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Improvement of Gluten-Free Sponge Cake with A Combination of Chia Seed Flour and Millet Flour

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ABSTRACT

Background: Celiac disease is an autoimmune disorder in which the patient is permanently intolerant to proteins containing gluten, and the only treatment is to use a gluten-free diet. The present study is designed to investigate physicochemical, antioxidant activity, textural, and sensory properties attributes of developed gluten-free sponge cake containing chia seed (*Salvia hispanica L.*) flour (CHF) along with millet (*Panicum miliaceum*) and corn flour. **Methods:** The cake was produced using CHF (4, 8, 12, 16%; w/w) with the replacement of millet flour (MF), and 50% corn flour (CF) was used equally in all the samples. **Results:** Applied CHF significantly increased moisture, ash, protein, and fat content of gluten-free cakes ($P<0.05$). Replacement of CHF with MF reduced specific volume (using colza grains) and porosity (using image processing method) by 64% and 76%, respectively. Texture analysis showed an increase in hardness in all the three-storage times with the addition of CHF. Color measurement indicated that CHF significantly reduced the amount of a*, and L* values of the crusts. Radical scavenging activity by DPPH was improved in a dose-dependent manner ($P<0.05$). The lowest amount of CHF in cake formula had the highest score in sensory evaluation with verbal hedonic scale. **Conclusion:** Based on the results, the samples containing 4% CHF, 46% MF, and 50% CF had the most desirable texture and palatability with reasonable radical scavenging activity among developed gluten-free sponge cakes.

Introduction

Celiac disease is an autoimmune disease that occurs in people allergic to the gluten protein found in some grains, and a gluten-free diet is approved as the most important medical therapy in improvements in symptomatology and small bowel histology (Pietzak and Kerner Jr, 2012). A gluten-free diet is one of approved therapies for these patients. The protein of gluten is made up of two peptides, prolamin (gliadin) and glutenin (glutelin) which plays a key role in the quality of baking products such as elasticity, water, and gas holding capacity, and also textural acceptance of confectionaries, especially cakes (Ortolan and Steel, 2017, Wilderjans *et al.*, 2008). Thus, optimizing the formula of gluten-free products for these patients has been a concern for current research.

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Millets contain nutritious ingredients such as various vitamins, minerals, proteins, fiber, and suitable fats, which are classified as a gluten-free grain that can be used without any worry for gluten-related disorders (Sruthi and Rao, 2021). Previously, several studies have used millet flour in gluten-free bakeries (Jyotsna *et al.*, 2016, Omran and Mahgoub, 2022). Moreover, chia is recognized as a valuable seed due to its nutritional components such as protein, vitamins, and minerals (Muñoz *et al.*, 2013). Having primary and synergistic natural antioxidants has made chia seeds unique (Ayerza and Coates, 2001). The seed also contains a considerable amount of insoluble fiber which has the ability to be absorbed several times its weight in water, and provide significant health benefits to the human being (Muñoz *et al.*, 2013). In addition to the above mentioned nutritional values, the seed is a natural source of omega-3 fatty acids, which is almost 75% of the total oil content of chia (Ixtaina *et al.*, 2011); the health benefits of these fatty acids were presented in a 2012 study (Jin *et al.*, 2012). Chia seeds have many medicinal effects; the crude chia mucilage affects the growth of some intestinal bacterial groups, such as *Enterococcus spp* and *Lactobacillus spp* (Tamargo *et al.*, 2018). In addition, chia seeds may be effective in glucose intolerance (Guevara-Cruz *et al.*, 2012), dyslipidemia (de Souza Ferreira *et al.*, 2015), and hypertension (Toscano *et al.*, 2014). Regarding their functional characteristics in food science, they are hydrophobic and have a mucilage state, and supplementation of cookies with chia seeds mucilage create an acceptable texture, color, and mouthfeel, hence the overall quality score of the products (Punia and Dhull, 2019). In a gluten-free layer cake, replacing 10% of pre-hydrated chia seed flour with whole flour showed the same overall acceptability, texture, taste, and aroma as the layer cake prepared with 100% rice flour and 100% wheat flour (Sung *et al.*, 2020). Some other studies also demonstrated that CHF can be successfully used in gluten-free bakery products (Silav-Tuzlu and Tacer-Caba, 2021, Tomić *et al.*, 2022). Considering the nutritional and health benefits of chia seed flour and its capacity to improve the texture of gluten-free products, the present study is designed to assess the effect of chia seed flour along with millet and corn flour on physicochemical (specific volume, crust color, proximate principals), antioxidant activity, textural (porosity, and hardness), and sensory characteristics of experimental gluten-free sponge cake.

Materials and Methods

Chia seeds (*Salvia hispanica L.)* and millet seeds (*Panicum miliaceum*) were obtained from the local market in Tehran, Iran. The seeds were ground by a coffee grinder into whole chia and millet flour. Ready corn flour was purchased from the local market (Natco Foods Ltd, Tehran, Iran). The nutritional characteristics of the flour are shown in **Table 1**.

Preparation of the experimental gluten-free sponge cake

Five types of samples were prepared in this study based on different millet/chia flour ratios (**Table 2**). The basic ingredients of control gluten-free cake (CGF) were elaborated using the following formulation: 28.4% corn and millet flour (50%+50%), 22% sugar, 17.5% sunflower oil, 22% whole eggs, 0.5% baking powder, 0.15% vanilla, 0.61% dried milk powder, and 7.61 boiled water. Egg yolk and sugar were initially mixed, and mixing procedure was continued for 4 min to achieve a creamy mixture. Other ingredients including vanilla, milk powder, oil, flours, and the rest were added, and mixed gently at low speed for 3 min to obtain a homogenous batter. Based on the determined formula, batters were prepared and batter portions of 30 g were dosed into paper mold (50 mm diameter × 35 mm high). The molds were placed on baking tray and baked in oven (Akhavan Industry, Iran) at 210 °C for 23 min. Baked sponge cakes were left to cool at room temperature, and after 1 hour, they were packed in a clip-on polyethylene bags and analyzed at the day of preparation (Mehraban Shendi *et al.*, 2017).

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Proximate Analysis

The moisture, protein, lipid, and ash contents of gluten-free cakes were determined by the following AACC methods: 44-15, 46-13, 30-25, and 08-01, respectively (American Association of Cereal Chemists (AACC), 2000).

Table1. Nutritional characteristics of chia, millet, and corn flour (g/100g).

Nutrient	Chia flour	Millet flour	Corn flour
Protein	16.54	10.75	7.11
Carbohyrate	42.12	73.05	79.45
Fiber	34.40	3.50	3.90
Fat	17.00	4.25	1.75
Energy(Kcal)	486	373	370

Table 2. Experimental design of gluten-free sponge cake.

Type of cake	Millet flour (%)	Chia seed flour (%)
CGF	50	0
CHF4	46	4
CHF8	42	8
CHF12	38	12
CHF16	34	16

*Corn flour was used equally in the amount of 50% in control and treatment groups;**CFG**: Control gluten-free cake; **CHF**: Chia seed flour*

Physicochemical characteristics

Gluten-free cakes were weighed within one hour after baking, volumes of cakes were measured using colza grains, and specific volumes were calculated (Gómez *et al.*, 2007). The color of crust of experimental cakes was evaluated using a Hunter Lab Color Flex (Hunter Associates Laboratory, Inc, Reston, VA, USA). The results were expressed under CIELAB system. CIELAB is a color space used to describe and quantify color. The parameters determined were L*(L* = 0 (black) and L*=100 (white)), a*(-a*=greenness and +a*=redness), and b*(-b*=blueness and +b*=yellowness). The parameters determined were L*(L* = 0 (black) and L*=100 (white)), a*(-a*=greenness and +a*=redness), and b*(-b*=blueness and +b*=yellowness). The porosity of the sponge cakes was measured by image processing method; cross sections of bisected cakes were imaged using a Sharp MX-M453N scanner with a resolution of 600 pixels. The image was then delivered to Image J software. By enabling the 8-bit segment, gray images were created. After that, the binary part of the software converted gray images to binary images. An index porosity of sample cakes was defined by the ratio of light to dark spots in images. The higher this ratio, the higher the numbers of pores and porosity (Sahraiyan *et al.*, 2013). The texture properties of samples were evaluated on day 1, day 3, and day 5. Hardness measurements were done by a texture analyzer, Hounsfield, Germany. Furthermore, a 5 cm diameter cylindrical probe with a velocity of 2 mm/s was used. The cake sample had a 3 cm thickness under the probe of the machine, and 80% overlapped with the probe. Finally, the amount of displacement and force was measured by computer software connected to the machine (Ayoubi, 2018).

Antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured to determine the antioxidant properties of gluten-free cakes. First, a 0.1- ml methanolic solution containing between 0.1 and 0.5 mg of the crude extract or its fractions was mixed with 2 ml of deionized water and then added to a test tube containing a methanolic solution of DPPH (0.25 ml). It was allowed to react in a dark room for 20 min. The absorbance at 517 nm was measured using a spectrophotometer (U-2000; Hitachi, Tokyo, Japan). A standard was also measured as a control using the same extract conditions except for the use of methanol instead of a cake extract (Amarowicz *et al.*, 1996). The DPPH radical scavenging activity was calculated as:

DPPH radical scavenging activity (%)=[(cake extract absorbance at 517 nm)-(control absorbance at 517 nm)/(control absorbance at 517 nm)]×100

All analyses were performed in triplicate.

Sensory evaluation

The sensory evaluation was carried out by a verbal hedonic scale consisting of five points (1: disliked extremely, 2: dislike, 3: neither like nor

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dislike, 4: like slightly, 5: like extremely) (Beikzadeh *et al.*, 2017). The panelists consisted of 15 semi-trained individuals from the laboratory staff (20-45 years old). Random three-digit numbers blinded samples. Panelists first evaluated the first sample; then, for each evaluation, they rinsed their mouths with water to minimize any residual effect. The acceptability of softness and hardness, porosity, color, flavor, appearance, and overall, acceptability were evaluated for each sample.

Data analysis

Data were analyzed by statistical software SPSS, version 25. The values were presented as mean ± standard deviation. The significance of differences among means of treatment groups was determined by one-way variance analysis using Duncan Post Hoc with P-value<0.05 set as a significant point.

Results

Chemical, nutritional, and physical characteristics of gluten-free cakes are presented in **Table 3**. Increasing the amount of CHF in cake formulation replacing millet flour raised the sample`s moisture and ash contents significantly ($P<0.05$). Fat and protein contents were similarly increased by adding CHF to 20.4% and 51.8%, respectively. The specific volume of the cake is the volume per unit weight of the cake sample. The specific volume of the cakes ranged from 3.05 to 4.64 cm3/g; CGF presented the highest value, significantly different from the others, and the replacement of CHF with MF caused a significant decline in cake volumes.

The results of the analysis of crust color (**Table 3**) have shown that parameters of L*, a* and b* for the crust of the control samples were significantly ($P<0.05$) higher compared to the chia seed supplemented cakes, and CHF16 had the lowest color values compared with the other samples. The addition of CHF significantly affected the porosity of cake crumbs ($P<0.05$); 12% and 16% replacements of CHF significantly lowered the porosity of gluten-free cakes compared to the other samples.

Antioxidant activities of gluten-free cakes were significantly increased via the replacement of CHF with millet flour; the highest value belonged to CHF16 (93.19±2.29%) based on **Table 3**.

Table 3. Proximate analysis and physical properties of gluten-free sponge cakes.

Variable	CGF	CHF4	CHF8	CHF12	CHF16
Moisture (%)	8.10±0.10^{c}	10.25±0.25^{b}	10.35±0.04^{bc}	10.65±0.22^{a}	10.48±0.22^{ab}
Ash (%)	1.77±0.17^{c}	1.84±0.11^{c}	2.43±0.47^{b}	2.81±0.37^{ab}	3.28±0.19^{a}
Fat (%)	24.82±0.11^{a}	24.94±0.05^{a}	27.39±1.16^{b}	28.09±0. 48^{b}	37.68±0.27^{c}
Protein (%)	6.02±0.07^{a}	6.36±0.16^{b}	6.52±0.12^{b}	6.55±0.15^{b}	7.25±0.10^{c}
Specific volume (cm^3/g)	4.64 ±0.04^{e}	2.63±0.03^{d}	2.22±0.02^{c}	1.71±0.01^{b}	1.65±0.04^{a}
a*	10.03±0.18^{a}	7.75±0.29^{b}	7.66±0.29^{b}	7.09±0.74^{bc}	6.66±0.28^{c}
b*	32.65±0.55^{a}	27.70±0.20^{b}	26.71±0.31^{c}	24.64±0.36^{d}	22.82±0.63^{e}
L*	57.42±0.2^{a}	55.47±0.31^{b}	51.76±0.75^{b}	51.49±1.04^{c}	48.60±0.39^{c}
Porosity (%)	13.25±1.13^{d}	6.23±1.32^{c}	5.72±0.23^{b}	3.90±0.36^{a}	3.23±0.58^{a}
Antioxidant activity (%)	28.76±0.28^{e}	43.75±0.20^{d}	50.77±0.01^{c}	89.10±0.17^{b}	93.19±2.29^{a}

CFG*: Control gluten-free cake;* ***CHF****: chia seed flour; Values are presented as mean±standard deviation. Data in the same row sharing a common lowercase letter are not significant ($P< 0.05$) by Duncan`s test.*

The three-day texture analysis of gluten-free cakes is shown in **Table 4**. As presented, CGF had the lowest force on days 1, 3 and 5 compared to other treatment cakes, and forces increased through the addition of CHF and the reduction of MF. Moreover, the hardness of all treatment samples increased significantly during the storage time due to dehydration and retrogradation reactions of the starch. These differences in the first, third, and fifth days were significant for the control sample, but for most samples containing chia seed flour, these differences were not

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significant in the second and third days.

The average values of panel perceptions of gluten-free sponge cakes prepared with CHF, MF, and CF were evaluated for their color, aroma and taste, softness and hardness in the mouth, porosity, appearance and overall acceptability using a 5-point Hedonic scale. Based on the results (**Table 5**), the highest score relates to control samples with corn and millet flour. For the other treatments, the best score in the overall acceptance belongs to the sample containing 4% CHF, 46% MF, and 50% CF which has the highest score in color, aroma and taste, porosity and appearance.

Table 4. Texture hardness of gluten-free sponge cake.

Type of cake	Texture hardness (N)		
	Day 1	Day 3	Day 5
CGF	27.16±1.04^{eC}	53.38±6.03^{dB}	76.74±2.26^{cA}
CHF4	52.83±1.35^{dB}	76.79±3.39^{cA}	81.33±1.22^{cA}
CHF8	65.13±4.09^{cB}	83.24±4.59^{cA}	129.25±29.25^{bA}
CHF12	131.83±2.53^{bB}	157.37±5.05^{bA}	182.73±2.94^{aA}
CHF16	197.13±2.80^{aC}	254.75±15.25^{aB}	271.25± 11.10^{aA}

***N**: Newton; **CFG**: Control gluten-free cake; **CHF**: Chia seed flour. Values are presented as mean±standard deviation. Data in the same column sharing a common lowercase letter are not significantly different, and data in the same row with a similar uppercase letter are not significantly different (P< 0.05) by Duncan`s test.*

.**Table 5.** Sensory properties of gluten-free sponge cake.

Sensory property	CGF	CHF4	CHF8	CHF12	CHF16
Color	4.69±0.10^{a}	3.24±0.00^{c}	3.42±0.00^{b}	2.71±0.00^{e}	3.14±0.00^{d}
Aroma and taste	3.71±0.00^{a}	3.57±0.00^{b}	3.28±0.00^{d}	3.42±0.00^{c}	3.42±0.00^{c}
Softness and hardness	3.57±0.00^{a}	3.28±0.00^{b}	3.28±0.00^{b}	3.28±0.00^{b}	3.28±0.00^{b}
Porosity	4.00±0.00^{a}	3.71±0.00^{b}	3.71±0.00^{b}	3.42±0.00^{c}	3.00±0.00^{d}
Shape	4.14±0.00^{a}	3.57±0.00^{b}	3.28±0.00^{d}	3.42±0.00^{c}	3.14±0.00^{e}
Overall quality	3.75± 0.55^{a}	3.66±0.64^{b}	3.48±0.67^{c}	3.39±0.71^{d}	3.50±0.67^{c}

***CFG**: Control gluten-free cake; **CHF**: Chia seed flour. Values are presented as mean±standard deviation. Data in the same row sharing a common lowercase letter are not significantly different (P< 0.05) by Duncan`s test.*

Discussion

The results of this study indicated that gluten-free cakes prepared with CHF in the highest concentrations had the highest amount of ash, moisture, fat, and protein contents among other samples. The addition of CHF significantly enhanced antioxidant activity. However, the findings pointed out that the best sample of the texture and appearance of cakes is using the least amount of chia seed flour. Likewise, the best score for sensory evaluation and overall acceptance belonged to adding 4% chia seed flour. Increasing moisture content is highly correlated to the fiber content of chia seeds with water-holding capacity. Similar to the results of this study, the presence of chia flour in the formulation of flour products leads to their freshness and prevents water removal during product storage (Rendón-Villalobos *et al.*, 2018). Chia seeds also have a high amount of oil, including α-linolenic acid (64.39% of total oil), linoleic acid (21.46%), and less than 10% of saturated fat (Timilsena *et al.*, 2017), which can well describe the rise in fat contents of gluten-free cakes enriched with CHF. Sponge cakes containing CHF as an egg-replacer had higher unsaturated fatty acids (including ω-3, ω-6) (Aljobair, 2022). Previous studies on chia-supplemented products similarly observed that ash and fat contents increased with adding chia flour (Levent, 2017, Pizarro *et al.*, 2015, Pizarro *et al.*, 2013). Adding

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CHF significantly increased the amount of protein in cake samples based on the high protein content of chia seeds (around 24 g/100g) (Felisberto *et al.*, 2015).

Regarding the specific volume, it was also Sung *et al.* found that chia seed flour could not restore volumes of gluten-free layer cake unless rehydrated chia seed flour were added (Sung *et al.*, 2020). Since the amount of chia flour is high in cake formulation, the decrease in the specific volume of cake samples through increasing the amount of chia flour might relate to increase in the thickness of air cell wall in the cake dough. The condensation might also have been related to the high fiber contents, which caused more water absorption. Overall, the thickness of the air bubbles' walls increased so that they could not expand well at oven temperature, so the volume did not improve. In the case of the color of the cakes, the decrease of lightness (L*) was first because of the color of chia seeds. Chia seed flour is darker than corn flour; the presence of more minerals and natural pigments justifies the dark color of the cake containing chia flour (Steffolani *et al.*, 2014). The reduction can then be explained by increasing the amount of protein and following Maillard reaction in the cakes by adding percentages of CHF (Felisberto *et al.*, 2015); during baking, the temperature of the cake surface reaches the temperature of Millard and caramelization reactions, so the brightness of the cake surfaces will decrease. Consistent with the results of this study, Felisberto *et al.* showed that L* and b* of the pound cake prepared with chia mucilage decreased (Felisberto *et al.*, 2015). Pizarro *et al.* also demonstrated similar results of color values by adding chia seed flour *(Pizarro et al., 2013)*.

As mentioned earlier, the porosity of cakes decreased by the addition of CHF. Higher fiber in samples containing chia flour probably reduces the possibility of producing carbon dioxide gas during baking, which contributes to the lower porosity of the samples containing CHF. Similar to the study's results, a weak structure was reported in cakes containing chia seed flour (Bulegon *et al.*, 2022).

Chia seed is well recognized for its naturally potent antioxidant activity due to the high content of phenolic compounds, flavonoid contents, chlorogenic acid, caffic acid, myristin, camphor, tocopherols, phytosterols and carotenoids (Scapin *et al.*, 2016, Steffolani *et al.*, 2014). The antioxidant activity of gluten-free noodles similarly was increased by higher concentrations of chia seeds flour (Levent, 2017).

The difference in tissue hardness during storage time can be explained by better water retention due to more fiber in samples. The differences of chia seed flour samples on the third and fifth days were related to starch retrogradation. MF itself decreased the hardness of gluten-free products (Azarbad *et al.*, 2019), so the substituting CHF with MF resulted in an increase in the hardness of gluten-free cakes. Changes in cake hardness were negatively correlated with specific volume, so applying the high amount of chia seed flour in the current work reduced the specific volume and increased the hardness of the samples. These changes might also be related to the lack of gluten network formation, hence the reduction of specific volume and porosity in the gluten-free samples. Aljobair *et al.* similarly showed that the hardness of the samples increased in a dose-dependent manner through the addition of CHF *(Aljobair, 2022)*.

Conclusion

Replacing millet grain flour with chia flour in gluten-free sponge cake resulted in an increase in ash, moisture, fat, and protein content, and improved the sponge cake's antioxidant activity. In contrast, the quality of gluten-free sponge cake could not be improved by replacing millet flour with chia seeds, as the color, texture, and sensory acceptability did not change positively. Based on the nutritional value of chia seed and findings from current work, the authors suggest the application of low concentrations of chia seed flour in formulations for gluten-free fortified cake samples. This is because even a 4% replacement enhances antioxidant activity by 50% in comparison with the control sample. These results

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represent a promising use of chia flour as a functional ingredient in manufacturing gluten-free products. Further studies regarding texture improvement and sensory acceptability with a higher percentage of chia seed flour and applying chia seed by-products in gluten-free cake formulation are recommended.

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Authors' contributions

Saberi A, Mojani Qomi MS and Zolfaghari MS designed the research; Saberi A conducted the research; Mojani Qomi MS analyzed the data, wrote the paper, and was responsible for the final content. All the authors read and approved the final manuscript.

Conflic of interest

There are no conflicts of interest.

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