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original scientific paper

Effect of Pumpkin Seed Protein Concentrate and Xanthan Gum on the Properties of Gluten-Free Frozen Batter and the Resultant Cake

Running title: Pumpkin Protein and Xanthan in Gluten-Free Frozen Batter

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SUMMARY

Research background. The increasing demand for gluten-free products has necessitated improved formulations, particularly under frozen storage conditions. Although freezing and thawing often lead to quality deterioration, limited research has explored the role of additives in enhancing gluten-free frozen batters. This study investigates the effects of pumpkin seed protein concentrate (PSPC) and xanthan gum (XG) on the properties of gluten-free frozen batter and the resulting cake.

Experimental approach. Batter samples were formulated with varying mass fractions of PSPC (0, 10 and 20 %) and XG (0, 0.1 and 0.2 %), then frozen at $-18\text{ }^{\circ}\text{C}$ for 30 and 60 days. After thawing,

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the batters were analyzed for physicochemical properties, including viscosity and density. The baked cakes were evaluated for ash, moisture, protein content, porosity, volume, crust color, and sensory characteristics.

Results and conclusions. Increasing PSPC mass fraction significantly enhanced the batter's viscosity and density, as well as the porosity and volume of the cakes ($p < 0.05$). Higher PSPC mass fractions also led to a significant decrease in crust lightness and redness ($p < 0.05$). Xanthan gum positively influenced most quality attributes, although it significantly increased cake hardness ($p < 0.05$). Sensory analysis showed that the formulation containing 10 % PSPC and 0.2 % XG, after 30 days of freezing, achieved the highest overall quality and consumer acceptance ($p < 0.05$).

Novelty and scientific contribution. The positive effect of PSPC and XG on the properties of gluten-free frozen batter and the resulting cakes was confirmed in the study. These findings contribute to the optimization of gluten-free formulations that could be used to develop high-quality gluten-free frozen bakery products.

Keywords: gluten-free; pumpkin seed protein concentrate; xanthan gum; frozen batter; cake quality; sensory evaluation

INTRODUCTION

The use of frozen batter in bakery production has emerged as an innovative strategy to maintain product freshness after thawing. This approach offers several benefits, including enhanced consumer satisfaction, reduced labor and equipment costs, and extended shelf life (1,2). However, freezing and thawing processes pose significant challenges to the final product quality, mainly due to the formation of large ice crystals that adversely affect texture and overall attributes (3).

To mitigate these issues, precise temperature control and advanced storage techniques are essential to minimize quality degradation during freezing and distribution (4). Previous research has demonstrated that storing batter at sub-zero temperatures significantly improves product quality compared to storage at higher temperatures. Furthermore, ingredient selection in batter formulations plays a crucial role in preserving the physicochemical and sensory properties of frozen baked goods. Additives such as hydrocolloids, emulsifiers, fats, and protein isolates can reduce freezing-induced damage, thereby enhancing texture and freshness (5–8). For instance, the incorporation of gums into frozen batter helps regulate water migration during thawing, minimizing structural damage (9).

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Hydrocolloids also delay staling, improving the quality of baked products made from frozen batter (10).

An important factor influencing frozen batter quality is the strength of the gluten network, with stronger networks typically yielding better texture and structure (11). However, the rising prevalence of celiac disease and gluten sensitivity has significantly increased demand for gluten-free bakery products. Currently, lifelong adherence to a gluten-free diet remains the only effective treatment for celiac disease (12,13). Consequently, extensive research has focused on developing high-quality, nutritionally balanced gluten-free baked goods.

To improve the functional properties of gluten-free products, gluten alternatives such as hydrocolloids and protein isolates are essential (14,15). Hydrocolloids are hydrophilic, long-chain polymers that form gels in aqueous environments, stabilizing batter and retaining gas during baking (16,17). Common hydrocolloids in gluten-free baking include hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), and xanthan gum (XG), all contributing to improved structure and volume in baked goods (18,19). XG, a heteropolysaccharide composed of repeating pentasaccharide units, is particularly valued for its high solubility and viscosity, making it an effective thickening and stabilizing agent (20).

Among gluten-free grains, rice is widely used due to its low sodium content, high digestibility, and nutritional value (21). Besides serving as a carbohydrate source, rice provides essential vitamins and is cost-effective, making it an ideal base for gluten-free formulations. Additionally, pumpkin seeds (*Cucurbita pepo*), known for their rich content of bioactive compounds and essential nutrients, have attracted attention for their health-promoting potential (22,23). These seeds contain 32–45 % protein and are rich in essential amino acids (24). The globulin fraction of pumpkin seed protein resembles that of legume proteins, highlighting its suitability as a functional and nutritious ingredient (16).

This study investigates the effects of pumpkin seed protein concentrate (PSPC), a protein-rich ingredient, and xanthan gum (XG), a hydrocolloid, on the quality of gluten-free frozen batter and cakes. Although previous studies have examined the impact of freezing on batter and cake quality, to date, no significant research has specifically addressed the role of additives such as PSPC and XG in gluten-free frozen batter. Therefore, the primary objective of this study is to fill this research gap by evaluating the physicochemical and sensory effects of these additives in gluten-free cakes made from frozen batter. The control sample consists of frozen batter without PSPC or XG. Ultimately, this study aims to develop optimized, consumer-acceptable formulations for gluten-free frozen bakery products.

MATERIALS AND METHODS

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Materials

Rice flour produced from the Tarom rice variety was purchased from a rice store in Gorgan, Golestan Province, Iran. The flour was sieved using a U.S. standard No. 80 sieve (aperture size approx. 180 μm) manufactured by Endecotts Ltd. (London, UK) to ensure uniform particle size. The sieved flour was then packed in triple-layered nylon bags and stored in a refrigerator at 4 °C. Fat-free pumpkin seed meal was obtained from Golestan Oil Factory (Gorgan, Golestan Province, Iran) and similarly packed in triple-layered nylon bags and refrigerated at 4 °C. Xanthan gum was supplied by Rhodia Food (Manchester, UK). Other ingredients, including sunflower oil (Rana Company, Tehran, Iran), powdered sugar (Golha Company, Mashhad, Iran), eggs (Telavang Company, Tehran, Iran), vanilla extract (Golha Company, Mashhad, Iran), dry milk powder (Pegah Golestan Dairy, Gorgan, Golestan Province, Iran), whey powder (Pegah Golestan Dairy, Gorgan, Golestan Province, Iran), and baking powder (Golha Company, Mashhad, Iran), were sourced from reputable domestic producers. Purified water was obtained from a water purification system (WOSON DRINK10, Jiangmen, China).

Preparation of pumpkin seed protein concentrate

Pumpkin seed protein concentrate (PSPC) was prepared according to the method of Wang *et al.* (25), with minor modifications. Defatted pumpkin seed meal was mixed with 99.8 % ethanol at a 1:10 (*m/V*) ratio and incubated at 25 °C for 20 min with periodic shaking to enhance protein extraction, using a shaker incubator (JSSI-070, JS Research Inc., Gongju, South Korea). After incubation, the mixture was centrifuged at 10 000 \times g for 15 min using a high-speed centrifuge (Combi R515, Hanil, Incheon, South Korea). The supernatant was discarded, and the precipitate was placed under a fume hood to remove any residual ethanol by evaporation. Subsequently, the precipitate was dried in a hot-air oven at 25–30 °C for approximately 24 h, ground into a fine powder, and sieved through a U.S. standard No. 80 sieve (aperture size \approx 180 μm ; Endecotts Ltd., London, UK) to obtain uniform particle size. The resulting concentrate was packed in polyethylene bags and stored at 4 °C until further use.

Preparation of gluten-free frozen batter

Batter samples were prepared using the sugar-batter method described by Binyon and Bamford (26), with slight modifications. In this process, the amount of rice flour was varied, and PSPC was incorporated at three mass fractions (0, 10 and 20 %). Additionally, XG was added at mass fractions of 0, 0.1 or 0.2 % based on the total mass of the rice flour and PSPC mixture. Initially, sunflower oil (57 g) and powdered sugar (72 g) were mixed using a medium-speed mixer for 5 min

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until a light-colored, creamy consistency was achieved. Eggs (72 g) were then gradually incorporated into the cream in three separate additions. The dry ingredients, *i.e.* flour (100 g), dry milk powder (2 g), whey powder (4 g), vanilla powder (0.5 g), and baking powder (2 g) were subsequently added to the mixture, and blending continued until a semi-smooth batter was obtained. Finally, water (30 g) was added, and mixing continued until a fully smooth and homogeneous batter was formed. The prepared batters were transferred into disposable containers, sealed tightly, and divided into two groups. The first group was stored in a freezer at $-18\text{ }^{\circ}\text{C}$ for 30 days, and the second group for 60 days.

Preparation of gluten-free cake from frozen batter

After being frozen for 30 and 60 days, the batter samples were removed from the freezer and thawed in an incubator at $30\text{ }^{\circ}\text{C}$ for 2 h. Subsequently, 50 g of each batter sample were transferred into separate cake moulds and baked at $180\text{ }^{\circ}\text{C}$ for 25 min in a conventional oven (Memmert UFP800TS, Schwabach, Germany), following the method described by Binyon and Bamford (26). After baking, the cakes were removed from the oven and allowed to cool at room temperature for 1 h. They were then packaged in polyethylene bags (Vazin Plastic, Mahdasht, Alborz, Iran) and prepared for physicochemical and sensory evaluations. All tests were performed in triplicate.

Chemical analysis

Chemical analyses were performed on rice flour, fat-free pumpkin seed meal, PSPC, and gluten-free cake prepared from frozen batter. Moisture content was determined in triplicate by drying approximately 5 g of sample in a drying oven (Memmert UFP800TS, Schwabach, Germany) at $105\text{ }^{\circ}\text{C}$ for 4 h until constant mass was achieved, following AOAC Official Method 925.09 (27). Protein content was measured in triplicate using the Kjeldahl method with a digestion apparatus (Nabertherm B150, Lilienthal, Germany). About 1 g of sample was digested with concentrated sulfuric acid and a catalyst, followed by distillation and titration of the released ammonia, according to AOAC Official Method 920.87 (27). Ash content was determined in triplicate by incinerating approx. 2 g of dried sample in a muffle furnace (Nabertherm B150, Lilienthal, Germany) at $550\text{ }^{\circ}\text{C}$ until white ash was obtained, indicating complete combustion, following AOAC Official Method 923.03 (27). All results are reported on a dry mass basis.

Viscosity and density of batter after thawing

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The viscosity of the batter sample was measured using a Brookfield rotational viscometer (Model DV-II equipped with a Helipath stand, Brookfield Engineering Laboratories, Middleboro, MA, USA). Based on the instrument manual and preliminary tests, the T-C spindle from the Helipath spindle set was selected. Viscosity measurements were recorded at a rotational speed of 20 rpm (28).

The density of batter samples was calculated by dividing the mass of a specific volume of batter samples by the mass of an equal volume of distilled water, according to standard methods (29).

Measurement of cake volume, porosity and hardness

The volume of cake samples was determined using the seed displacement method, according to AACC Method 10-05.01 (30). Initially, the volume of an empty aluminum container was measured by completely filling it with millet seeds. After baking and cooling, each cake sample was removed, and the container was refilled with millet seeds to determine the displaced volume. The difference between the initial and refilled measurements was recorded as the volume of each cake sample (30).

The porosity of the gluten-free cake crumb was evaluated 2 h after baking using a digital image processing technique (31). A 2 cm×2 cm slice of the cake crumb was scanned using an HP Scanjet G3010 scanner (Hewlett-Packard, Palo Alto, CA, USA) at a resolution of 600 dpi. The obtained image was processed using ImageJ software (31). First, the image was converted to grayscale and then binarized. The resulting binary image consisted of light and dark pixels, with the ratio of light to dark pixels used as an index of porosity. A higher ratio indicated greater porosity in the cake texture. The porosity percentage was calculated using the Analyze function in ImageJ (32).

The hardness of the cake samples, measuring 2 cm×2 cm×2 cm, was evaluated 24 h after baking using a TA.XT Plus Texture Analyzer (Stable Microsystems, Surrey, UK). A 2 cm cubic piece of the cake crumb, excluding the crust, was selected for testing. The P/36R probe (36 mm diameter) was used to compress the sample by 1 cm (50 % of its original height). Pre-test, test, and post-test speeds were set at 2, 1, and 2 mm/s, respectively. The maximum force applied during compression, recorded in Newtons, represented the sample's hardness. The testing procedure and parameters followed standard methods (33).

Evaluation of cake crust color

The color of the cake crust was evaluated using the color indices L^* , a^* , and b^* . The L^* value indicates the lightness of the sample, ranging from 0 (black) to 100 (white). The a^* value represents the color range from red (+120) to green (-120), while the b^* value covers the range from yellow

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(+120) to blue (-120). Two hours after baking, images of the crust were captured using an HP G3110 scanner (Hewlett-Packard, Palo Alto, CA, USA) at a resolution of 600 dpi (34). The images were then analyzed using ImageJ software (32).

Sensory evaluation of gluten-free cake prepared from frozen batter

The sensory evaluation of the cake samples was conducted by a panel of 15 semi-trained assessors (10 females and 5 males), aged between 24 and 29 years. All assessors were graduate students of Gorgan University of Agricultural Sciences and Natural Resources who voluntarily participated in the study. The panel was classified as "semi-trained" because participants received brief training and instructions on the evaluation procedures, including the use of a 9-point hedonic scale, but they were not professionally trained sensory analysts. The evaluation was performed in a controlled environment with standardized lighting, ambient room temperature, and absence of interfering odors or noises. Each sample was coded with a random three-digit number and presented in a randomized order to minimize bias. Water was provided for palate cleansing between samples. Assessors rated sensory attributes including appearance, aroma, texture, taste, and overall acceptability. The test followed general guidelines for hedonic testing as described by Lawless and Heymann (35).

Statistical analysis

All tests were conducted in triplicate. Data analysis was performed using a completely randomized factorial design with generalized linear models (GLM) in SAS software v. 9.1.3 (36). Significant differences among treatments were determined by one-way analysis of variance (ANOVA), and means were compared using Duncan's multiple range test at a 95 % confidence level (36).

RESULTS AND DISCUSSION

Chemical composition of rice flour, fat-free pumpkin seed meal, and PSPC

The chemical composition of rice flour, fat-free pumpkin seed meal, and PSPC, including moisture, ash, and protein content, is summarized in (Table 1). The fat-free pumpkin seed meal initially contained 48.57 % protein and 3.35 % ash. After the protein enrichment process, the protein content increased to 59.78 %, while the ash content decreased to 7.05 %. This notable increase in protein content (by 11.21 %) highlights the efficiency of the protein extraction method. Additionally, the reduction in ash content suggests the effective removal of non-protein components. The high

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protein content of PSPC makes it a promising ingredient for enhancing the nutritional profile of gluten-free formulations.

Table 1.

Moisture content of cake prepared from frozen batter

The results (Fig. 1) demonstrated that the addition of XG and PSPC significantly ($p < 0.05$) improved moisture retention in the cakes. These additives resulted in higher moisture content compared to the control samples. Furthermore, the duration of freezing negatively affected the moisture quality of the cakes, as those frozen for 30 days retained higher moisture content than those frozen for 60 days. Among the experimental samples, the highest moisture content was observed in the sample containing 20 % PSPC and 0.2 % XG. XG is widely recognized for its high water-holding capacity, which prevents the formation of large ice crystals during freezing and reduces water migration during thawing (37). Previous studies have shown that XG significantly aids moisture retention during both baking and post-baking stages, thereby extending the shelf life of frozen bakery products (38). Additionally, the incorporation of PSPC, which is rich in proteins and natural fibers, enhances the water-holding capacity and moisture retention in cakes (39).

Fig. 1.

Ash and protein content of cake prepared from frozen batter

The ash content of the cake samples was significantly influenced by the incorporation of PSPC. Specifically, increasing levels of PSPC in the formulations led to a statistically significant increase in ash content ($p < 0.05$). This increase can be attributed to the higher mineral content of pumpkin seed flour compared to rice flour. As shown previously, the ash content of PSPC is substantially higher than that of rice flour, which explains the observed increase in overall ash levels with higher PSPC substitution. Additionally, Fig. 2a clearly illustrates the upward trend in ash content corresponding to increasing mass fractions of PSPC in the cake formulations. In contrast, the addition of XG had no significant effect on the ash content ($p > 0.05$), indicating that XG does not contribute meaningfully to the mineral composition of the final product. Furthermore, no statistically significant differences were observed in ash content between samples prepared from dough stored for 30 days and those prepared from dough stored for 60 days. These findings suggest that the incorporation of pumpkin seed flour can effectively enhance the mineral content and overall nutritional profile of gluten-free baked products (40).

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According to the results shown in Fig. 2, cakes formulated with higher levels of PSPC exhibited a significant increase in protein content compared to the control samples ($p < 0.05$). This increase can be primarily attributed to the higher protein content of PSPC (59.78 %) compared to rice flour (8.20 %). Replacing rice flour with PSPC effectively enhanced the protein content of the cakes, which is particularly important for improving the nutritional profile of gluten-free products. These findings are consistent with previous studies by Voučko *et al.* (41) and Tomić *et al.* (42). On the other hand, the addition of XG did not significantly affect the protein content in this study. Additionally, the duration of dough storage under frozen conditions did not have a significant impact on the protein content of the cakes. This lack of effect may be attributed to the characteristics of the Kjeldahl method, which measures protein content based on total acids during storage. Therefore, the absence of changes in protein content after frozen dough storage could be related to the limitations of this method in detecting such structural alterations.

Fig. 2

Viscosity and density changes in batter after thawing

According to the results presented in Fig. 3a, the addition of XG significantly ($p < 0.05$) increased the viscosity of the cake batter. Similarly, PSPC also contributed to improving viscosity, and the synergistic effect of these two ingredients further enhanced this property. However, no significant difference in the viscosity of the frozen batters was observed between samples frozen for 30 and 60 days. Freezing and thawing processes generally reduce batter quality due to the physical stresses imposed on the matrix, which disrupt its ability to retain moisture. This leads to an increase in free water and, consequently, a reduction in viscosity (28,43). However, the addition of hydrocolloids such as XG significantly increases batter viscosity and prevents its reduction under freezing conditions (44). This effect is attributed to the high water-binding capacity of hydrocolloids, which compete with the batter's polymers (such as starch and proteins) to reduce water activity and prevent moisture migration (16). PSPC, with its high protein content and water-holding capacity, combined with XG, which dissolves in cold water and forms gel-like networks, effectively contributed to increasing batter viscosity (20).

Batter density plays a crucial role in air bubble retention, which directly influences the final cake volume (45). Lower batter density is generally associated with better air retention and, consequently, a higher final product volume. In this study, the effects of different levels of PSPC and XG on batter density were evaluated after 30 and 60 days of frozen storage. As shown in Fig. 3b, the addition of PSPC up to 10 % in combination with XG up to 0.2 % significantly reduced batter density

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($p < 0.05$). This reduction was more pronounced after 60 days of storage compared to 30 days. Specifically, the combination of 10 % PSPC and 0.1 % XG resulted in the lowest batter density after 60 days, indicating a stronger batter matrix, optimal viscosity, and improved air bubble retention. In contrast, increasing the PSPC level to 20 %, particularly when combined with 0.2 % XG, led to an increase in batter density. This increase is likely due to excessive viscosity, which hindered proper air incorporation during mixing, thereby reducing aeration and increasing density. The sample containing 20 % PSPC and 0.2 % XG exhibited one of the highest densities across both storage durations. Furthermore, comparison of the two storage periods revealed that batter density generally increased after 60 days. This trend may be attributed to microstructural changes in the batter caused by ice crystal formation and recrystallization during prolonged frozen storage, which could negatively impact air bubble stability. These findings are consistent with previous studies reporting that low concentrations of hydrocolloids such as XG tend to reduce batter density, while higher concentrations may have the opposite effect due to increased viscosity that interferes with air incorporation (28,46).

Fig. 3

Changes in volume, porosity and hardness of cake from frozen batter

Cake volume is a critical quality attribute that directly influences consumer acceptance of baked products (28). It reflects not only the visual appeal of the final product but also the batter's ability to retain gas during baking, thereby indicating the effectiveness of leavening and structural development processes. As shown in Fig. 4a, the incorporation of PSPC and XG generally led to a significant increase in cake volume compared to the control sample ($p < 0.05$). The highest volume was observed in the formulation containing 10 % PSPC and 0.2 % XG ($p < 0.05$), suggesting a synergistic interaction between these ingredients in enhancing batter structure and gas retention. However, this trend was not consistent across all formulations. Notably, the sample containing 20 % PSPC alone exhibited a greater volume than the sample with 10 % PSPC and 0.1 % XG ($p < 0.05$), indicating that, in certain cases, a higher PSPC mass fraction alone may be more beneficial than its combination with a low level of XG. In contrast, the formulation containing 20 % PSPC and 0.2 % XG showed the lowest volume among all enriched samples ($p < 0.05$). This reduction in volume appears to be multifactorial. Excessive batter viscosity likely hinders air incorporation during mixing and destabilizes air bubbles during freezing, thus limiting volume expansion during baking (28). Furthermore, at high mass fractions, both PSPC and XG may compete with rice starch for available water, thereby impairing starch gelatinization and weakening the cake's structural integrity. Additionally, the high protein content in PSPC can promote protein aggregation, forming a rigid

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network that restricts gas cell expansion during baking. Overall, moderate increases in batter viscosity due to PSPC and XG can support air bubble stability and enhance cake volume. However, when viscosity becomes excessively high, especially at elevated mass fractions of these additives, it may lead to insufficient aeration and compromised starch functionality, ultimately reducing final cake volume. In addition to formulation, frozen storage duration had a significant impact on cake volume. Cakes produced from batter stored at $-18\text{ }^{\circ}\text{C}$ for 30 days consistently exhibited higher volume compared to those stored for 60 days ($p < 0.05$). The observed reduction in volume after 60 days of frozen storage is likely attributed to gradual degradation of the batter matrix due to ice crystal formation and recrystallization, which diminishes the batter's ability to retain air post-thawing and restricts expansion during baking (28).

The results of porosity analysis, as shown in Fig. 4b, demonstrate that increasing the PSPC level significantly enhances cake porosity compared to the control sample ($p < 0.05$). Similarly, the addition of XG improves porosity, corroborating previous findings that XG facilitates better gas distribution within the batter matrix (45). The synergistic effect of PSPC and XG is particularly evident in the sample containing 10 % PSPC and 0.2 % XG, which exhibits the highest porosity after 30 days of frozen storage. This suggests that the combination of PSPC and XG contributes to a more stable batter structure, enhancing effective gas cell entrapment during baking. However, although the overall trend indicates increased porosity with the addition of PSPC and XG, this effect is not consistently maintained over time. Notably, samples stored for 60 days show a decline in porosity, likely due to ice crystal formation and recrystallization, which can damage gas cells and compromise the structural integrity of the cake matrix. This highlights the impact of prolonged frozen storage on the physical properties of batter systems. These findings are consistent with previous studies reporting the positive influence of pumpkin seed flour on porosity (38), as well as the functional benefits of hydrocolloids like XG in maintaining aeration through improved gas retention and viscosity modulation (45).

The cakes produced from frozen batter, 24 h after baking, exhibited a significant reduction in hardness with the addition of PSPC (Fig. 4c), while the inclusion of XG significantly increased cake hardness ($p < 0.05$). The reduction in hardness observed in samples containing pumpkin seed protein concentrate was attributed to the formation of larger pores within the cake structure. However, Shi *et al.* (46) reported that the addition of protein peptides to frozen batter resulted in reduced cake hardness after freezing and improved texture. Storage time also had a significant impact on cake hardness (47). Prolonged storage, particularly in samples stored for 60 days, led to increased hardness. On the other hand, the addition of XG significantly increased the number of pores in the cakes, contributing to the formation of smaller, more uniform holes. This prevented excessive air

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expansion and helped maintain the cake structure under the forces applied during texture analysis. These results indicate that the formulation of frozen batter, particularly with the addition of protein isolates and hydrocolloids, has a significant impact on the structural and textural properties of baked products during frozen storage.

Fig. 4

Changes in crust color of cake from frozen batter

The color characteristics of the crust of gluten-free cakes produced from batter stored under freezing conditions for 30 and 60 days were investigated. The results in Fig. 5 show that the addition of PSPC significantly decreased the lightness (L^*) of the samples compared to the control ($p < 0.05$). This reduction in lightness is likely due to increased Maillard reaction intensity during baking. However, the addition of XG to the formulation reduced the darkening effect caused by PSPC and significantly increased the lightness of the samples ($p < 0.05$). This effect is mainly attributed to the role of hydrocolloids in promoting more uniform moisture distribution and reducing the intensity of Maillard browning reactions (48). Since pumpkin seeds contain high levels of sulfur-containing amino acids, the darkening effect observed with PSPC addition is likely related to reactions between free amino acids and reducing sugars during baking. Regarding color indices, the a^* (redness) value indicated that the addition of PSPC and XG significantly reduced the redness of the samples compared to the control ($p < 0.05$). Furthermore, the b^* (yellowness) value significantly increased in samples containing PSPC and XG compared to the control ($p < 0.05$). These results align with previous findings reporting significant changes in the color and texture of gluten-free breads when plant flours such as pumpkin seed flour were added (40,49). Additionally, freezing duration had a significant effect on the darkening of the cake crust. Longer freezing periods, especially 60 days, led to darker final products. These findings suggest that both freezing duration and the additives used can considerably impact the color characteristics and overall quality of gluten-free cakes.

Fig. 5.

Evaluation of sensory properties of cake from frozen batter

The sensory evaluation results of cakes prepared from frozen batter stored for 30 to 60 days demonstrated that the addition of XG significantly improved sensory attributes, including color, appearance, aroma, taste, and texture ($p < 0.05$). Conversely, increasing the mass fraction of PSPC resulted in decreased sensory scores across all evaluated attributes. Based on these findings, the sample containing 0.2 % XG without PSPC, derived from batter stored for 30 days, was identified as

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the best in terms of sensory acceptance. Among the PSPC-containing samples, the formulation with 10 % PSPC and 0.2 % XG received the highest sensory scores and was determined to be the optimal substitution level. Furthermore, storage duration of the frozen batter significantly influenced sensory attributes, with cakes stored for 30 days exhibiting markedly superior sensory characteristics. Due to the large number of treatments (which includes 18) and multiple sensory attributes evaluated, only the overall acceptability scores are presented for clarity and better visualization. The results illustrated in Fig. 6 indicate that the addition of XG to the frozen batter formulation, particularly after 30 days of both PSPC and XG achieved higher sensory scores compared to other formulations with varying levels of PSPC and XG. A recent study by Galenko *et al.* (50) also investigated the effect of pumpkin seed protein concentrate in cakes. Although that study did not focus on frozen batter, it demonstrated that the inclusion of 10 % PSPC led to higher sensory acceptance regarding taste, texture, and overall appeal. To the best of our knowledge, this study is the first to explore the use of PSPC in cakes prepared from frozen batter. The findings clearly emphasize that careful selection of the optimal substitution level and ingredient combination significantly affects the sensory attributes and overall quality of baked products.

Fig. 6

CONCLUSIONS

This study demonstrates the potential of PSPC and XG as effective functional ingredients in gluten-free cake formulations designed for frozen storage. By partially replacing rice flour with PSPC and incorporating XG, it was possible to enhance the nutritional value, structural integrity, and sensory properties of the cakes. The optimal formulation comprising 10 % PSPC and 0.2 % XG offered a balanced improvement in appearance, texture, and consumer acceptance after 30 days of frozen storage. Importantly, the findings highlight the influence of freezing and storage on the performance of hydrocolloids and protein concentrates in batter systems, emphasizing the need to consider these factors in product development. These results contribute new insights into optimizing gluten-free products for long-term frozen storage, offering practical solutions for both health-focused consumers and the food industry.

ACKNOWLEDGEMENTS

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FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The manuscript is original and has strictly followed all ethical procedures. No part of the manuscript has been published before, nor is any part under consideration for publication in another journal. The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The sensory evaluation of gluten-free cakes containing PSPC and XG was carried out with voluntary adult participants at Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Iran). All participants provided informed consent before participation. The study involved tasting ordinary food products that are completely safe for human consumption and posed no risk, discomfort, or harm. No invasive procedures were involved, and all steps followed internationally accepted ethical principles for human sensory evaluation. According to institutional and national guidelines, formal ethics committee approval was not required for this type of harmless sensory study.

AUTHORS' CONTRIBUTION

Both authors fulfilled all four ICMJE authorship criteria. H. Malekitabrizi contributed mainly to conceptualization, investigation, formal analysis, and writing the original draft. M. Aalami was primarily responsible for resources, supervision, and project administration. Both authors contributed to reviewing and editing the manuscript, approved the final version, and are accountable for all aspects of the work.

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Table 1. Chemical composition of rice flour, pumpkin seed meal, and PSPC (dry mass basis)

Component	w/%		
	Moisture	Ash	Protein
Rice flour	9.29±0.07	0.63±0.02	8.20±0.11
Fat-free pumpkin seed meal	(7.35±0.06) ^a	(3.35 ±0.12) ^b	(48.57±0.51) ^b
PSPC	(6.24 ± 0.52) ^b	(7.05± 0.17) ^a	(59.78 ± 0.23) ^a

Values are expressed as mean±standard deviation (S.D.). Different superscript letters within the same column indicate statistically significant differences (p≤0.05) according to Duncan’s multiple range test. **Note:** Rice flour was not included in the statistical analysis due to its compositional differences compared to the pumpkin-based samples

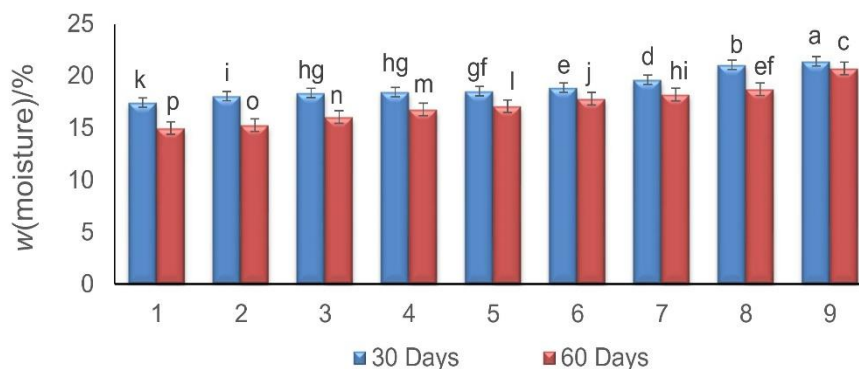
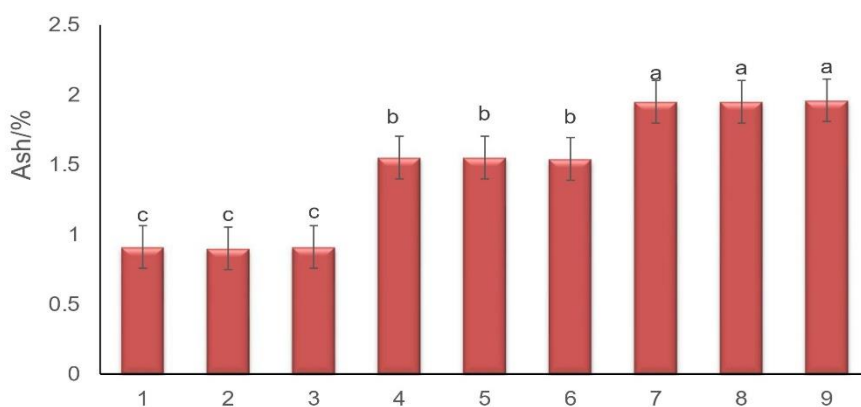


Fig. 1. Effect of PSPC and XG on the moisture content of cake samples from frozen batter after 30 and 60 days of storage. Treatments numbered on the x-axis correspond to the following formulations: 1=control, 2=0 % PSPC+0.1 % XG, 3=0 % PSPC+0.2 % XG, 4=10 % PSPC+0 % XG, 5=10 %

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PSPC+0.1 % XG, 6=10 % PSPC+0.2 % XG, 7=20 % PSPC+0 % XG, 8=20 % PSPC+0.1 % XG, 9=20 % PSPC+0.2 % XG. Values with different letters indicate significant differences according to Duncan’s test ($p \leq 0.05$)

a)



b)

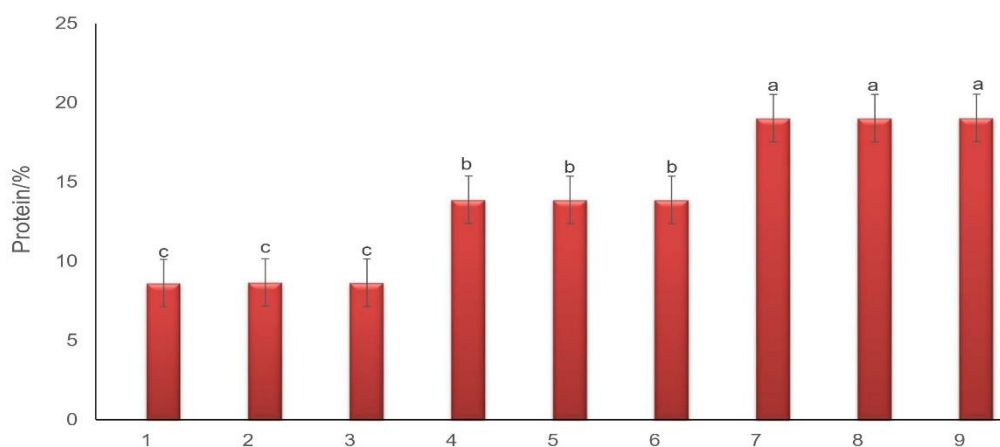
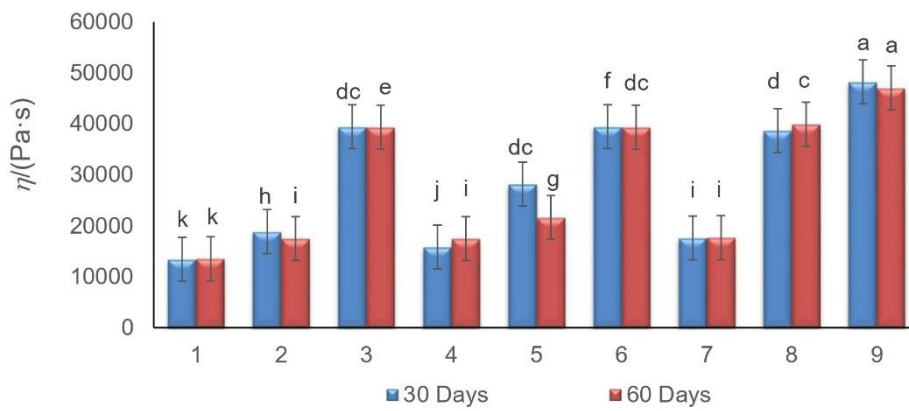


Fig. 2. Effect of PSPC and XG on: a) ash content, and b) protein content of cake samples from frozen batter. Treatments numbered on the x-axis correspond to the following formulations: 1=control, 2=0 % PSPC+0.1 % XG, 3=0 % PSPC+0.2 % XG, 4=10 % PSPC+0 % XG, 5=10 % PSPC+0.1 % XG,

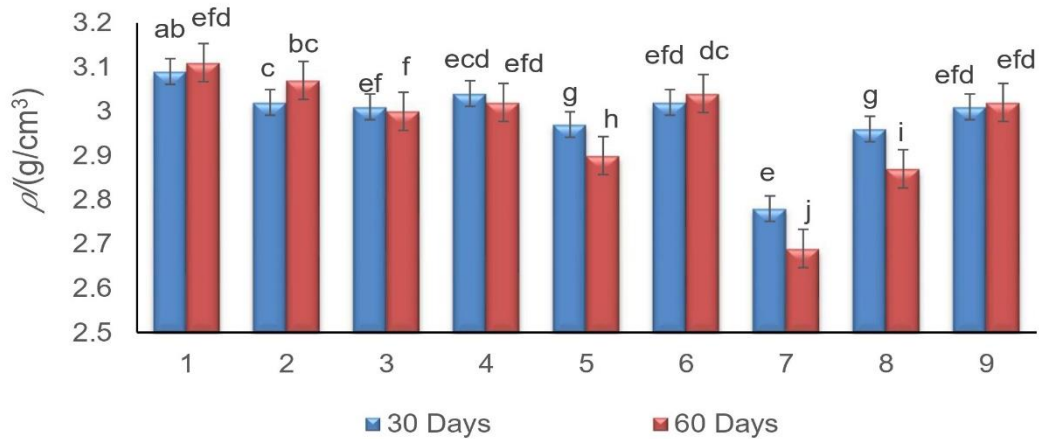
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6=10 % PSPC+0.2 % XG, 7=20 % PSPC+0 % XG, 8=20 % PSPC+0.1 % XG, 9=20 % PSPC+0.2 % XG.. Values with different letters indicate significant differences according to Duncan’s test ($p \leq 0.05$)

a)



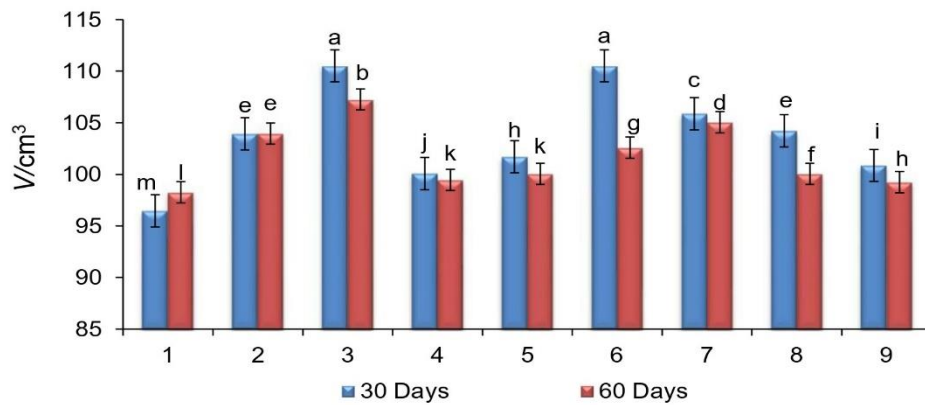
b)



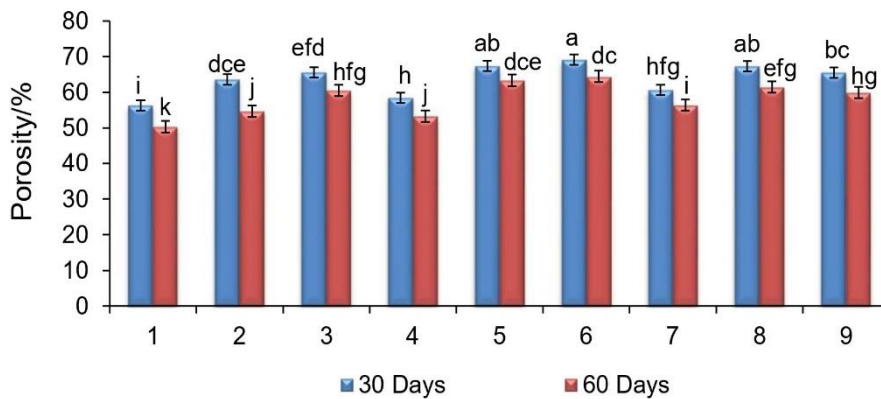
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Fig. 3. Effect of PSPC and XG on: a) viscosity, and b) density of frozen batter samples after 30 and 60 days of storage. Treatments numbered on the x-axis correspond to the following formulations: 1=control, 2=0 % PSPC+0.1 % XG, 3=0 % PSPC+0.2 % XG, 4=10 % PSPC+0 % XG, 5=10 % PSPC+0.1 % XG, 6=10 % PSPC+0.2 % XG, 7=20 % PSPC+0 % XG, 8=20 % PSPC+0.1 % XG, 9=20 % PSPC+0.2 % XG. Values with different letters indicate significant differences according to Duncan’s test ($p \leq 0.05$)

a)



b)



c)

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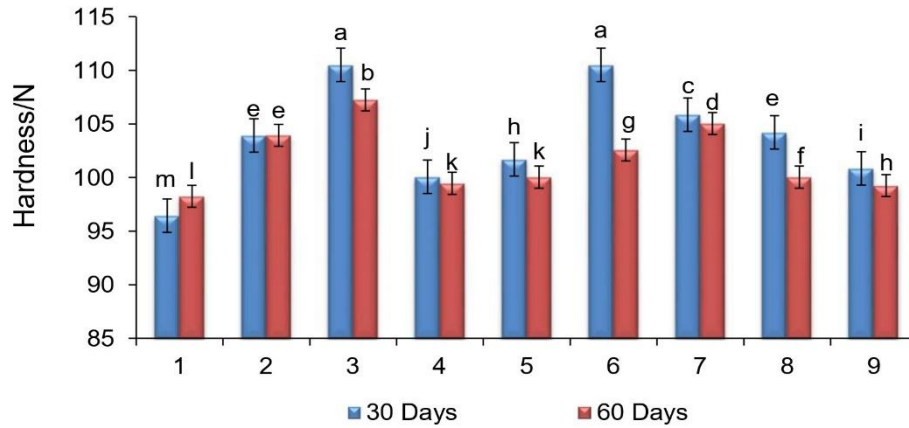
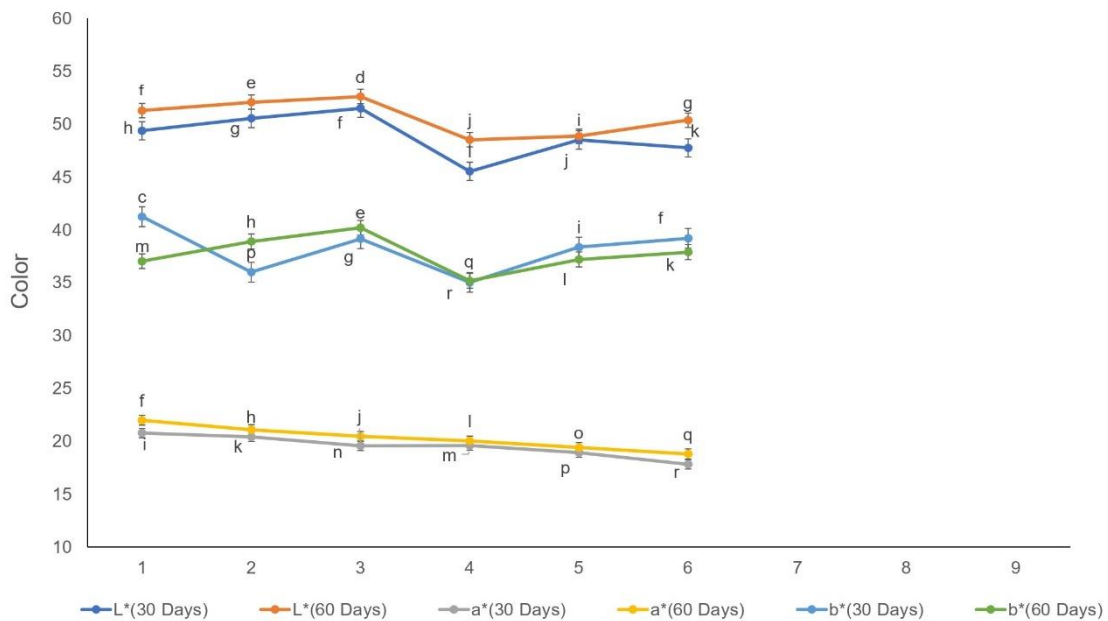


Fig. 4. Effect of PSPC and XG on: a) volume, b) porosity, and c) hardness of cake samples from frozen batter after 30 and 60 days of storage. Treatments numbered on the x-axis correspond to the following formulations: 1=control, 2=0 % PSPC+0.1 % XG, 3=0 % PSPC+0.2 % XG, 4=10 % PSPC+0 % XG, 5=10 % PSPC+0.1 % XG, 6=10 % PSPC+0.2 % XG, 7=20 % PSPC+0 % XG, 8=20 % PSPC+0.1 % XG, 9=20 % PSPC+0.2 % XG. Values with different letters indicate significant differences according to Duncan’s test ($p \leq 0.05$)



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Fig. 5. Effect of PSPC and XG on the color (L , a^* , b^*) of cake samples from frozen batter after 30 and 60 days of storage. Treatments numbered on the x-axis correspond to the following formulations: 1=control, 2=0 % PSPC+0.1 % XG, 3=0 % PSPC+0.2 % XG, 4=10 % PSPC+0 % XG, 5=10 % PSPC+0.1 % XG, 6=10 % PSPC+0.2 % XG, 7=20 % PSPC+0 % XG, 8=20 % PSPC+0.1 % XG, 9=20 % PSPC+0.2 % XG. Values with different letters indicate significant differences according to Duncan's test ($p \leq 0.05$)

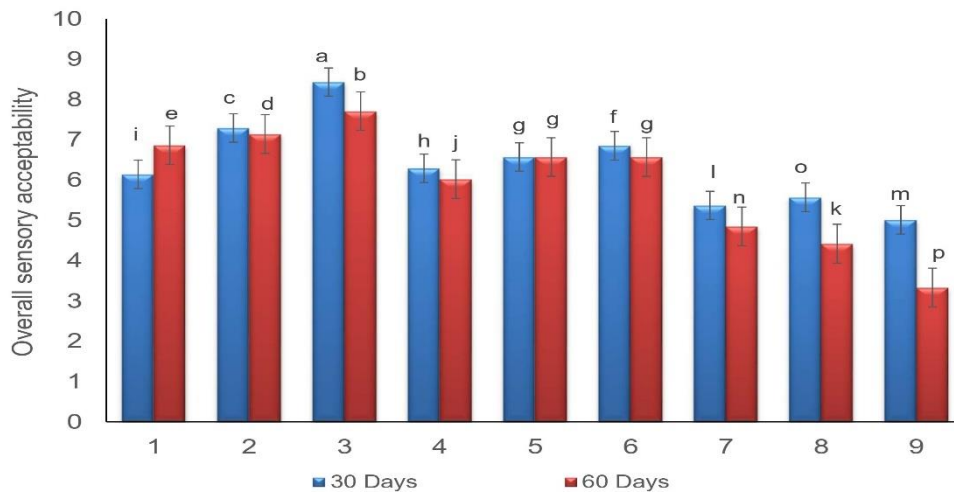


Fig. 6. Effect of PSPC and XG on overall sensory acceptability of cake samples prepared from frozen batter after 30 and 60 days of storage. Blue bars represent samples after 30 days and red bars after 60 days. Treatments numbered on the x-axis correspond to the following formulations: 1=control, 2=0 % PSPC+0.1 % XG, 3=0 % PSPC+0.2 % XG, 4=10 % PSPC+0 % XG, 5=10 % PSPC+0.1 % XG, 6=10 % PSPC+0.2 % XG, 7=20 % PSPC+0 % XG, 8=20 % PSPC+0.1 % XG, 9=20 % PSPC+0.2 % XG. Values with different letters indicate significant differences according to Duncan's test ($p \leq 0.05$)