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Preparation and quality characteristics of gluten-free sponge cake using alfalfa seed (*Medicago sativa* L.) flour

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ABSTRACT

Celiac disease is an autoimmune disorder in which the patient is reacted to the protein gluten. Lifelong adherence to a strict gluten-free diet seems to be an effective treatment. The flour made from gluten-free legumes such as alfalfa (*Medicago sativa L.*) seeds can be used in bakery products. In this study, alfalfa seed flour (ASF) was incorporated into the formulation of a gluten-free (GF) sponge cake, then the quality, nutritional, and sensory characteristics were evaluated. Rice flour (RF) and corn flour (CF) were replaced in equal proportions with four different ratios of ASF (2.5, 5, 7.5, and 10%). The findings indicated that substituting with ASF led to a significant reduction in the specific volume and porosity of the gluten-free (GF) samples. On the contrary, by increasing the amount of ASF, the hardness of the GF cakes increased ($p < 0.05$). Replacing the flours with ASF led to a significant increase in moisture, protein, and ash ($p < 0.05$). The amount of carbohydrates in the final product was reduced by 22%, and there were no significant changes in the fat content ($p > 0.05$). The addition of ASF resulted in a significant decrease in the L* value, while the a* and b* values for both the cake's crumb and crust increased ($p < 0.05$). The iron and calcium contents exhibited a linear increase by ASF substitution ($p < 0.05$). The Sensory properties were maintained by 2.5 and 5% ASF. To attain the overall quality and sensory properties, the replacement of a small amount of ASF in GF sponge cake is recommended.

Keywords: Alfalfa seed; Gluten-free sponge cake; Mineral; Texture

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1. Introduction

Cereals play a significant role in the diets of people in developing countries due to their economic value as a food group (Laskowski et al., 2019). Among various cereal-based products, cakes are particularly popular, and those of high quality are predominantly made from wheat (Delcour et al., 2012). In light of the sensitivity to gluten in inflammatory diseases, particularly celiac disease, the production of gluten-free products has gained significant attention worldwide (Jnawali et al., 2016). The exclusive means of managing autoimmune gastrointestinal disorders and the consequent inflammation in individuals with celiac disease is by adhering to a gluten-free diet (Arendt & Dal Bello, 2011). Alfalfa (*Medicago sativa L.*) is an agricultural product belonging to the Leguminosae family and is one of the most valuable forage legumes in the world, which can be used in the formulation of cereal-based bakery products (such as cakes, biscuits, and bread) to improve the nutritional quality. Alfalfa seeds are a rich source of protein (about 30.1%) with an average of 7.4% moisture, 3.6% fat, 6.9% fiber, 3.9% ash, and 48.1% free-nitrogen extract (Ullah et al., 2016). Higher concentrations of polyunsaturated fatty acids (PUFAs), especially essential fatty acids such as linoleic acid (omega-6 or 18: 2C) and linolenic acid (omega-3 or 18: 3C) along with higher levels of riboflavin, vitamin E, and vitamin C and iron have been reported in alfalfa grain compared to wheat grain (Márton et al., 2010). Alfalfa seed demonstrates a robust ability to scavenge radicals (Zagórska-Dziok et al., 2020), and the phenolic content of whole alfalfa seed is comparable to green tea extract, rosemary, and grape seed (Yanishlieva & Heinonen, 2001). Previous studies have shown that alfalfa seed powder and its extract had the potential to mitigate hyperglycemia and dyslipidemia (Mölgaard et al., 2006; Farsani et al., 2016). The antimicrobial evaluation of alfalfa extract against

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different bacterial strains revealed that it exhibited the highest level of inhibition against gram-positive bacteria. (Joy & George, 2014).

It seems there are only a limited number of studies on the potential application of ASF in cereal-based products. Giuberti et al. (2018) found that raising the substitution levels of rice flour with ASF could enhance nutritional value, hardness, total phenolic content, in vitro antioxidant capacity, and the resistant starch of gluten-free rice cookies ($p < 0.05$), whereas the starch hydrolysis index decreased linearly ($p < 0.05$). To contribute to the existing literature and emphasize the significance of a gluten-free diet for individuals with celiac disease, this study aimed to assess the feasibility of producing gluten-free sponge cake by substituting rice and corn flour with ASF at varying levels. Newly developed samples were then compared with the control sample for changes in technological quality, textural, nutritional, and sensory properties.

2. Material and Methods

2.1. Raw material

In order to conduct this study, the commercial RF and CF (Golha Food Industries, Tehran, Iran) were purchased from the local market in Tehran. Alfalfa seeds were purchased from an online organic market (Attarak Online Ltd) and subsequently ground in a laboratory mill fitted with a 1-mm screen (Retsch grinder model ZM1; Brinkman Instruments, Rexdale, ON, Canada).

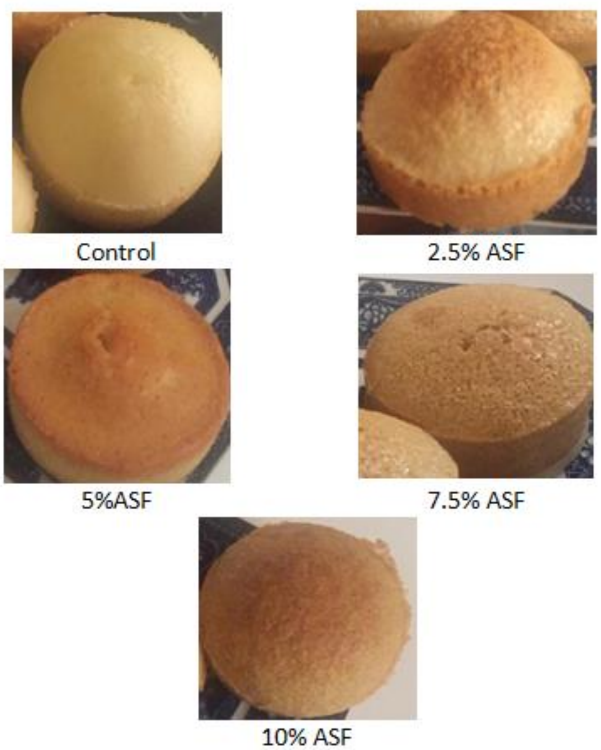

Fig. 1. Example pictures of the appearance of experimental gluten-free sponge cakes with alfalfa seed flour. ASF: Alfalfa seed flour.

2.2. Sponge cake formulation

The cake recipe used in this study was adapted from Drabińska et al. (2018). After making certain adjustments, the gluten-free control sample sponge cake was prepared using a combination of RF (79.7%) and CF (20.3%). Additional ingredients included 69.8% whole egg, 22.7% sugar, 6% sunflower oil, 0.3% salt, 0.5% xanthan gum, and 1% baking powder. For the experimental GF sponge cakes, RF and CF were equally substituted with the ASF in four different ratios (2.5, 5, 7.5, and 10% w/w). Following the preparation of the dough formula, batters were dosed into paper molds (50 mm diameter x 35 mm high), and baked in a household oven (Feller, KM600, China) at a temperature of 180±4°C for 30 min. After baking, the gluten-free cakes were allowed to cool and were then stored individually in airtight plastic bags at room temperature until they were ready for analysis. To ensure consistency, three separate batches were produced for each treatment on the same day.

2.3. Qualitative analysis

2.3.1. Specific volume

The specific volume of the produced cakes was determined by the rapeseed substitution method and compared with the control sample. The samples used had the same weights and were prepared from the geometric center of the cakes (Sahin & Sumnu, 2006). A specific volume was then calculated as the ratio of volume and weight (Gómez et al., 2007).

2.3.2. Color measurement

Image J graphics software (National Institute of Health, Bethesda, MD) was used to measure the color of the test specimens. A 2×2 cm slice of the cake was separated. Photography equipment included a digital camera (Canon, SX230 HS, Japan) and a wooden box. A 12-watt fluorescent lamp (Master, made in China) was used to provide the light needed for photography. After preparing the required photos, the photos were transferred to a computer and uploaded to the relevant software to measure color indicators. Three color components including L^* (brightness index), a^* (redness index), and b^* (yellowness index) were measured for crumbs and crusts (Mokhtarian et al., 2017).

2.3.3. Porosity

To measure the porosity of samples, Image J graphic software (National Institute of Health, Bethesda, MD) was used via imaging from the center of the cakes (Sahin & Sumnu, 2006). For this purpose, a cut of 2×2 cm size was prepared from the inside part of the cake. An index porosity of sample cakes was defined by the ratio of light to dark spots in images. The higher this ratio shows the higher the number of pores and porosity.

2.3.4. Texture evaluation

Textural characteristics of cakes were measured with a Texture Analyzer (Stable Microsystems, TAXT-2i Texture Analyzer, Godalming, Surrey, UK) (Gómez et al., 2007). A 5-cm diameter cylindrical aluminum probe with a velocity of 2 mm/s was used. A cake piece sample measuring 3×3×3 cm was carefully taken from the center of each treatment and positioned on the probe, with an overlap of up to 80%. A time interval of the 30 s between the first and the second compression was considered to determine the hardness, springiness, chewiness, and cohesiveness.

2.3.5. Proximate analysis and mineral content

Moisture, fat, protein, and ash levels of the experimental samples were determined according to AOAC (1980). Following the determination of moisture (M), fat (F), protein (P), and ash (A) percentages, the carbohydrate content was calculated using the following equation:

$$C\ (\%) = 100 - (M + P + A + F) \quad (1)$$

Atomic Absorption Spectrophotometer (AAS Vario6, Analytik Jena AG, Germany) was recruited to measure the iron and calcium contents of the samples. The cake was converted into ash in a muffle furnace. Then, 15 mL of 3 N HCl was added to the crucible. The crucible containing ash material and HCl was boiled, and the volume reached 2–3 mL. Next, it was made up to 50 mL in a volumetric flask, and mineral content was estimated by the atomic absorption spectrophotometer by flame mode and results were expressed as mg/100g of the sample (Gupta et al., 2009).

2.4. Sensory evaluation

Sensory evaluation of gluten-free sponge cake was performed by a 21-member group of the panelist (staff laboratory members and students, 50% males, in the age range of 22 to 40 years old). The members had previous experience in the sensory evaluation of bakery products. All the evaluations were performed on a five-point hedonic scale (0-dislike, 5-like). Questionnaires were prepared and distributed among the assessment team. For each question, 5 options were considered as answers: (1: disliked extremely, 2: dislike, 3: neither like nor dislike, 4: like slightly, 5: like extremely) (Beikzadeh et al., 2017). Approximately 30 g of each formula which was previously labeled with three-digit codes was offered to each member of the panelist. Then, the evaluated sensory characteristics (including aroma, taste, crust color, texture, and overall acceptability) were recorded in the relevant questionnaire.

2.5. Statistical analysis

Statistical analysis of data was performed using factorial experiment in a completely randomized design. Mean data were compared using Duncan's post hoc test at a 95% confidence level. SAS software version 1/3/9 was used for the statistical analysis. All qualitative tests were conducted with three replicates to ensure the reliability and consistency of the results.

3. Results and Discussion

3.1. Quality and nutritional evaluations

The qualitative and nutritional properties of GF cakes are presented in Table 1. Specific volume and porosity are two important physical parameters that have a significant impact on the acceptability of the market cakes. The higher of these two indices indicate the better quality of technology and baking process, hence the popularity of the product (Sahin & Sumnu, 2006). However, by increasing ASF concentrations, both the specific volume and porosity of the experimental cakes were decreased. In fact, the highest ratio of ASF led to reductions of approximately 35% and 13% in the specific volume and porosity respectively, compared to the control group.

The decline in the specific volume of samples that underwent ASF replacement can be attributed to the elevated protein content of ASF in comparison to CF and RF (30% versus 6% and 7%) (Altındağ et al., 2015; Ullah et al., 2016). The gluten-free and non-elastic nature of ASF's protein content results in reduced specific volume and porosity. Our results indicated lower specific volume and porosity compared with regular cakes containing wheat flour in other studies (Baeva et al., 2003; Jahanbakhshi & Ansari, 2020). Ultimately, it is clear that incorporating ASF did not effectively address the absence of a gluten network.

The hardness of the cakes varied between 9.07 and 16.61 N. The inclusion of ASF resulted in a notable increase in hardness. This rise in the hardness of cereal products appears to be associated with the dietary fiber content present in the ingredients. (Ktenioudaki & Gallagher, 2012). In addition to higher protein content, ASF contains a greater amount of crude fiber compared to corn and rice flour (Altındağ et al., 2015; Ullah et al., 2016). Meanwhile, the decrease in specific volume and porosity had an impact on the hardness of gluten-free ASF-containing cakes. Lower cohesiveness and higher springiness were observed, which were associated with larger cake volumes.

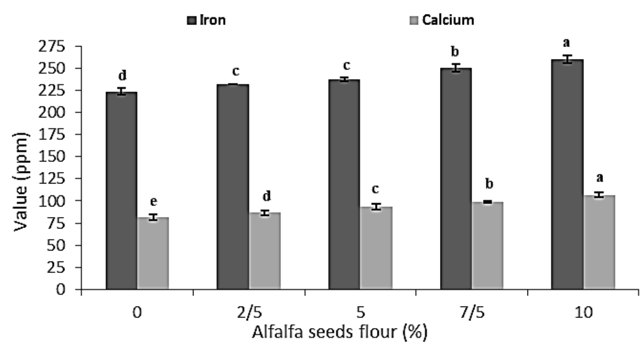

Fig. 2. The effect of alfalfa seed flour on mineral contents of gluten-free cake. Different lower cases letters above same color error bars represent statistically significant difference ($p < 0.05$).

The results of the analysis of variance of the effect of ASF replacement on the color parameters (L^* a^* b^*) of the crusts and crumb of gluten-free cakes showed that the L^* of both were significantly reduced through the addition of ASF, and the control sample had the highest value compared with the experimental cakes. Whereas, the two other parameters (a^* and b^*) had significant raise with increasing ASF concentration. Changes in color parameters in our study were in line with previous findings by Giuberti et al. (2018). The decrease in crumb and crust L* (lightness) with the addition of ASF could be attributed to the pigments found in alfalfa seeds, as suggested by Zouari et al. (2016). This is likely due to the significant carotenoid content in alfalfa seeds, which imparts a slightly yellowish color (resulting in an increase in the b* value) (Agrahar-Murugkar et al., 2018). The presence of reducing sugars and amino acids from alfalfa seed powder in the cake sample led to non-enzymatic browning through the Maillard reaction during cooking. This, in turn, resulted in the darkening of the color of the samples, as evidenced by the increase in the* value (Mancebo et al., 2015). The increase in the redness of the cake crusts can be attributed to both caramelization and the higher protein content achieved through the addition of ASF. Caramelization is known to contribute to the development of red tones in baked products (Bozdogan et al., 2019). The general appearance of gluten-free sponge cakes is demonstrated in Fig. 1.

The substitution of RF and CF with ASF resulted in a linear increase in the total protein and ash content of the cakes ($p < 0.01$). Conversely, the amounts of carbohydrates in the sponge cakes showed decreasing trends. These alterations were related to the nature of chemical compounds of different flours and their replacement in their recipes. Similar results have been reported by Ullah et al. (2016) in ASF-supplemented wheat flour cookies. The moisture content of gluten-free cakes changed similarly with the replacement with ASF. The incorporation of ASF had a significant positive impact on the moisture content of the cakes ($p < 0.01$). Among the samples, those containing 10% alfalfa seed flour exhibited the highest moisture content. The high fiber content present in alfalfa seeds helps retain moisture within the cake texture, preventing excessive evaporation of moisture during the baking process (Jaldani et al., 2017). There was no significant difference in the fat content between the control and treatment samples, as the fat amounts in all three flours were relatively similar, ranging from 1.5% to 2.5% (Altındağ et al., 2015; Ullah et al., 2016).

The results of changes in mineral contents of gluten-free cakes are presented in Fig. 2. The gradual substitution of RF and CF with ASF resulted in increased levels of iron and calcium in the products. Specifically, gluten-free cakes containing 10% ASF exhibited approximately 16.2% higher iron content and 30.8% higher calcium content compared to the control samples. This indicates that incorporating ASF into the cakes can be an effective way to enhance the iron and calcium nutritional profile of the final product.

3.2. Sensory evaluation

The sensory evaluation of gluten-free sponge cakes was determined by a 5-point hedonic scale (Table 2). In all cases, gluten-free cakes with 2.5% ASF obtained the highest score. However, as the percentage of ASF in the recipe increased, the rating decreased, leading to a significant decline in the scores of samples with 10% ASF ($p < 0.05$). The findings regarding the use of high quantities of ASF in this study align with previous research in the field (Ullah et al., 2016; Giuberti et al., 2018). The color of ASF, its distinctive beany flavor, and subsequent bitter-feeling taste was responsible for less acceptability by the panelists. Therefore, the lowest ratios of ASF (2.5% and 5%) in gluten-free sponge cakes had the highest scores among other experimental cakes, and their ratings were similar to or even better than those of control samples.

Table 1. The effect of alfalfa seed flour on the qualitative properties and the gluten-free sponge cakes.

	Treatments				
	Control	2.5% ASF	5% ASF	7.5% ASF	10% ASF
Specific Volume (cm^3/g)	1.99 ± 0.14^{a}	1.77 ± 0.07^{b}	1.55 ± 0.04^{c}	1.39 ± 0.03^{d}	1.29 ± 0.03^{d}
Porosity (%)	21.89 ± 1.61^{a}	20.68 ± 0.87^{ab}	20.19 ± 1.32^{ab}	19.59 ± 0.99^{b}	18.95 ± 0.64^{b}
Hardness (N)	9.07 ± 1.76^{c}	12.76 ± 0.61^{b}	13.01 ± 0.14^{b}	15.09 ± 1.36^{ab}	16.61 ± 1.85^{a}
Cohesiveness	0.62 ± 0.01^{a}	0.57 ± 0.00^{b}	0.57 ± 0.02^{b}	0.54 ± 0.06^{c}	0.54 ± 0.00^{c}
Springiness	0.95 ± 0.00^{a}	0.91 ± 0.01^{b}	0.91 ± 0.05^{b}	0.89 ± 0.00^{bc}	0.86 ± 0.01^{c}
Chewiness	4.30 ± 0.01^{c}	4.90 ± 0.03^{bc}	5.10 ± 0.01^{b}	6.10 ± 0.00^{ab}	6.30 ± 0.02^{a}
Crust color					
L*	56.35 ± 0.42^{a}	55.13 ± 0.97^{ab}	54.24 ± 1.49^{b}	52.20 ± 0.97^{c}	50.54 ± 0.95^{c}
a*	8.13 ± 0.31^{d}	9.22 ± 0.14^{c}	10.32 ± 0.21^{b}	12.07 ± 0.77^{a}	12.99 ± 0.91^{a}
b*	10.42 ± 0.41^{d}	16.31 ± 0.98^{c}	19.60 ± 0.71^{b}	22.66 ± 0.43^{a}	22.63 ± 2.07^{a}
Crumb color					
L*	66.42 ± 0.17^{a}	63.39 ± 1.08^{b}	60.84 ± 0.30^{c}	60.08 ± 0.34^{cd}	58.92 ± 1.13^{d}
a*	-2.47 ± 0.64^{c}	-0.42 ± 0.86^{b}	1.37 ± 1.01^{a}	1.41 ± 0.71^{a}	2.25 ± 0.39^{a}
b*	4.15 ± 0.98^{d}	6.28 ± 0.92^{c}	7.73 ± 0.58^{bc}	9.32 ± 1.18^{b}	13.19 ± 0.60^{a}
Proximate analysis					
Moisture (%)	26.04 ± 0.07^{e}	27.11 ± 0.26^{d}	28.38 ± 0.39^{c}	29.29 ± 0.51^{b}	30.10 ± 0.58^{a}
Carbohydrate (%)	30.04 ± 0.06^{a}	28.41 ± 0.05^{b}	26.45 ± 0.12^{c}	24.79 ± 0.11^{d}	23.47 ± 0.17^{e}
Protein (%)	11.88 ± 0.12^{e}	12.41 ± 0.19^{d}	12.98 ± 0.00^{c}	13.53 ± 0.06^{b}	14.05 ± 0.14^{a}
Ash (%)	1.05 ± 0.01^{e}	1.09 ± 0.01^{d}	1.14 ± 0.01^{c}	1.27 ± 0.01^{b}	1.29 ± 0.01^{a}
Fat (%)	30.99 ± 0.32^{a}	30.98 ± 0.08^{a}	31.05 ± 0.03^{a}	31.12 ± 0.04^{a}	31.09 ± 0.19^{a}

ASF: alfalfa seed flour; data are presented as mean ± standard deviation. Data in the same column sharing a lowercase common letter are not significantly different ($p < 0.05$).

Table 2. Effects of alfalfa seed flour on sensory characteristic of gluten free sponge cakes.

	Treatments				
	Control	2.5% ASF	5% ASF	7.5% ASF	10% ASF
Aroma	4.67±0.57^{a}	5.00±0.00^{a}	4.00±1.00^{ab}	3.00±1.00^{b}	3.05±0.89^{b}
Taste	4.00±1.00^{ab}	4.67±0.57^{a}	4.33±0.57^{ab}	3.67±0.57^{ab}	3.37±1.00^{b}
Color	3.67±0.57^{ab}	4.67±0.57^{a}	4.33±0.57^{a}	3.67±0.57^{ab}	3.00±1.06^{b}
Texture (mouth fell)	3.67±0.57^{ab}	4.33±1.15^{a}	4.00±1.00^{a}	3.67±0.59^{ab}	3.19±1.05^{b}
Overall Quality	4.00±0.66^{ab}	4.67±0.5^{a}	4.17±0.78^{ab}	3.50±0.66^{ab}	3.15±1.00^{b}

ASF: alfalfa seed flour; data are presented as mean ± standard deviation. Data in the same column sharing a lowercase common letter are not significantly different ($p < 0.05$).

4. Conclusion

In line with global interest in gluten-free food formulas, the present study formulated and evaluated new recipes in the bakery industry. To develop a gluten-free sponge cake that is both nutritious and appealing, various combinations of rice, corn, and alfalfa seed flour were investigated in terms of quality, nutritional content, and sensory acceptability. Our analyses revealed that ASF-substituted sponge cakes contained higher protein, ash, iron, and calcium contents compared with the control samples. The specific volume and porosity were decreased and hardness increased through the addition of ASF. Likewise, the inclusion of ASF caused changes in the color characteristics of the experimental cakes. Both the crusts and crumbs exhibited a decrease in the lightness index (L*), while the values of a* and b* increased. The sensory evaluation indicated that among the gluten-free sponge cakes, those prepared with 2.5% and 5% incorporation of ASF were found to be more acceptable by the panelists compared to the other samples. It can be concluded that the use of ASF up to a maximum amount of 5% might represent a good combination with other flours in terms of technological quality, nutritional value, and sensory attributes.

Further studies are recommended to investigate the impact on the staleness of gluten-free cakes by assessing the texture and moisture at different intervals. This research would provide valuable insights into the long-term quality and shelf stability of such cakes. Additionally, it is recommended to conduct further research on the effects of incorporating ASF in other bakery products, focusing on their nutritional health benefits, such as antioxidant activity and increased fiber content. Exploring the potential of ASF in enhancing the nutritional profile of various baked goods would provide valuable insights into their positive impact on human health. Moreover, considering the industrial dimensions and scalability of incorporating ASF in bakery production is also suggested, as it would provide practical implications for large-scale implementation in the baking industry.

Acknowledgment

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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