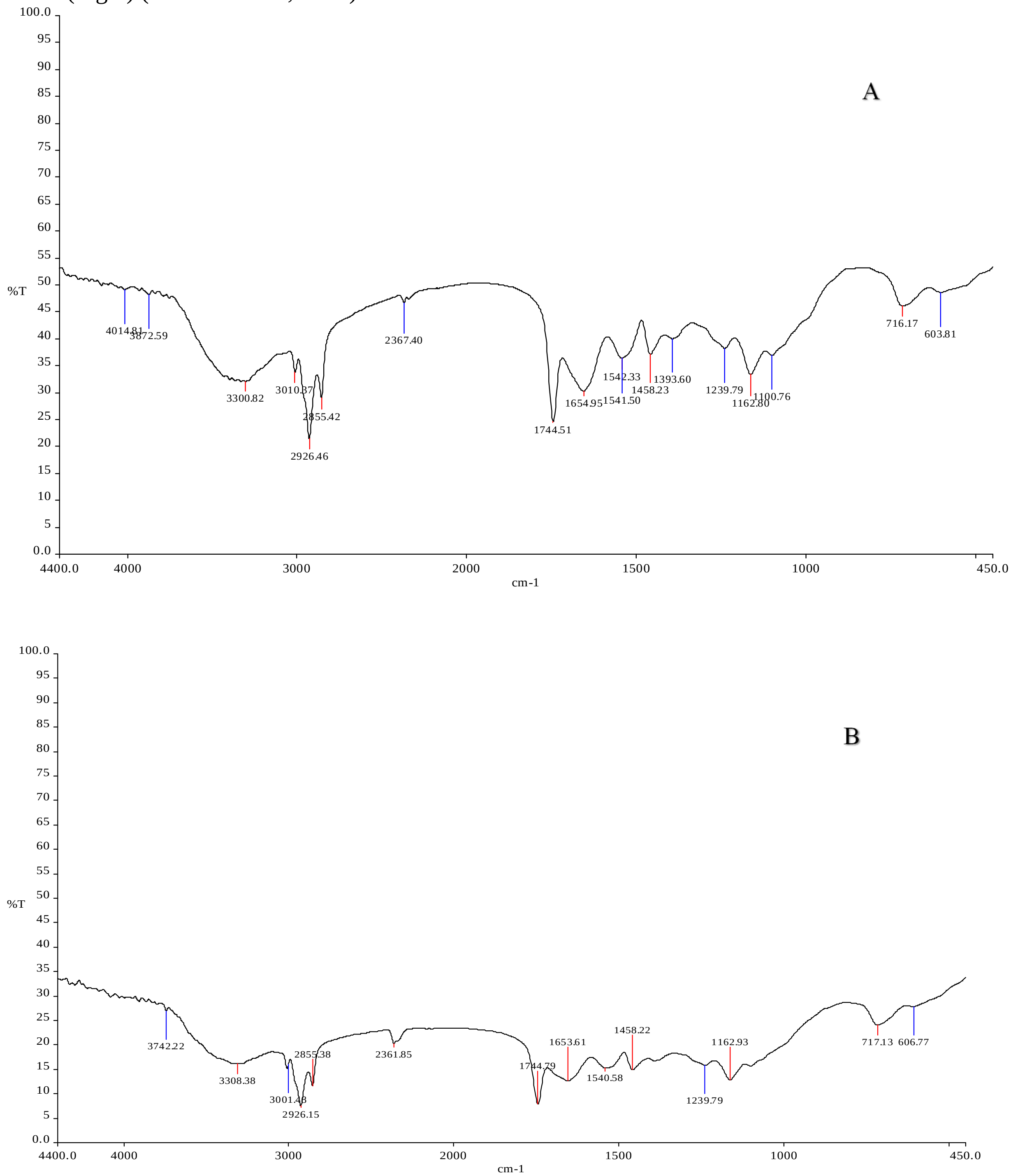

Fig 1. FTIR of (A) raw and (B) roasted of chia seed

Wave number range of 3200- 3600 cm^{-1} was due to hydroxyl groups (OH) of phenols and available N-H in amines II. The existence of peaks in the wave number range of 2800- 3000 cm^{-1} indicates the characteristic of stretching C-H bonding in Methyl groups. A peak with a centrality of 1748 cm^{-1} indicates the presence of stretching C=O bonding (Carbonyl) in esters of lipids and fatty acids in the sample. The existence of a peak in the range of 1220-1800 cm^{-1} indicates the stretching C=O bonding (Amide I) in samples. A peak in the range of 1500- 1600 cm^{-1} indicates the presence of stretching C=O bonding (Carboxylic group) probably due to the presence of Uronic acid in the sample structure. The observed peak in 1246 cm^{-1} is probably because of amide III in the structure. According to the comparison of FTIR Spectra of control and roasted samples, roasting reduced the amount of polysaccharide (1740 -1750 cm-1) and protein/ lipid (2800-3000 cm-1) in samples. However, the intensity and sharpness of peaks increased in 1500-1600 cm-1 and 1200- 1300 cm-1 indicating the effect of roasting on amounts of amides, amino acids, aldehydes and esters. The creation of melanoidins during the roasting process is probably the most important reason for changes in amounts and intensity of peaks in the FTIR spectrum.

Conclusion

This study evaluated different treatments on antioxidant and nutritional properties of chia seed. The results showed roasting improves functional properties (such as total phenolic content, antioxidant activity) of the chia seed. So, the modified chia seed is rich in total phenolic content and antioxidant activity and relieve good functional characterizes that could be used in food formulation like bread, sponge cake, muffins and etc.

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تأثیر تیمار حرارتی و غیرحرارتی بر میزان مواد معدنی، ترکیبات فعال زیستی و فعالیت آنتی‌اکسیدانی دانه چیا (*Salvia hispanica* L)

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چکیده

در این پژوهش، اثر تیمارهای حرارتی (برشته کردن و اتوکلاو) و تیمارهای غیرحرارتی (خیساندن و جوانه‌زنی) بر میزان فنل کل، مقدار فلاونوئید کل، فعالیت آنتی‌اکسیدانی و میزان مواد معدنی دانه چیا بررسی شد. نتایج نشان داد که تیمارهای حرارتی باعث افزایش میزان فنل کل در نمونه ها شد به‌طوری که میزان فنل کل در نمونه‌ها از (mg (GAE/g 0/1 ±0/95 در نمونه شاهد تا mg (GAE/g) 0/12 ±1/32 در نمونه برشته شده و mg (GAE/g) 0/1± 1/11 در نمونه اتوکلاو شده، افزایش یافت. خیساندن سبب کاهش میزان ترکیبات فنلی کل در نمونه‌ها شد در حالی که جوانه زدن باعث افزایش میزان ترکیبات فنلی کل در نمونه‌ها شد. برشته کردن اثر معناداری بر میزان ترکیبات فلاونوئید کل در نمونه داشت در حالی که اتوکلاو کردن، خیساندن و جوانه‌زنی سبب کاهش میزان ترکیبات فلاونوئید کل در نمونه‌ها شد. تیمارهای حرارتی (برشته کردن و اتوکلاو کردن) سبب افزایش فعالیت آنتی‌اکسیدانی در نمونه‌ها شد در حالی که خیساندن سبب کاهش میزان فعالیت آنتی‌اکسیدانی نمونه ها شد و جوانه‌زنی اثر معناداری بر میزان فعالیت آنتی‌اکسیدانی نمونه‌ها نداشت. جوانه‌زنی باعث افزایش میزان مواد معدنی در نمونه‌ها شد در حالی که تیمار حرارتی (برشته کردن) اثر معناداری بر میزان مواد معدنی نداشت و تنها میزان آهن در نمونه‌ها افزایش یافت. طیف به‌دست آمده از FTIR نشان داد، تیمار حرارتی سبب کاهش مقدار پلی‌ساکارید ($1740-1750\ cm^{-1}$) و نسبت پروتئین/ لیپید ($2800-3000\ cm^{-1}$) در نمونه‌ها شد.

واژه‌های کلیدی: دانه چیا، میزان فنل کل، میزان فلانوئید کل، FTIR

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