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Improvement of the Quality Characteristics of Gluten-Free Bread Using Fava Bean Protein Isolate and *Balangu Shirazi* (*Lallemantia royleana*) Seed Mucilage

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ABSTRACT

Bread is considered one of the most important food products in many countries. The presence of gluten protein in wheat is the main factor responsible for creating the desirable dough structure and improving bread quality. However, removing gluten from bread formulations—due to nutritional requirements or health considerations for individuals with celiac disease—poses the challenge of decreased bread quality. Therefore, extensive research has focused on the use of hydrocolloid substitutes to compensate for the functional role of gluten. Accordingly, the aim of the present study was to improve the quality attributes of gluten-free bread using broad bean protein isolate and *Balangu Shirazi* seed mucilage. To determine the experimental treatments, four independent variables were used, including different levels of soy protein isolate, broad bean protein isolate, carboxymethyl cellulose (CMC), and *Balangu Shirazi* seed mucilage. After incorporating these components into the formulation and preparing the samples, physicochemical, textural, and sensory evaluations were performed. The results of the batter textural properties showed that increasing the levels of protein isolates and *Balangu Shirazi* seed mucilage led to an increase in batter firmness. The highest and lowest firmness values were observed in sample 5 (2% soy protein isolate, 2% broad bean protein isolate, 0.25% CMC, 0.25% *Balangu* mucilage) with 508 g, and sample 9 (4.75% soy protein isolate and 0.25% CMC) with 19 g, respectively. Using broad bean protein isolate compared to soy protein isolate resulted in reduced baking loss (the lowest baking loss belonged to sample 3 containing 4% soy protein isolate, 0.5% CMC, and 0.5% *Balangu Shirazi* mucilage, with 21.56%) and increased porosity (the highest porosity was observed in sample 7 containing 4.5% broad bean protein isolate and 0.5% *Balangu* mucilage, with 31.6%). Additionally, the use of CMC compared to *Balangu Shirazi* mucilage led to a greater reduction in baking loss. However, regarding porosity, a considerable increase was observed when soy protein isolates and *Balangu* mucilage were used simultaneously. Soy protein isolate increased bread firmness (the highest firmness was recorded for sample 11, containing 4.75% soy protein isolate and 0.25% CMC, with 1328.27 g), while broad bean protein isolates decreased firmness (the lowest firmness belonged to sample 12, containing 5% broad bean protein isolate, with 140.17 g). Simultaneous use of soy protein isolates and *Balangu* mucilage improved the cohesiveness of the gluten-free bread samples. Regarding crust browning index, the effect of soy protein isolate was greater than that of broad bean protein isolate. Finally, to determine the optimal gluten-free bread formulation, the optimization section of the software indicated that 0.981% soy protein isolate, 3.59% broad bean protein isolate, and 0.422% *Balangu Shirazi* seed mucilage constituted the best formulation for producing gluten-free bread in this study.

1- INTRODUCTION

Bread is one of the most important staple foods in the human diet; however, the presence of gluten in its composition can cause serious health problems for certain individuals. Gluten, which is found in cereals such as wheat, barley, and rye, can trigger adverse immune and gastrointestinal reactions in individuals with celiac disease or gluten sensitivity. In recent years, increasing awareness of these conditions has led to the development and production of gluten-free breads that can serve as suitable alternatives for individuals with such dietary restrictions [1]. Gluten-free breads are typically produced using alternative ingredients such as rice flour, corn flour, potato starch, and other gluten-free materials. The main challenge in producing these breads is achieving a soft and voluminous texture similar to that of gluten-containing breads, since gluten plays a crucial role in the formation of dough structure and elasticity. To overcome this limitation, the incorporation of ingredients such as proteins, gums, enzymes, and natural improvers has been proposed [2].

Faba bean (*Vicia faba* L.) is an annual plant belonging to the Fabaceae family and is considered a nutritious legume due to its high content of protein, dietary fiber, B-group vitamins, and minerals. Depending on the cultivar, faba beans contain approximately 21–41% protein, and their protein is particularly rich in the amino acid lysine. Faba bean protein also exhibits desirable functional properties, including emulsifying, foaming, and gelling capacities. These characteristics make faba bean protein a suitable ingredient for use in the formulation of gluten-free products [3].

Balangu Shirazi seed gum (*Lallemantia royleana* L.) is a natural hydrocolloid extracted from the seeds of the Balangu plant. Due to its functional properties, including gel-forming ability, viscosity enhancement, water-holding capacity, emulsion stabilization, and texture improvement, it is considered a suitable alternative to some commercial gums such as xanthan and guar. This gum has various applications in the production of low-fat products, gluten-free foods, dairy products, sauces, and plant-based beverages, and it can play an important role in improving product quality and extending the shelf life of food products [4].

Proteins and gums play an important role in improving the quality of gluten-free breads, as these products lack gluten, the unique component in conventional breads that is responsible for dough structure, elasticity, and gas retention. The absence of gluten in gluten-free breads often leads to a weakened crumb structure and accelerated staling. Numerous studies have therefore investigated the use of proteins and gums in gluten-free bread formulations. In this regard, protein sources such as soy protein [5], pea protein [6], egg protein [7], and milk protein [8], as well as gums including xanthan [9], guar [10], and carboxymethyl cellulose [11], or combinations of proteins and gums, have been used to improve the quality characteristics of gluten-free bread.

Accordingly, considering the need of celiac patients for high-quality gluten-free breads comparable to conventional wheat breads, the aim of this study was to improve the quality characteristics of gluten-free bread using faba bean protein isolate and Balangu Shirazi seed gum.

2-Materials and Methods

Materials

Rice flour (Domsiah cultivar), carboxymethyl cellulose (Sandoz, China), soy protein isolate (Yu-Wang, China), faba bean protein isolate (Mehta cultivar), Balangu Shirazi seed mucilage, yeast (Razavi, Mashhad, Iran), vegetable oil (Rana, Golestan, Iran), sugar (Golha, Iran), salt (Golha, Iran), sodium hydroxide (Mojallali, Iran), and hydrochloric acid (Mojallali, Iran) were used in this study.

Methods

Preparation of Rice Flour

Rice flour was prepared using Domsiah rice cultivated during the 2023 growing season at Shahid Rajaei Agricultural School in Gonbad-e Kavus. Initially, 3 kg of rice grains were soaked in water for 1 h. After soaking, the rice was drained and, following complete removal of excess water, ground using a laboratory hammer mill (Mini Bosch, Iran). The obtained flour was then spread in a thin and uniform layer in an oven (Behnoud Gostar Taban, Iran) at 35 °C until it reached an appropriate moisture content. Subsequently, the flour was passed through an 80-mesh sieve and finally stored in moisture-proof packages until further use.

Preparation of Dried Faba Bean Flour

For the preparation of dried faba beans, 300 kg of fresh faba beans (Mehta cultivar) were obtained from the Golestan Agricultural Research Center. The seeds were removed from the pods and the seed coats were separated. The dehulled seeds were then dried at 40 °C for 12 h using a semi-industrial dryer (Tam Sanat, Iran). After drying, the seeds were milled using a laboratory hammer mill (Mini Bosch, Iran). The resulting flour was passed through an 80-mesh sieve and subsequently packed in moisture-proof containers and stored under refrigeration until further use [12].

Extraction of Balangu Shirazi Seed Mucilage

Balangu Shirazi seeds were purchased from a medicinal herb store in Gorgan, Iran. The seeds were cleaned to remove impurities and then soaked in distilled water at a ratio of 1:20 (w/v) at 50 °C and pH 7 for 1 h. After complete hydration, the mucilage was separated using an extractor equipped with a rotating plate. The extracted material was then filtered through a cloth to remove residual solids and subsequently dried in a freeze dryer (Operon, South Korea) for 24 h. The dried mucilage was ground using a laboratory grinder (coffee grinder, Iran) and finally stored in moisture-proof polyethylene bags at −20 °C until further use [14].

Formulation of Gluten-Free Bread

The formulation of gluten-free bread based on rice flour is presented in Table 1. A combination of carboxymethyl cellulose and Balangu Shirazi seed mucilage (as stabilizers) was used at varying levels ranging from 0 to 1%, while a mixture of faba bean protein isolate and soy protein isolate was incorporated at levels ranging from 4 to 5%. It should be noted that, based on preliminary experiments, carboxymethyl cellulose and soy protein isolate were used in the gluten-free bread formulation to produce the control sample. Subsequently, other samples were prepared by partially replacing these hydrocolloids with faba bean protein isolate and Balangu Shirazi seed mucilage.

Table 1. Formulation of gluten-free bread based on rice flour

No.	Ingredients	Amount (%)
1	Rice Flour	100
2	Water	150
3	Stabilizer	0-1
4	Protein Isolate	4-5
5	Salt	2
6	sugar	8
7	Instant dry yeast	1
8	Vegetable oil	6

Preparation of Batter and Production of Gluten-Free Bread

To prepare gluten-free bread, the ingredients were first weighed according to the formulation presented in Table 1. The hydrocolloids, including the stabilizers and protein isolates used in the formulation, were initially mixed with half of the required water and stirred to ensure proper hydration and dispersion. Subsequently, the dry ingredients, including rice flour, sugar, salt, and yeast, were added to the mixture. The remaining water was then incorporated to achieve complete mixing and to form the batter, which was mixed for 3 min at medium speed using a mixer. Vegetable oil was added at the final stage and the mixture was further mixed for 1 min at high speed. The resulting batter was poured into molds (45 g per mold) and placed in a fermentation chamber (Bake Sanat, model BS-716F) at 35 °C and 85% relative humidity for 35 min for proofing. After the final fermentation, the molds were baked in a hot-air oven (Unox, Rosella model) at 190 °C with saturated steam injection for 30 min. After baking, the breads were removed from the molds and allowed to cool at room temperature for 1 h. The samples were then packed in moisture-proof polyethylene bags and stored until further analyses were conducted [15].

Cooking Loss

To determine the cooking loss of gluten-free bread, the reduction in the weight of the batter placed in the molds after baking was measured relative to the initial weight of the batter. For this purpose, the weight of the batter poured into the molds and the weight of the bread after baking and cooling at room temperature were recorded. The cooking loss was then calculated using the following equation [16].

$$\text{Cooking loss (\%)} = \frac{\text{Batter weight} - \text{Bread weight after baking and cooling}}{\text{Batter weight}} \times \text{١٠٠}$$

Specific Volume

The specific volume of the bread samples was determined using the rapeseed displacement method according to AACC standard method 10-72 (AACC, 2000). In this method, the bulk density of rapeseed was first determined. The bread sample was then placed in a container with known dimensions and volume, and the remaining space was filled with rapeseeds. The container was subsequently weighed. Finally, the specific volume was calculated by dividing the volume of the bread by its initial weight [17].

Batter Texture Evaluation

The texture of the batter was evaluated using the reverse extrusion test with a texture analyzer (TA-XT Plus, Stable Micro Systems Ltd., UK). The prepared batter samples were poured into cylindrical Perspex containers (69 mm in height) specifically designed for the instrument. The test was performed using a transparent disc probe with a diameter of 35 mm attached to an aluminum adapter. The pre-test, test, and post-test speeds were set at 1, 5, and 1 mm/s, respectively. As the disc penetrated into the sample and subsequently returned, the parameters of firmness (N), adhesiveness (N), and consistency (N·s) were obtained from the force–time curve [18].

Texture Evaluation of Gluten-Free Bread

The textural properties of gluten-free bread were measured at 2, 24, and 48 h after baking using a texture analyzer and the Texture Profile Analysis (TPA) method according to Ronda et al. (2005). For this purpose, bread samples were cut into cubes of approximately 2 cm and subjected to the TPA test. A cylindrical probe (P/36R) with a diameter of 36 mm was used to compress the samples. The textural parameters, including firmness and cohesiveness, were determined from the obtained force–time curves [6].

Porosity

The porosity of the bread crumb was evaluated 2 h after baking using the image analysis method. For this purpose, a transverse slice of the bread crumb was prepared and photographed. The obtained image was analyzed using ImageJ software. By activating the 8-bit option, the image was converted into a grayscale image. Subsequently, the binary option of the software was applied, producing binary images consisting of bright and dark pixels, where the bright pixels represent the pores within the crumb structure. A higher proportion of bright pixels indicates greater porosity. Finally, by using the Analysis function of the software, the ratio of pore area to total area was calculated and the porosity percentage of the samples was recorded [19].

Color Measurement (Crust Browning Index)

To evaluate the browning index of gluten-free bread samples, color parameters were measured 3 h after baking, after the samples had cooled to room temperature. The color indices L*, a*, and b* were determined. For this purpose, the bread samples were scanned using a scanner, and the obtained images were analyzed using the portable version of ImageJ software.By converting the color space from RGB to LAB in the Plugins section of the software, the color parameters were calculated. Finally, the browning index (BI) was determined using the following mathematical equations [20].

$$X = \frac{a^* + \text{۱.۷۵}\, L^*}{\text{۵.۶۵۴}L^* + a^* - \text{۳.۰۱۲}\, b^*}$$

$$BI = \frac{(X - \text{۰.۳۱})\times \text{۱۰۰}}{\text{۰.۱۷۲}}$$

Sensory Evaluation

Sensory evaluation of the prepared samples was conducted by a panel of 13 trained assessors aged between 17 and 40 years. The evaluated sensory attributes included crust and crumb color, porosity, appearance, flavor, firmness, texture, and overall acceptability. Finally, the scores of overall acceptability were used for statistical analysis of the results [21].

Statistical Analysis

In this study, a D-optimal mixture design was applied to evaluate the feasibility of using faba bean protein isolate and Balangu Shirazi seed mucilage as substitutes for soy protein isolate and carboxymethyl cellulose in the formulation of gluten-free bread. The experimental design consisted of four independent variables, including faba bean protein isolate, soy protein isolate, Balangu Shirazi seed mucilage, and carboxymethyl cellulose. The combination of these variables resulted in 15 experimental treatments (Table 2). To describe the relationships between the dependent variables and the independent variables, appropriate regression equations were fitted, and the results were illustrated as mixture contour plots using Design-Expert software. The coefficient of determination (R^2) and the P-value were used to evaluate the adequacy of the fitted models. In the applied experimental design, linear, quadratic, and cubic models were used to fit the obtained responses for the optimization of gluten-free bread formulation. The significance of the treatments in each model was determined by analysis of variance (ANOVA) at the 95% and 99% confidence levels.

Table 2. Experimental treatments according to formulation variables of gluten-free bread in the mixture design

Treatment	Fava bean Protein Isolate (%)	Soy Protein Isolate (%)	CMC (%)	Balabgu Shirazi Mucilage (%)
1	4.5	0	0.5	0
2	2.375	2.375	0.25	0
3	0	4	0.5	0.5
4	0	4.5	0	0.5
5	2	2	0.5	0.5
6	0	4.75	0	0.25
7	4.5	0	0	0.5
8	0	4.25	0.5	0.25
9	4.75	0	0.25	0
10	2.375	2.375	0	0.25
11	0	4.75	0.25	0
12	5	0	0	0
13	4	0	0.5	0.5
14	0	5	0	0
15	4.5	0	0.25	0.25

3-Results and Discussion

Textural Properties of Batter

Firmness

The results of the batter firmness test for gluten-free bread are presented in Table 3. As observed, the highest batter firmness was recorded for sample 5, containing 2% soy protein isolate, 2% faba bean protein isolate, 0.5% carboxymethyl cellulose, and 0.5% Balangu Shirazi seed mucilage, with a value of 2073 g. In contrast, the lowest firmness was observed in sample 9, containing 4.75% faba bean protein isolate and 0.25% carboxymethyl cellulose, with a value of 19 g. Overall, the interaction effects of faba bean and soy protein isolates, as well as Balangu Shirazi seed mucilage and carboxymethyl cellulose (Figure 3), indicate that increasing the proportion of soy protein isolate and Balangu Shirazi seed mucilage led to an increase in batter firmness. As clearly illustrated in Figures 1A and 1B, increasing the level of soy protein isolate and stabilizers resulted in higher batter firmness. Furthermore, Figures 1C and 1D show that increasing the proportion of stabilizers in the batter formulation also contributed to a significant increase in firmness. Among the tested hydrocolloids, Balangu Shirazi seed mucilage exhibited a greater effect on increasing batter firmness compared with carboxymethyl cellulose. This difference is clearly evident when comparing samples 6 and 11. Although both samples contained similar levels of soy protein isolate and hydrocolloid, the firmness of sample 6 (containing Balangu Shirazi seed mucilage) reached 508 g, whereas sample 11 (containing carboxymethyl cellulose) showed a lower firmness value of 279 g**.** In general, gums and proteins increase batter firmness through water absorption and the formation of complexes with other components present in the system. The findings of the present study are consistent with those reported by other researchers.

In this regard, Nourila et al. [22] attributed the increase in batter firmness of gluten-free cakes containing xanthan gum and carboxymethyl cellulose to enhanced water absorption and interactions between the gums and the proteins present in the formulation. In another study, the increase in cake batter firmness with higher levels of whey proteins and soy protein isolate was attributed to the structural properties of proteins in aqueous systems, which promote water absorption and the formation of a gel network within the batter. Therefore, increasing the levels of gums and proteins in the batter system strengthens the gel network, ultimately leading to increased firmness of the batter texture [23]. According to the analysis of variance (ANOVA) results presented in Table 4, each of the variables had a significant effect on the firmness of the samples, with the greatest effects attributed to Balangu Shirazi seed mucilage and carboxymethyl cellulose ($P < 0.01$). The quadratic model was found to be the most appropriate model for fitting the firmness data. The coefficient of determination ($R^2 = 0.948$) indicates a good fit of the model and demonstrates its adequate ability to predict the changes in firmness based on variations in the independent variables.

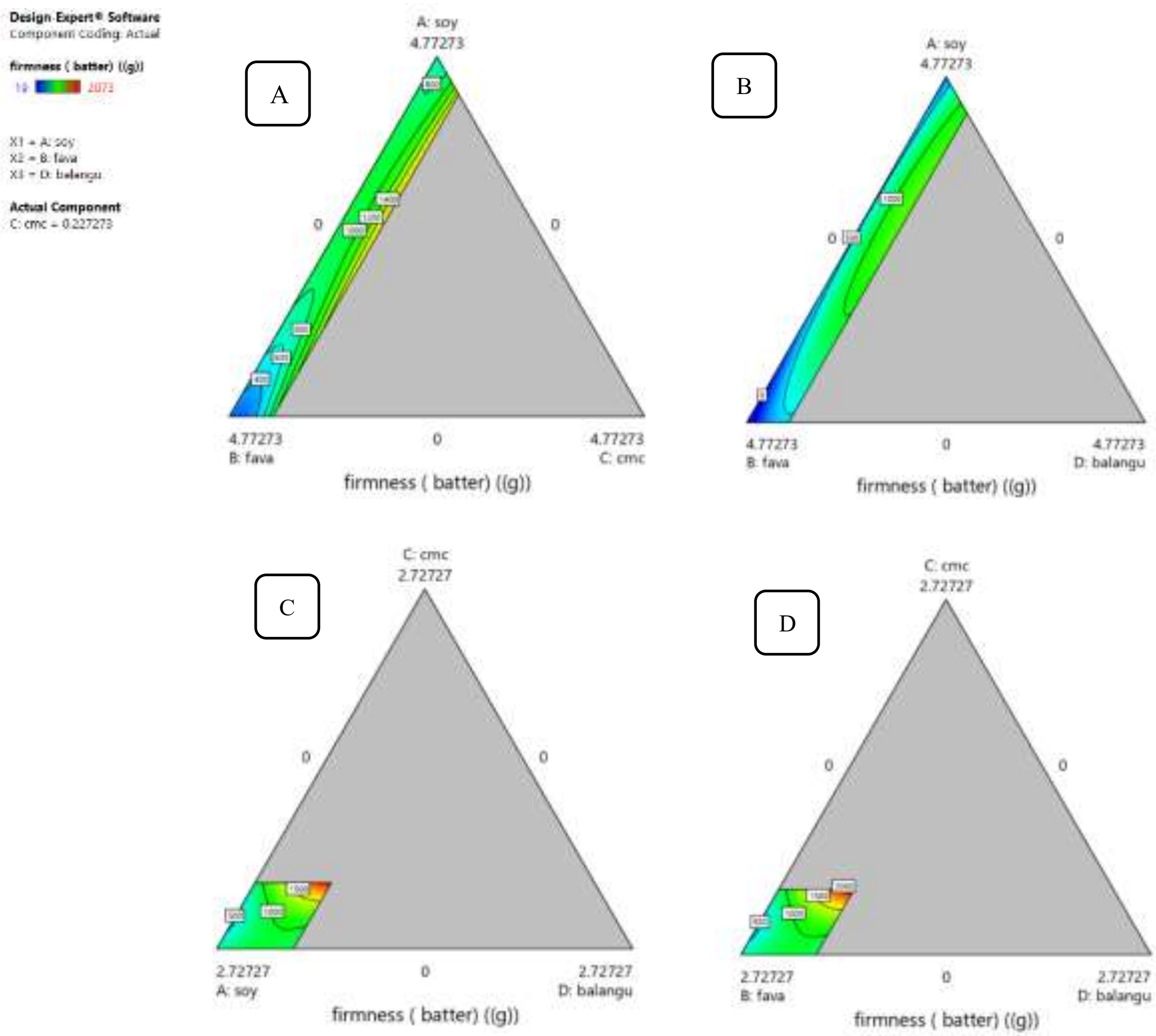


Figure 1. Contour plot of the combined effect of independent variables on the Firmness of gluten-free batter.

Table 3. Experimental results of gluten-free bread samples

Dependent variables						Independent variables				
Batter Consistency)g.s(	**Batter Adhesiveness)g(**	**Batter Firmness)g(**	**Porosity)%(**	**Specific Volume)MLg^{-1}(**	**Baking Loss)%(**	**Balabgu Shirazi Mucilage)%(**	**CMC)%(**	**Fava bean Protein Isolate)%(**	**Soy Protein Isolate)%(**	**Treatment**
459	18	24	20.9	2.93	25.51	0	0.5	4.5	0	1
8267	624	348	22.4	3.87	29.20	0	0.25	2.375	2.375	2
44954	2963	1790	10.6	2.86	21.56	0.5	0.5	0	4	3
21201	1104	844	19.9	4.91	31.22	0.5	0	0	4.5	4
53749	3197	2037	22	3.27	22.06	0.5	0.5	2	2	5
12649	581	508	17.5	2.97	27.42	0.25	0	0	4.75	6
1142	49	49	31.6	3.89	28.4	0.5	0	4.5	0	7
45440	2996	1763	24.7	4.38	21.86	0.25	0.5	0	4.25	8
381	14	19	20.8	3	28.95	0	0.25	4.75	0	9
27734	1561	1110	21.8	3.6	26.4	0.25	0	2.375	2.375	10
5851	310	279	13.3	2.36	31.6	0	0.25	0	4.75	11
397	11	21	19.1	3.02	34.35	0	0	5	0	12
43250	2460	1750	23.4	2.91	23.4	0.5	0.5	4	0	13
5619	237	226	16.4	3.13	30	0	0	0	5	14
2441	143	101	13.7	4.16	24.26	0.25	0.25	4.5	0	15

Table 3 (continued). Experimental results of gluten-free bread samples

Dependent variables				Independent variables				
Overall acceptance	Browning Index	Cohesiveness	Bread Hardness)g(	Balabgu Shirazi Mucilage)%(	CMC)%(	Fava bean Protein Isolate)%(	Soy Protein Isolate)%(	Treatment
3	44.62	0.68	383.68	0	0.5	4.5	0	1
4	60.49	0.758	520.09	0	0.25	2.375	2.375	2
3	51.37	0.726	384.99	0.5	0.5	0	4	3
5	64.97	0.813	686.39	0.5	0	0	4.5	4
5	48.80	0.772	466.18	0.5	0.5	2	2	5
4	61.81	0.756	746.9	0.25	0	0	4.75	6
2	53.75	0.789	450.3	0.5	0	4.5	0	7
5	44.96	0.751	492.2	0.25	0.5	0	4.25	8
3	43.83	0.752	325.69	0	0.25	4.75	0	9
5	53.85	0.615	622.88	0.25	0	2.375	2.375	10
3	34.65	0.759	1328.27	0	0.25	0	4.75	11
2	44.64	0.323	140.17	0	0	5	0	12
4	44.71	0.700	847.13	0.5	0.5	4	0	13
3	55.26	0.692	881.94	0	0	0	5	14
3	52.93	0.673	505.57	0.25	0.25	4.5	0	15

Table 4. Predictive model for the dependent variables of gluten-free bread samples

Model coefficients										Measured parameters
CD	BD	BC	AD	AC	AB	D	C	B	A	
**-17091.6	**-2075.6	-216.98	**-2080.8	-191.15	2.28	**1878.7	**180.94	**30.26	**34.03	**Baking Loss (%)**
**-8716.6	**-417.8	*-139.99	**-406.1	*-143.2	**5.63	**387.09	**128.18	**3.08	**3.06	**Specific Volume (MLg^{-1})**
*-4336.5	*188.7	*2192.9	266.2	*2051.04	21.58	303.68	2020.66	19.62	16.57	**Porosity (%)**
81592.4	82347	-119000	85208	117100	*2070.8	**72724	**108600	**-17.9	**352.9	**)g(Batter Firmness**
116900	138000	-190000	146600	-181000	**3269.1	**122500	**173200	**-26.28	**270.56	**Batter Adhesiveness)g(**
203700	223600	-314600	231900	-3087700	*53851	**197700	**287000	**-604.7	**7607	**)g.s(Batter Consistency**
53662.35	-15896	76374	-24052.3	70786.6	-389.9	**18562.6	**-65746	**86.08	**1075.6	**Bread Hardness (g)**
-4.3	-13.23	-11.27	*42.15	*10.95	0.0861	**14.53	**-34.27	**0.361	**0.64	**Cohesiveness**
**-1710	712	**969	737	**1321.6	**63.37	**-508.66	**917.07	**92.44	**54.96	**Browning Index**
327.09	248.39	-8.89	290.65	-32.42	5.89	**250.62	**24.3	**1.81	**3.3	**Overall acceptance**

A- Soy Protein Isolate, B Fava bean Protein Isolate, C -CMC, D Balangu Shirazi Seed Mucilage

Table 4 (continued). Predictive model for the dependent variables of gluten-free bread samples

Model coefficients						Measured parameters
R^2(adj)	R^2	BCD	ACD	ABD	ABC	
0.932	0.987	*18541.07	*17720.4	11.32	-106.84	**Baking Loss (%)**
0.940	0.981	**10083.1	**1011.6	-33.11	-0.167	**Specific Volume (MLg^{-1})**
0.58	0.78	-	-	-	-	**Porosity) %(**
0.901	0.948	-	-	-	-	**Batter Firmness(g)**
0.921	0.958	-	-	-	-	**Batter Adhesiveness (g)**
0.902	0.948	-	-	-	-	**Batter Consistency (g.s)**
0.717	0.851	-	-	-	-	**Bread Hardness(g)**
0.8	0.859	-	-	-	-	**Cohesiveness**
0.99	0.997	**20953	**23095	**-1496.8	**647	**Browning Index**
0.614	0.797	-	-	-	-	**Overall acceptance**

A- Soy Protein Isolate, B Fava bean Protein Isolate, C -CMC, D Balangu Shirazi Seed Mucilage

Adhesiveness

Controlling adhesiveness in gluten-free product formulations require a delicate balance in the selection of functional ingredients. The choice of the appropriate type and quantity of hydrocolloids plays a crucial role in this aspect. Gums and proteins are particularly important in improving the batter properties of gluten-free bread, especially in enhancing adhesiveness and elasticity. Figure 4 illustrates the interactive effect of proteins and stabilizers on the adhesiveness of gluten-free bread batter. In line with Figures 2A and 2B, increasing the amount of soy protein and stabilizers clearly leads to an increase in adhesiveness. Among the protein sources, soy protein isolate had a greater impact on adhesiveness compared to faba bean protein isolate. This could potentially be attributed to the high-water absorption capacity and superior gel-forming properties of soy protein isolate relative to faba bean protein isolate. Consistent with this finding, Ghaemi et al. [24] reported that the addition of soy protein isolate, whey protein, and basil seed gum to gluten-free cake formulations increased batter adhesiveness. These researchers suggested that the addition of hydrocolloids enhances water absorption, leading to the formation of a gel-like network through strong water–protein interactions, which in turn increases the adhesive force. Conversely, as shown in Figures 2C and 2D, increasing the amount of stabilizers had a direct effect on increasing adhesiveness. The highest adhesiveness was observed when both carboxymethyl cellulose and Balangu Shirazi seed mucilage were used concurrently (sample 5, 3179 g). The lowest adhesiveness was recorded in sample 12, containing 5% faba bean protein isolate, with a value of 11 g. Generally, in gluten-free bread formulations, due to the absence of a gluten network, the use of gums and hydrocolloids is a suitable strategy for improving texture and regulating batter adhesiveness. Gums enhance the water-holding capacity of the batter, leading to better moisture absorption and retention, which increases batter adhesiveness. As indicated in Figure 4, carboxymethyl cellulose had a greater influence on the formulation's adhesiveness compared to Balangu Shirazi seed mucilage. The results of this study are in agreement with those of Sahraian et al. [25], who observed a decrease in batter adhesiveness with the addition of Balangu Shirazi seed mucilage at concentrations of 0.25–0.5%. However, at a higher concentration of 0.75%, they observed an increase in sample adhesiveness. They attributed this phenomenon to the increased viscosity of the aqueous phase and the formation of a three-dimensional matrix by the Balangu Shirazi seed mucilage, which effectively traps free water and prevents excessive batter flow and stickiness. According to the ANOVA results presented in Table 4, all independent variables had a significant effect on batter adhesiveness. The quadratic model, with a coefficient of determination ($R^2 = 0.958$), was identified as the most appropriate model for fitting the adhesiveness data. Therefore, this model can be reliably used to predict changes in adhesiveness as a function of the independent variables.

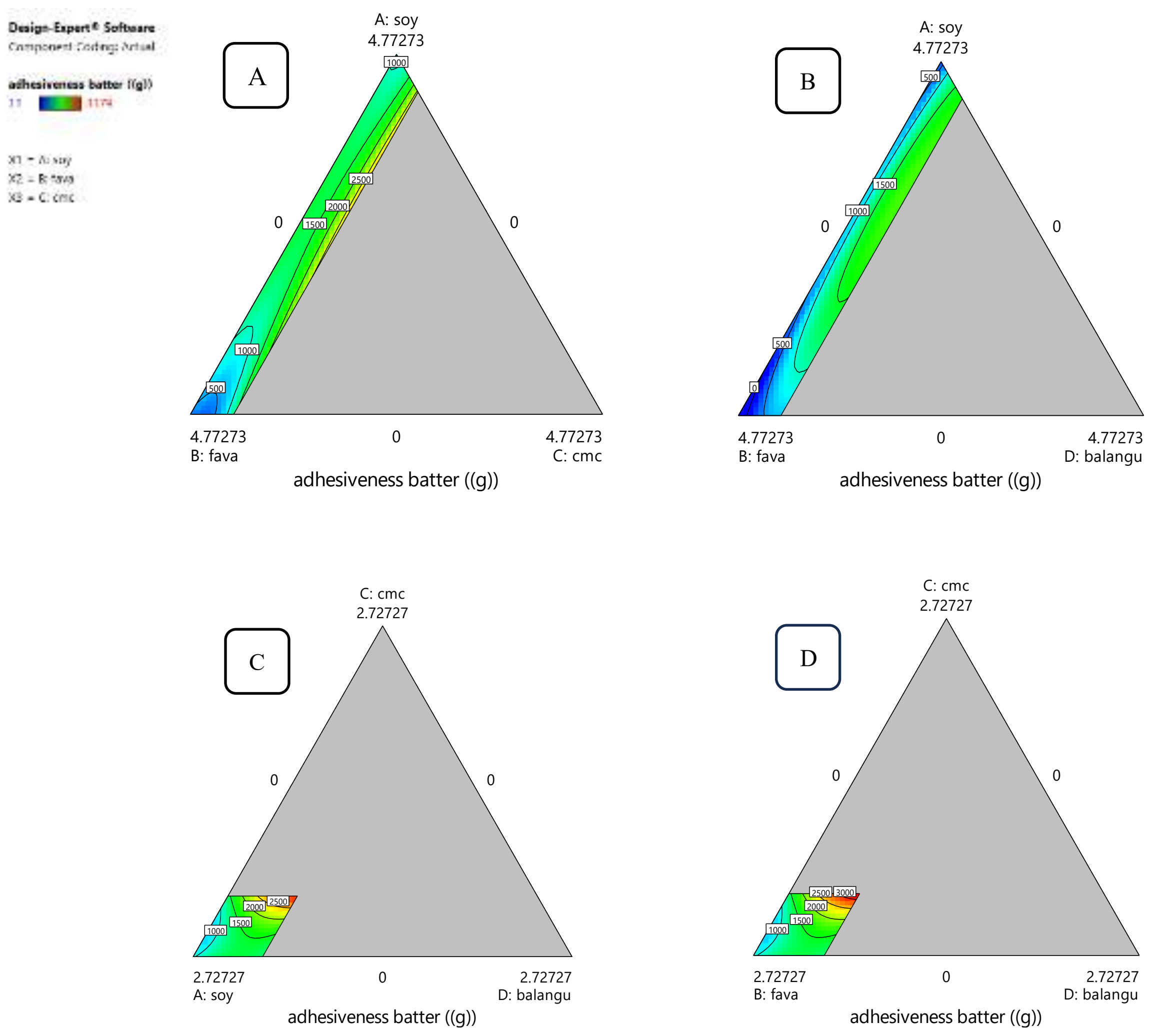


Figure 2. Contour plot of the combined effect of independent variables on the adhesiveness of gluten-free batter.

Consistency

In gluten-free breads, batter consistency is key to achieving a product with an appropriate texture, good volume, and high sensory acceptability; therefore, it is of particular importance. Based on the results in Table 3, the highest consistency was observed in sample 5 (2% soy protein isolate, 2% faba bean protein isolate, 0.5% carboxymethyl cellulose, and 0.5% Balangu Shirazi seed mucilage) at 53794 g·s, while the lowest consistency was recorded for sample 9 (4.75% faba bean protein isolate and 0.25% carboxymethyl cellulose) at 381 g·s. As shown in Figure 3 (A and B), increasing the level of stabilizers in the formulation, as well as increasing the amount of soy protein isolate, leads to an increase in consistency. However, a decrease in consistency was observed when faba bean protein isolate was used to replace soy protein isolate in the formulation.

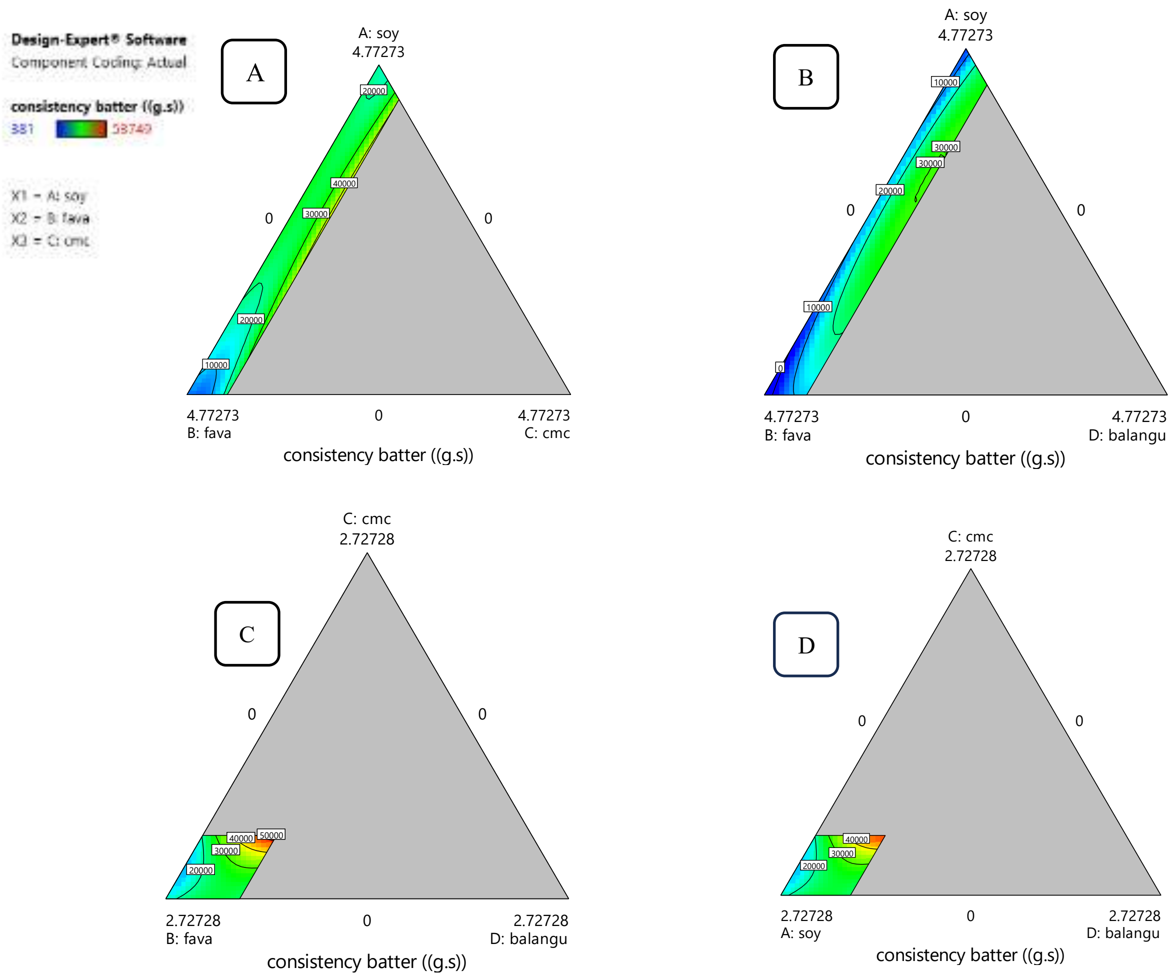


Figure 3. Contour plot of the combined effect of independent variables on the Consistency of gluten-free batter.

The increase in batter consistency is primarily attributed to the higher water-holding capacity of the stabilizers compared to the protein isolates. An optimal batter consistency enhances gas retention, ultimately leading to a well-maintained structure and increased volume in gluten-free bread. In this regard, Arndt and Ragaee [26] stated that the addition of xanthan and guar gums to gluten-free bread formulations improved viscosity and consistency, thereby increasing bread volume. However, excessive consistency can lead to a dense crumb structure and may inhibit bread expansion [27]. Ghaemi et al. [24] also reported an increase in the consistency of gluten-free cake batter upon the addition of soy protein isolate, whey protein concentrate, and xanthan gum, noting that increasing the xanthan gum level significantly boosted consistency. According to the analysis of variance (Table 4), the addition of soy protein isolates and faba bean protein isolate had a significant effect on the consistency of the samples ($P < 0.01$). Furthermore, the addition of carboxymethyl cellulose and Balangu Shirazi seed mucilage significantly increased batter consistency. Regarding the gluten-free bread batter prepared in this study, Balangu Shirazi seed mucilage exhibited a greater influence on consistency compared to carboxymethyl cellulose ($P < 0.01$). The quadratic model was determined to be the most appropriate for fitting the consistency results, yielding a coefficient of determination (R^2) of 0.948. Given this high R^2 value, the predictive model derived from these results is highly capable of accurately predicting changes in this parameter.

Baking Loss

Baking loss in gluten-free bread refers to the reduction in weight resulting from the evaporation of water and lipids during the baking process. Generally, minimizing baking loss is desirable in order to preserve moisture, maintain a soft texture, and extend the shelf life of gluten-free bread. In this context, hydrocolloids play a fundamental role. The evaluation of the combined effects of Balangu Shirazi seed mucilage, carboxymethyl cellulose (CMC), soy protein isolate, and faba bean protein isolate on baking loss in the gluten-free breads produced in this study revealed that the lowest baking loss was observed in sample 3 (4% soy protein isolate, 0.5% carboxymethyl cellulose, and 0.5% Balangu Shirazi seed mucilage) at 21.56%, whereas the highest value was recorded in sample 12 (5% faba bean protein isolate) at 34.35%. These differences clearly reflect the functional performance of the hydrocolloid combinations used in this research. As shown in Figure 4A, increasing the proportion of faba bean protein isolate, compared with soy protein isolate, reduced baking loss. On the other hand, increasing the level of Balangu Shirazi seed mucilage in the gluten-free bread formulation led to an increase in baking loss (Figure 4B). Furthermore, with increasing levels of carboxymethyl cellulose and Balangu Shirazi seed mucilage in the gluten-free batter formulation, a reduction in baking loss was observed (Figures 4C and 4D). As illustrated in these figures, the use of carboxymethyl cellulose showed a more pronounced effect on reducing baking loss compared with Balangu Shirazi seed mucilage. The reduction in baking loss in gluten-free breads may be attributed to the enhanced water-holding capacity of hydrocolloids, as well as the increased viscosity and stability of the batter in the presence of hydrocolloidal compounds such as faba bean protein isolate, Balangu Shirazi seed mucilage, and carboxymethyl cellulose. Under these conditions, the batter is better able to retain moisture, thereby reducing evaporation during baking. The results reported by Marco and Rosell (2008) are consistent with the findings of the present study; these researchers observed that the addition of xanthan gum and hydroxypropyl methylcellulose reduced baking loss in gluten-free bread [13]. Similarly, Lazariado et al. [28] reported that the combined use of xanthan gum, guar gum, and carboxymethyl cellulose exhibited synergistic effects, resulting in the lowest baking loss in gluten-free breads. They attributed this reduction to increased viscosity, improved water retention, and greater stability at high temperatures, which ultimately decreased the rate of water evaporation during baking. In another study, the combined use of hydroxypropyl methylcellulose and corn zein protein reduced baking loss and increased the volume of gluten-free bread. This effect was attributed to the thermal gelation of hydroxypropyl methylcellulose, which stabilizes gas bubbles, as well as the non-covalent interactions between corn zein protein and water molecules [27]. According to the analysis of variance presented in Table 4, each of the variables had a significant effect on the formulation. A cubic model was found to be the most appropriate for predicting the response, with a high coefficient of determination ($R^2 = 0.987$), indicating strong predictive capability for describing the effects of the independent variables on the dependent responses.

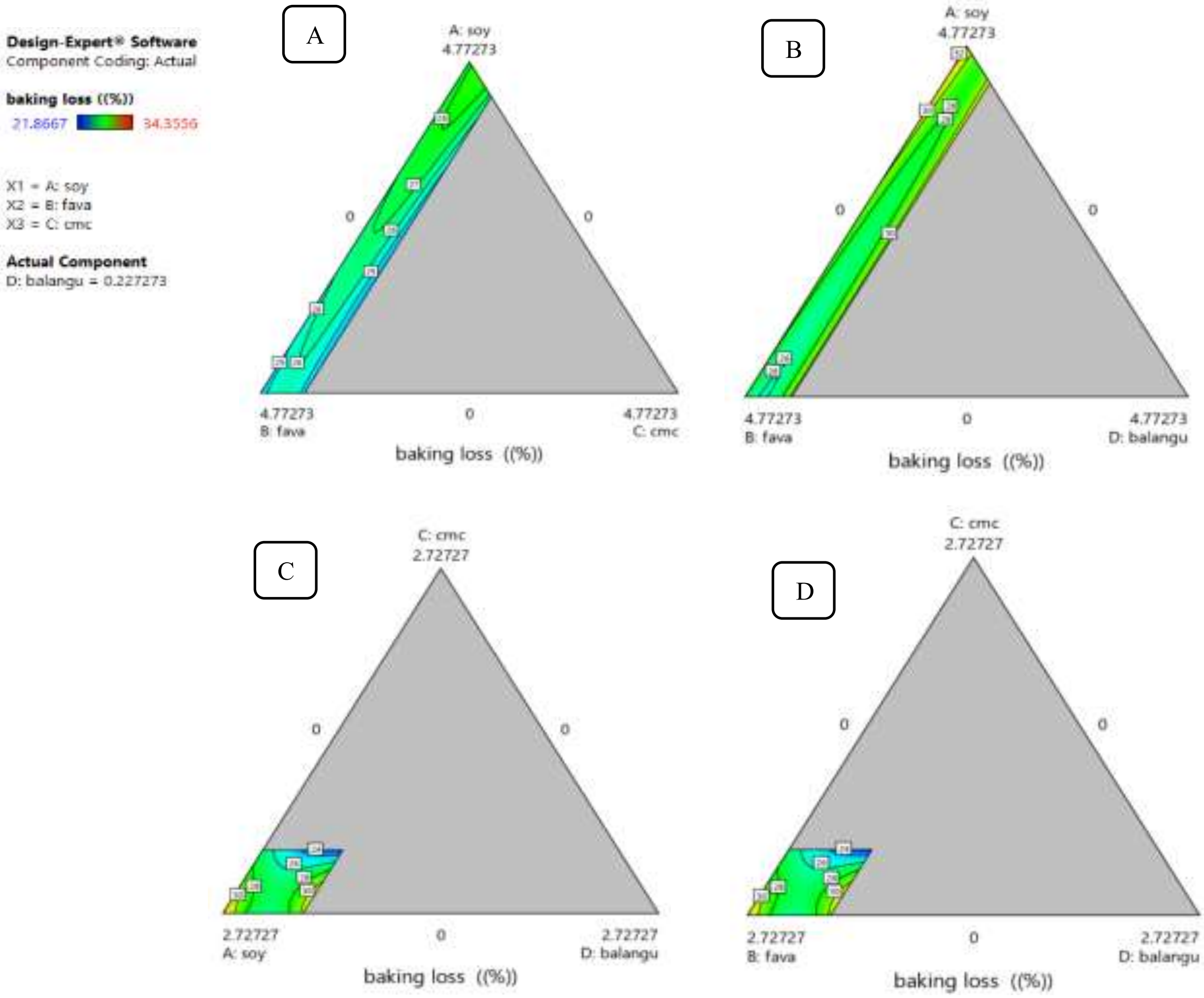
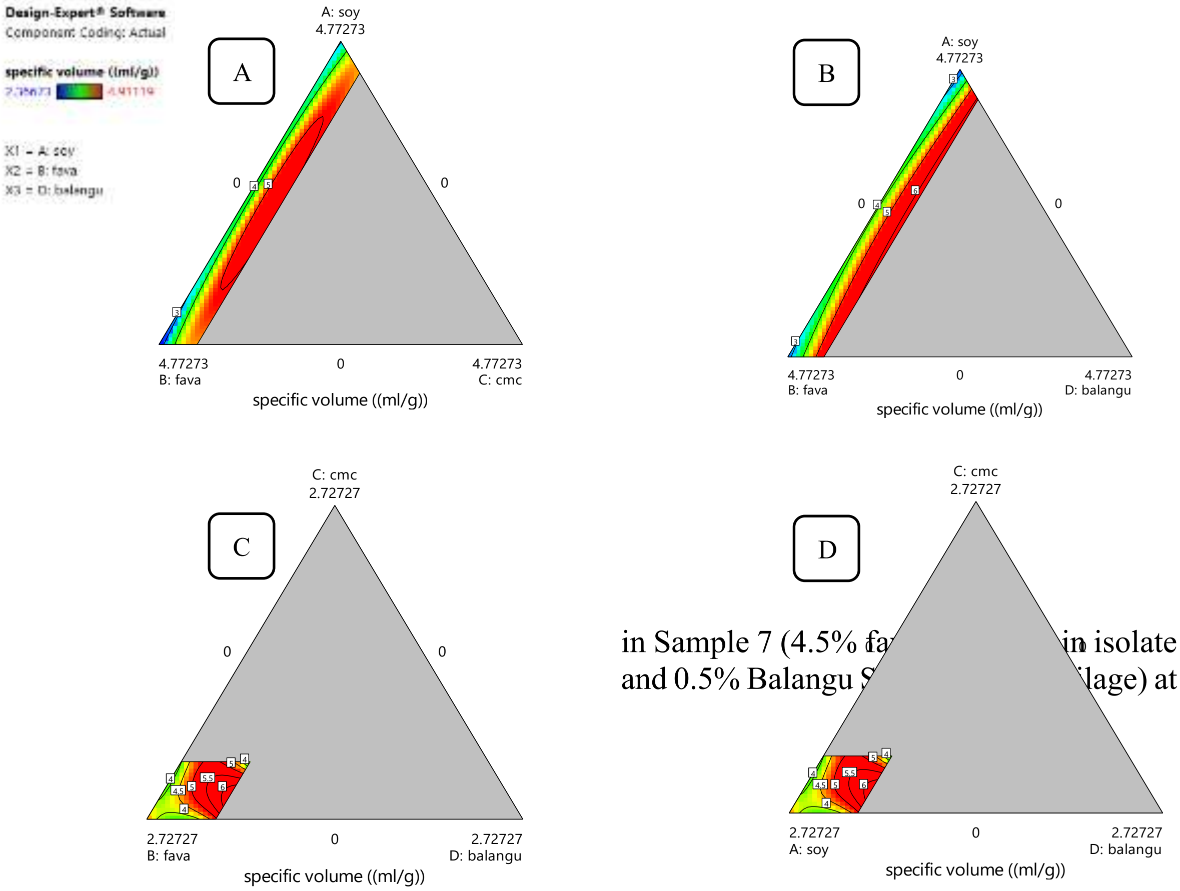


Figure 4. Contour plot of the combined effect of independent variables on the baking loss of gluten-free bread.

Specific Volume

Specific volume is one of the most fundamental and influential parameters affecting the quality of gluten-free bread from the consumer's perspective. According to the results of this study, the highest specific volume was observed in sample 4 (4.5% soy protein isolate and 0.5% Balangu Shirazi seed mucilage) at 4.91 mL g^{-1}, whereas the lowest specific volume was recorded in sample 11 (4.75% soy protein isolate and 0.5% carboxymethyl cellulose) at 2.36 mL g^{-1} (Table 3). Notably, the simultaneous combination of soy protein isolates and Balangu Shirazi seed mucilage played an effective role in increasing batter viscosity, improving structural stability, and consequently enhancing the specific volume of gluten-free bread. Figure 6 presents the mixture contour plot illustrating the effects of different hydrocolloids on specific volume. As shown in the figure, increasing the proportion of soy protein isolate relative to faba bean protein isolate resulted in an increase in specific volume. Moreover, increasing the level of stabilizers in combination with protein isolates led to a significant increase in the specific volume of the samples ($P < 0.01$) (Figures 5C and 5D). In agreement with these findings, Rosell and Arendt [29] reported a considerable increase in specific volume following the addition of xanthan gum and whey protein under the influence of the enzyme transglutaminase. They attributed this improvement to the increased viscosity of the batter and the formation of stable protein networks. Other studies have also emphasized the crucial role of protein–gum combinations in improving the physical properties of gluten-free bread. For instance, Sahin et al. [30] demonstrated that the combination of corn zein and hydroxypropyl methylcellulose (HPMC) increased the volume of gluten-free breads. They attributed this improvement to the thermal gelation of HPMC and the non-covalent interactions between zein and water, which helped maintain batter structure during baking. Similarly, in the present study, the presence of hydrocolloidal compounds such as CMC and Balangu Shirazi seed mucilage, together with protein isolates, significantly enhanced water-holding capacity, prevented gas escape, and consequently increased bread volume. These effects highlight the important role of the gel-like structure and rheological properties of these compounds in improving the overall quality of gluten-free.

According to the analysis of variance (Table 4), all ingredients used in this study—both individually and in combination—had a significant effect on specific volume. Among all the hydrocolloids tested, Balangu Shirazi seed mucilage exhibited the most pronounced effect. Furthermore, a significant increase in volume ($P < 0.01$) was observed when carboxymethyl cellulose and Balangu Shirazi seed mucilage were used in combination with protein isolates. Upon evaluating the statistical model employed in this study, the proposed cubic model, with a high coefficient of determination ($R^2 = 0.978$), demonstrated a strong ability to predict the trend of changes and successfully identified the most effective combinations for optimizing batter structure and increasing bread volume.



in Sample 7 (4.5% fa in isolate
and 0.5% Balangu S lage) at

Figure 5. Contour plot of the combined effect of independent variables on the Specific Volume of gluten-free bread.

Porosity

Porosity is one of the most critical quality indicators in bread, particularly in gluten-free products. In gluten-containing breads, the gluten protein network traps fermentation gases, creating a porous and voluminous structure. However, in gluten-free breads, this natural network is absent; therefore, the bread's porosity is heavily influenced by the type of substitute ingredients used and the processing technology employed. As shown in Table 3, the highest level of porosity was observed 31.6%, while the lowest value was recorded in Sample 3 (4% soy protein isolate, 0.5% carboxymethyl cellulose, and 0.5% Balangu Shirazi seed mucilage) at 10.6%.

According to Figures 6A and 6B, the impact of fava bean protein isolate on the porosity of gluten-free breads is greater than that of soy protein isolate. In fact, moving toward the side of the graph representing a higher amount of fava bean protein isolate leads to an increase in porosity (Point B), whereas moving toward the opposite side, which represents an increase in soy protein isolate, results in a decrease in porosity (Point A). This effect may be attributed to differences in the structure and functional behavior of these proteins in interaction with other batter components. Fava bean protein appears to form a more stable foam structure than soy protein, which helps generate and retain more stable gas bubbles during fermentation and baking. In addition, within the pH range of fermentation, fava bean protein isolate shows higher solubility and greater surface activity than soy protein isolate. These properties likely improve foam stability in the batter and promote the formation of greater porosity in the final bread. A study by Sozer et al. [31] similarly reported that gluten-free bread containing fava bean flour showed 82% porosity and a specific volume of 2.4 mL g^{-1}, whereas the soy-containing sample had only 61% porosity and a specific volume of 1.5 mL g^{-1}. This difference was probably related to the superior foaming properties, emulsifying capacity, and stronger protein network formation associated with fava bean protein. On the other hand, increasing the amount of carboxymethyl cellulose and Balangu Shirazi seed mucilage in the gluten-free bread formulation led to a reduction in porosity (Figures 6C and 6D). This decrease may be explained by the excessive increase in batter viscosity caused by these hydrocolloids, which can restrict bubble expansion and reduce the formation of an open porous structure in the bread crumb. In this context, Sahraeeian et al. [32] reported that adding Balangu seed gum to gluten-free sorghum bread up to a level of 0.5% increased porosity. However, they observed that further increasing the gum concentration led to a significant reduction in porosity, which they attributed to excessive batter viscosity and cohesiveness. In contrast, the present study demonstrated that the combined application of protein isolates and stabilizers resulted in a significant increase in porosity (Figure 7). This positive effect can be attributed to the synergistic interaction between the proteins and the hydrocolloids. While the proteins form the primary structural network, the stabilizers increase the viscosity of the continuous phase, thereby stabilizing the protein network and preventing gas bubble coalescence. This synergy ultimately facilitates the formation of uniform and well-distributed pores within the crumb structure. In this regard, Marco and Rosell [13] investigated the simultaneous effect of adding soy protein isolate, hydroxypropyl methylcellulose (HPMC), and transglutaminase on the structural properties of gluten-free bread. They reported that a triple combination consisting of 13% soy protein isolate, 4% HPMC, and 1% transglutaminase enzyme led to a more open structure and higher crumb porosity. These researchers stated that HPMC, as a hydrocolloid with gelling properties that increases batter viscosity, plays a key role in trapping and stabilizing fermentation gases. Conversely, while soy protein isolate alone was capable of reinforcing the protein network, it could not retain gas within the batter due to low viscosity. Consequently, the simultaneous presence of both the protein and HPMC is essential to create a more stable environment for retaining the gases produced in the batter. According to the analysis of variance (Table 4), the combined use of soy protein isolate–carboxymethyl cellulose, fava bean protein isolate–Balangu Shirazi seed mucilage, and carboxymethyl cellulose–Balangu Shirazi seed mucilage had a significant effect on porosity ($P<0.05$). Regarding the predictive model, the quadratic model was determined to be suitable for predicting the effect of each component on the porosity of the gluten-free bread due to its high coefficient of determination ($R^2=0.78$).

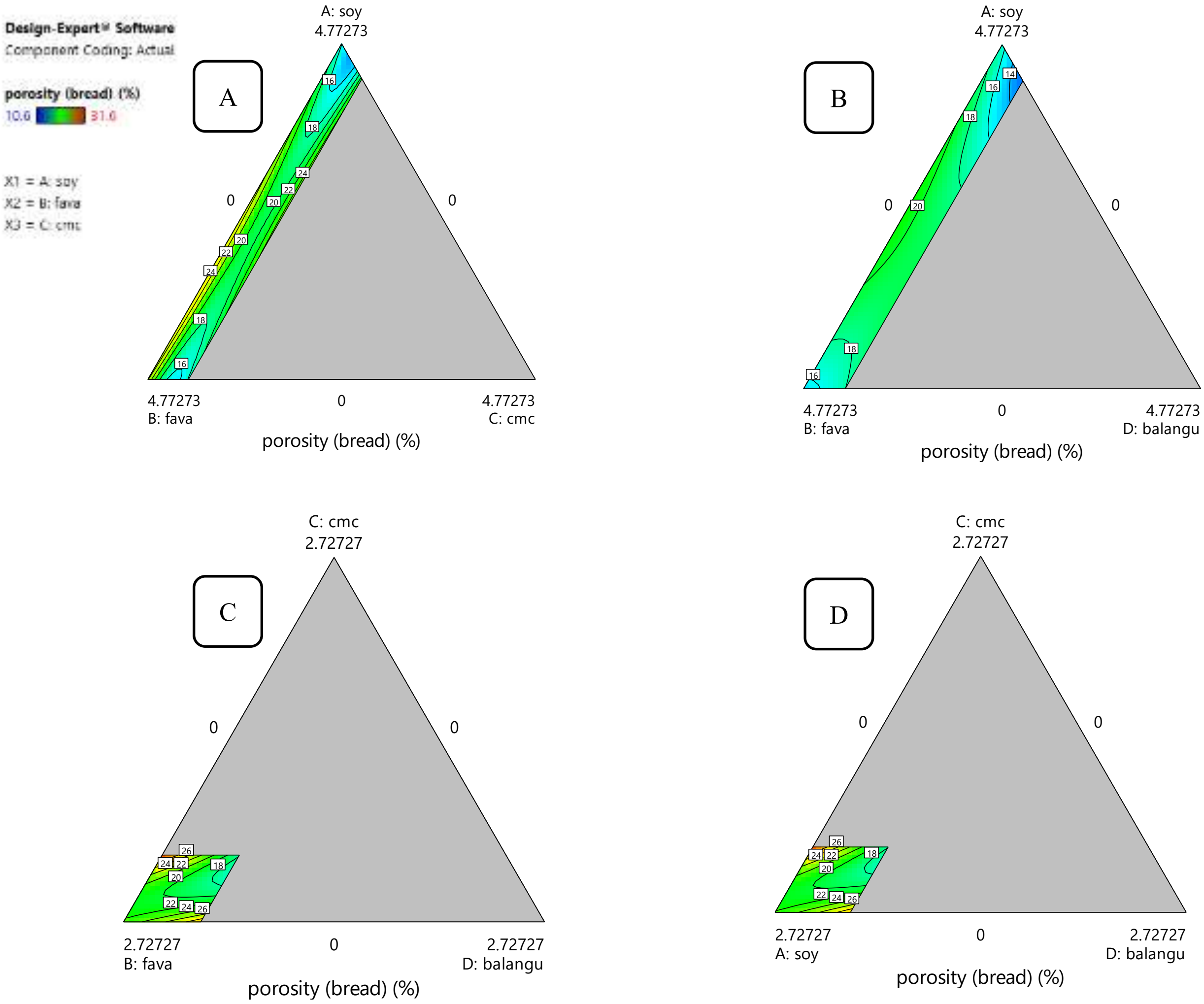


Figure 6. Contour plot of the combined effect of independent variables on the Porosity of gluten-free bread.

Texture Firmness

Firmness is one of the key parameters in evaluating the quality of gluten-free bread. The results of the texture firmness test for the gluten-free bread samples produced in this study are presented in Table 3. As shown, the highest and lowest firmness values were observed in Sample 11 (4.75% soy protein isolate and 0.25% carboxymethyl cellulose) and Sample 12 (5% fava bean protein isolate), with values of 1328.27 g and 140.17 g, respectively. According to Figure 7A, when fava bean protein isolate was used at higher levels (Point B), the firmness of the bread decreased. In contrast, increasing the proportion of soy protein isolate in the formulation—moving toward Point A—resulted in a marked increase in firmness. These findings suggest that fava bean protein isolate contributes to a softer crumb structure, whereas higher levels of soy protein isolate may promote a denser and firmer texture in gluten-free bread. This effect may be attributed to differences in the functional properties of the two protein sources. The major soy proteins, namely glycinin and β-conglycinin, have a strong tendency to form hard and compact gels under heat treatment. Such gel formation can reduce water-holding capacity and consequently increase crumb firmness. In contrast, fava bean proteins form a softer gel structure, which contributes to reduced bread firmness. The findings of the present study are in agreement with those reported by Makinen et al. [33]. These researchers investigated the incorporation of legume proteins, including fava bean, lentil, chickpea, and soy proteins, into bread formulations and observed lower crumb firmness in breads containing fava bean protein isolate compared with soy protein isolate. They attributed this effect to the relatively soft gel structure formed by fava bean protein isolate and the firmer heat-induced gel network produced by soy protein isolate. Furthermore, increasing the proportion of carboxymethyl cellulose and Balangu Shirazi seed mucilage in combination with the protein isolates resulted in different effects on the firmness of the gluten-free bread samples, as illustrated in Figures 7A and 7B. For example, in Figure 7A, moving from Point B toward Point C (which represents an increasing proportion of carboxymethyl cellulose in the formulation) leads to a significant increase in firmness ($P<0.01$). Conversely, at Point A—where the baseline firmness is high—increasing the share of carboxymethyl cellulose (moving from Point A toward Point C) results in a decrease in firmness; however, according to the analysis of variance (Table 4), this reduction is not statistically significant ($P>0.05$). A similar trend is visible in Figure 7B regarding the application of Balangu Shirazi seed mucilage. Specifically, combining Balangu Shirazi seed mucilage with fava bean protein isolate led to an increase in firmness, whereas its combination with soy protein isolate resulted in a reduction in crumb firmness. The interactive effect between carboxymethyl cellulose and Balangu Shirazi seed mucilage on the texture firmness of the gluten-free bread was not statistically significant ($P>0.05$). As shown in Table 4, the firmness responses of the gluten-free bread samples prepared in this study were well-fitted to a quadratic model. The predictive equation obtained for this parameter exhibited a high coefficient of determination ($R^2=0.85$), indicating that the model is highly reliable for predicting variations in this quality index.

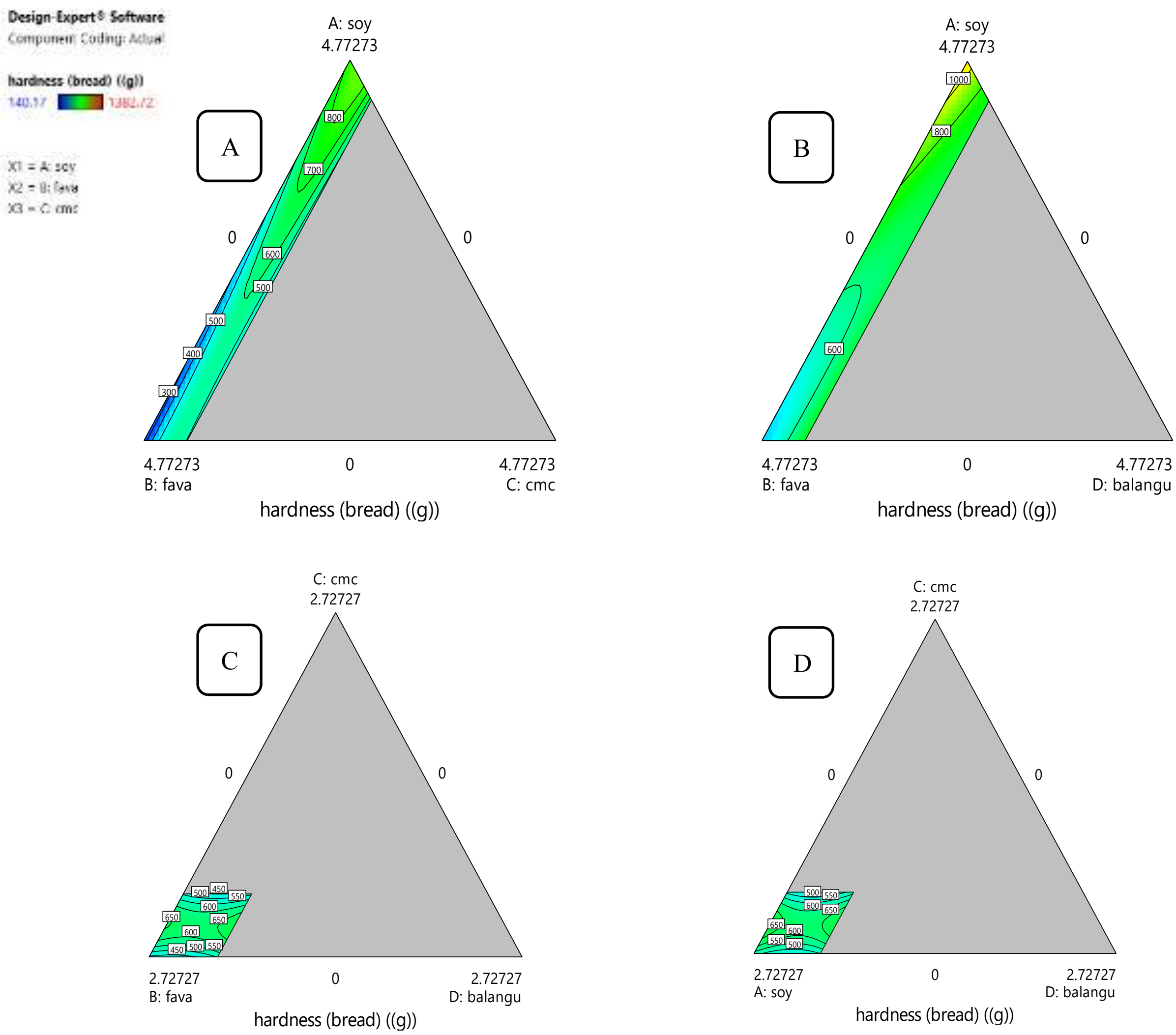


Figure 7. Contour plot of the combined effect of independent variables on the hardness of gluten-free bread.

Crumb Cohesiveness

Cohesiveness in bread refers to the crumb's capacity to resist crumbling and disintegration under stress. In conventional wheat breads, gluten creates a cohesive, continuous, and unified structure. However, in gluten-free formulations, the lack of a cohesive network makes the crumb highly susceptible to crumbling and fracturing. As shown in Table 3, the highest and lowest values of crumb cohesiveness were observed in Sample 4 (4.5% soy protein isolate and 0.5% Balangu Shirazi seed mucilage) and Sample 12 (5% fava bean protein isolate), with values of 0.813 and 0.323, respectively. The trend of crumb cohesiveness shown in Figures 8A and 8B indicated that increasing the proportion of soy protein isolate together with Balangu Shirazi seed mucilage in the gluten-free bread formulation significantly improved cohesiveness. This result may reflect a synergistic effect between soy protein isolate and Balangu Shirazi seed mucilage in strengthening the structural network of gluten-free bread. Moreover, when comparing the two stabilizers used in the formulation, Balangu Shirazi seed mucilage showed a greater effect than carboxymethyl cellulose in enhancing crumb cohesiveness (Figure 8D). The higher cohesiveness observed with Balangu Shirazi seed mucilage, compared with carboxymethyl cellulose, may be attributed to the ability of this native gum to form hydrogen bonds and retain water within the batter system, leading to a more integrated crumb structure and reducing the tendency of the bread to crumble. In agreement with these findings, Arora et al. [34], in the production of gluten-free bread using gluten-free flours such as pregelatinized starch, pearl millet, and buckwheat together with soy protein isolate, reported that the addition of soy protein significantly increased crumb cohesiveness in the produced breads. In those samples, cohesiveness was positively correlated with swelling capacity and water absorption capacity. According to the findings of Crockett and Vodovotz [35], the protein present in soy isolate plays an important role in mimicking the function of gluten in trapping fermentation gases in gluten-free breads. As shown in the analysis of variance (Table 4), the treatments had a significant effect on cohesiveness ($P<0.01$). Among the studied variables, Balangu Shirazi seed mucilage was identified as the most effective factor in increasing cohesiveness. In addition, both soy protein isolates and fava bean protein isolate had positive effects on improving cohesiveness. In contrast to the other components, carboxymethyl cellulose showed a negative effect on cohesiveness compared with the other ingredients (Table 4). Furthermore, evaluation of the combined effects of the ingredients revealed that the simultaneous use of soy protein isolates and Balangu Shirazi seed mucilage had the greatest positive effect on improving crumb cohesiveness ($P<0.05$). According to the analysis of variance, the quadratic model, with a coefficient of determination of $R^2=0.859$, was found to be an appropriate model for fitting and predicting the cohesiveness results of the gluten-free bread samples produced in this study.

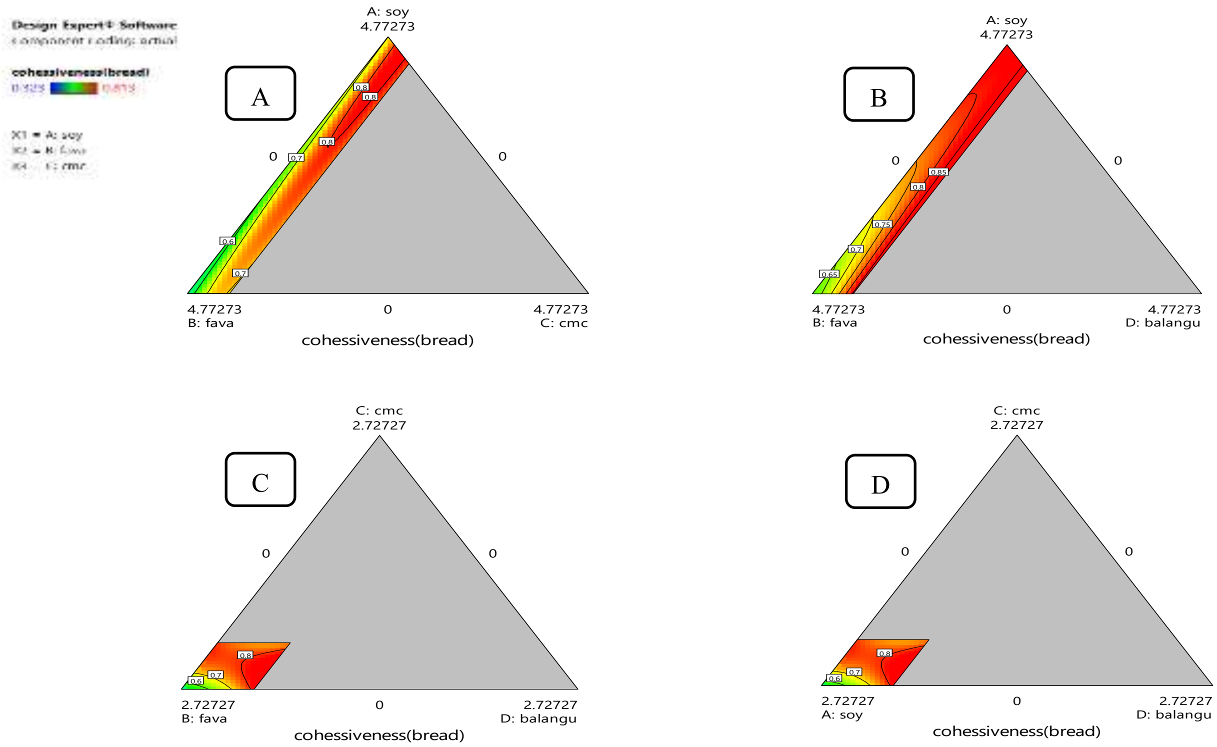


Figure 8. Contour plot of the combined effect of independent variables on the Cohesiveness of gluten-free bread.

Crust Browning Index

The browning index in gluten-free bread is considered one of the important criteria for evaluating the product's visual quality and sensory acceptance. This index is generally associated with crust color and is directly related to the chemical reactions occurring during baking as well as the composition of the dough or batter. The results for the browning index (Table 3) showed that the highest and lowest values were observed in Sample 4 (4.5% soy protein isolate and 0.5% Balangu Shirazi seed mucilage) and Sample 11 (4.75% soy protein isolate and 0.25% carboxymethyl cellulose), with values of 64.97 and 34.6, respectively. The use of each of the independent variables had a significant effect on the crust browning index (P<0.01). As shown in Figure 9A, when comparing the protein isolates, soy protein isolate had a greater effect on the browning index than fava bean protein isolate, such that increasing the proportion of soy protein isolate led to a higher browning index. However, under conditions where the two protein isolates were used simultaneously at equal proportions, an increase in the browning index was also observed. This finding suggests that both the type and combination of protein sources can influence crust color development in gluten-free bread. Zibro et al. [36] reported that the use of soy protein isolates and collagen led to a darker color in gluten-free bread, attributing this effect to the enhancement of Maillard reactions due to the higher protein content of the formulation. In another study, the incorporation of soy flour into gluten-free bread was also found to shift crust color from a lighter appearance toward brown as the level of soy flour increased. This effect was attributed to the presence of pigments in soy flour, as well as its natural sugars and amino acids participating in the Maillard reaction [37]. According to Figures 9C and 9D, when carboxymethyl cellulose and Balangu Shirazi seed mucilage were used in combination with the protein isolates, the browning index increased noticeably, particularly in the presence of carboxymethyl cellulose. In addition, the simultaneous use of soy protein isolates and Balangu Shirazi seed mucilage also resulted in an increased browning index. Overall, however, the effect of carboxymethyl cellulose on increasing the browning index was greater than that of Balangu Shirazi seed mucilage. The results of this section were inconsistent with some previous studies. It has been reported that the use of carboxymethyl cellulose in gluten-free bread formulations can produce a lighter crust color due to its ability to increase viscosity and water-holding capacity [38]. Additionally, it has been reported that the utilization of *Lallemantia royleana* (Balangu Shirazi) seed mucilage in gluten-free bread formulation increased crust lightness. This effect was attributed to moisture retention in the product and the enhanced water-holding capacity of the bread crust. Regarding the impact of carboxymethyl cellulose (CMC) and Balangu Shirazi seed mucilage on the increase in the browning index and crust darkness in the present study, it could be hypothesized that while the increased moisture content delays the drying of the crust, the formulation of a more uniform structure and the reduction of surface cracking facilitate more effective heat transfer during baking. On the other hand, the presence of free sugars in the hydrocolloids used in this study may have increased the likelihood of the Maillard reaction. Ultimately, according to the analysis of variance (ANOVA) table (Table 4), the independent variables investigated in this study had a significant effect on the browning index. Furthermore, due to its high coefficient of determination (R^2=0.997), the cubic model was identified as the appropriate model for fitting the crust browning index data.

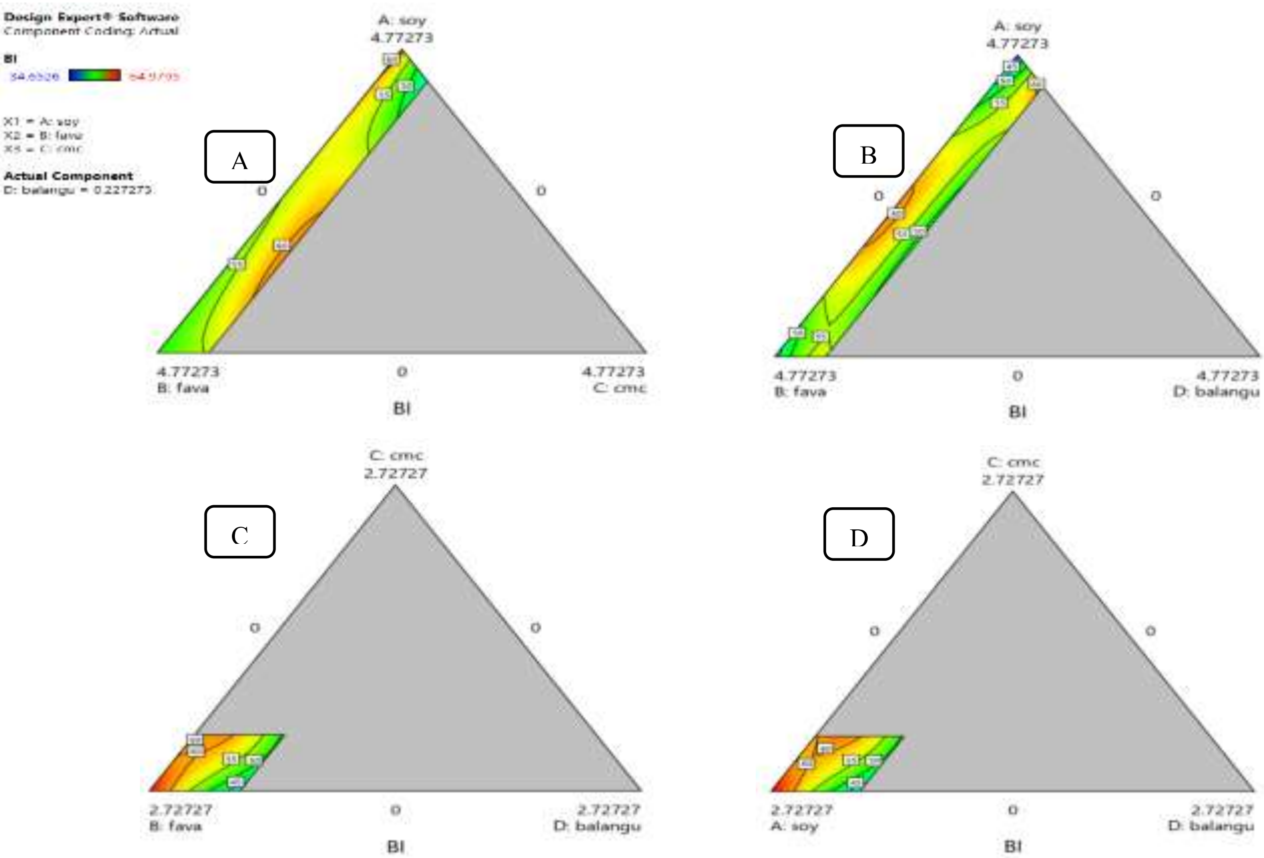


Figure 9. Contour plot of the combined effect of independent variables on the Browning Index of gluten-free bread.

Overall Acceptance

Overall acceptance in the sensory evaluation of gluten-free breads serves as a key indicator, playing a decisive role in predicting the market success of the product and measuring consumer satisfaction. This metric is a crucial tool for product developers to assess the extent to which formulation modifications are acceptable and valued from the consumers' perspective. Table 3 presents the overall results of the sensory evaluation of the gluten-free bread samples prepared in this study. As can be observed, samples 4, 5, 8, and 10 received the highest score (score 5) from the panelists, whereas samples 7 and 12 obtained the lowest score (score 2). The trend of the mixture contour plot for the overall acceptance of the gluten-free bread samples indicates that increasing the proportion of soy protein isolate in the formulation led to an increase in overall acceptance (Figure 10-A); however, its excessive incorporation resulted in a decline in overall acceptance. This finding is in agreement with the results reported by Yang et al. [39]. These researchers, who incorporated soy protein isolate into a rice flour-based gluten-free bread formulation, reported that adding soy isolate improved the volume and rheological properties of the product. However, increasing the level of soy protein isolate to 9% led to a significant decrease in the overall acceptance of the samples, which was attributed to the development of an undesirable soy off-flavor [25]. On the other hand, investigating the interaction between the protein isolates and the stabilizers used in this study (*Lallemantia royleana* seed mucilage and carboxymethyl cellulose) revealed an increase in the overall acceptance score (Figures 10-A and 10-B). Additionally, according to Figures 10-C and 10-D, the effect of *Lallemantia royleana* seed mucilage on increasing overall acceptance was greater than that of carboxymethyl cellulose. In a study investigating the effect of *Lallemantia royleana* seed mucilage on the physicochemical and sensory characteristics of gluten-free bread made from corn flour, the results showed that the addition of 2% Balangu seed mucilage improved the physical and sensory properties of the product (Jalali et al., 2019). These researchers stated that due to its hydration and gelling properties, *Lallemantia royleana* seed mucilage acts as a functional replacement for the gluten network in gluten-free bread, thereby increasing the overall acceptance score of the bread by improving its moisture content, volume, porosity, and texture. The results of the analysis of variance (ANOVA) for overall acceptance (Table 4) also indicated that each of the independent variables had a significant effect on overall acceptance ($P<0.05$However, the greatest effect was attributed to the use of *Lallemantia royleana* seed mucilage. Examination of the ANOVA table further revealed that the quadratic model was the most appropriate model for fitting the overall acceptance data. Moreover, considering its coefficient of determination (R^2=0.797) this model was deemed suitable for predicting the trend of changes in this parameter.

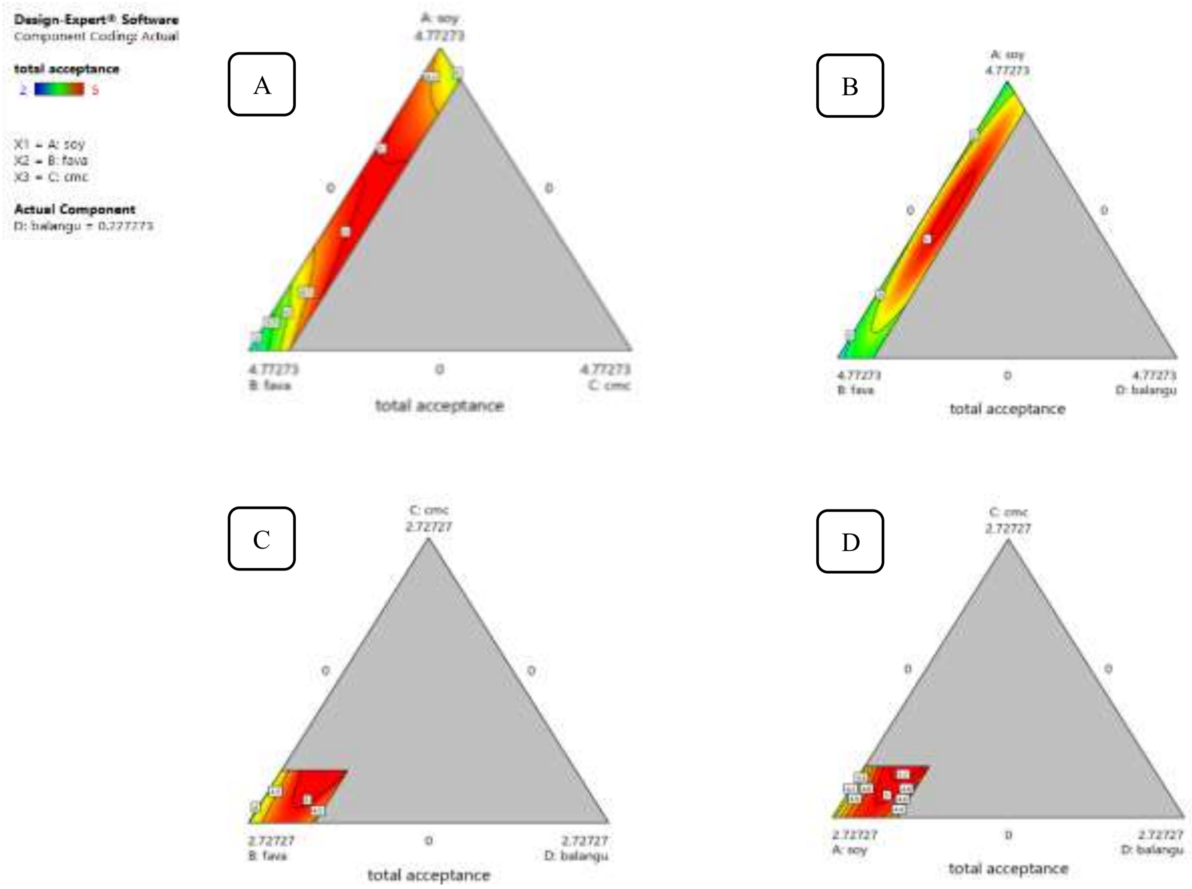


Figure 10. Contour plot of the combined effect of independent variables on the Overall Acceptance of gluten-free bread.

Formulation Optimization

To optimize the formulation of the gluten-free breads prepared in this study, the graphical optimization tool of the Design-Expert software was utilized. Accordingly, the optimization targets were first defined, and the independent and dependent variables were subsequently configured in the software. Among the dependent variables, batter firmness, adhesiveness, and consistency, as well as crust browning index and bread firmness, were set within specific target ranges, while the remaining parameters were set to maximum. Ultimately, the optimized formulation obtained from the software was determined to be 0.981% soy protein isolate, 3.59% faba bean protein isolate, and 0.422% *Lallemantia royleana* (Balangu Shirazi) seed mucilage. The results of the dependent variables obtained from the software are illustrated in the overlay contour plot in Figure 11. To validate the model, experimental tests were conducted on bread samples baked using the optimized formulation, and the results were compared with the predicted optimal values. It was observed that the model derived from the mixture design methodology was highly capable of predicting the experimental results.

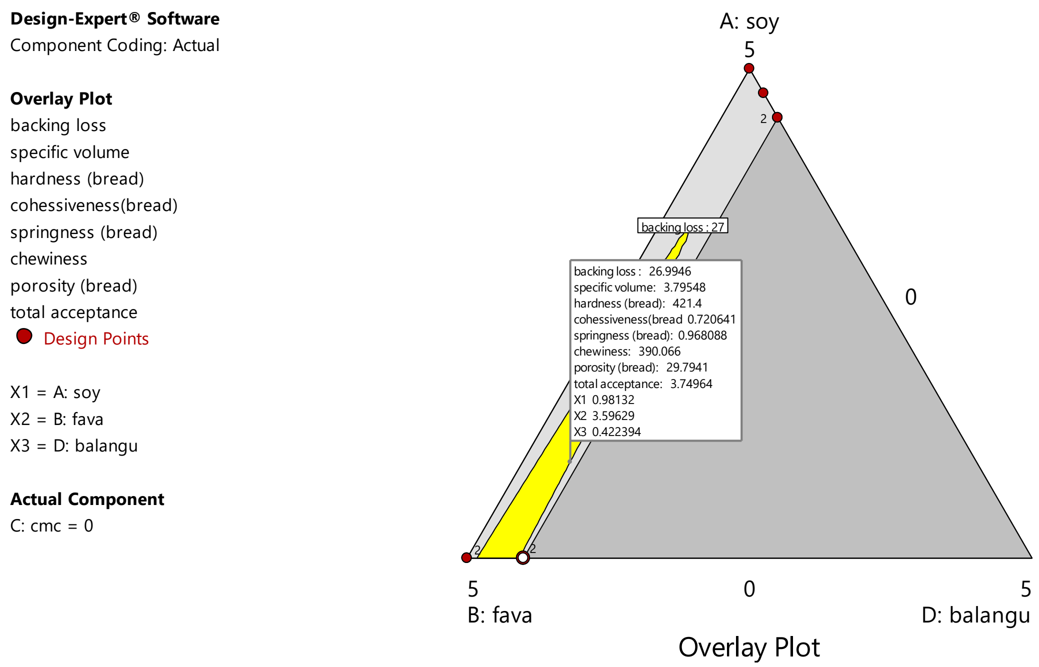


Figure 11. Overlay contour plot for the optimization of gluten-free bread formulation

4-Conclusion

Based on the results of this study, it was found that the use of hydrocolloid compounds can significantly improve the physical and sensory properties of gluten-free breads. Increasing the proportion of soy protein isolate and the natural stabilizer *Lallemantia royleana* (Balangu Shirazi) seed mucilage led to an increase in batter firmness and consistency and a reduction in adhesiveness. This effect can be attributed to the high-water absorption capacity of these components and the formation of a stable gel network within the batter. In contrast, the use of faba bean protein isolate reduced firmness and increased the porosity of the samples, indicating the formation of a more stable foam structure and improved retention of gas cells within the batter. Industrial stabilizers such as carboxymethyl cellulose also played an important role in reducing baking loss and increasing the specific volume of the breads; however, their effects on porosity and crumb cohesiveness were different. In addition, the crust browning index increased with higher levels of soy protein isolate and certain stabilizers, which can be associated with Maillard reactions and moisture retention during baking. The results of the sensory evaluation further indicated that the optimal combination of proteins and gums—particularly the simultaneous use of soy protein isolate and *Lallemantia royleana* seed mucilage in the formulation—resulted in the highest overall consumer acceptance. The statistical models developed in this study, with high coefficients of determination, were able to accurately predict the variations in textural and sensory parameters. Overall, the combined use of plant proteins and hydrocolloids can be considered an effective strategy for improving the structural quality and sensory acceptance of gluten-free breads and may contribute to the development of products with characteristics closer to conventional gluten-containing breads. Furthermore, the results demonstrated that *Lallemantia royleana* seed mucilage can potentially replace carboxymethyl cellulose in the formulation used in this study. Such substitution not only preserves functional properties such as viscosity and stability but also, due to its natural origin and gel-forming ability, may enhance the texture and sensory quality of the product. Additionally, the results indicated that replacing a large portion of soy protein isolate with faba bean protein isolate is feasible. This modification may provide several advantages, including improved nutritional value due to the favorable amino acid profile of faba bean protein isolate, reduced risks of soy-related allergies, greater utilization of local and more economical protein sources, and improved textural characteristics of the final product. Therefore, the combination of faba bean protein isolate and *Lallemantia royleana* seed mucilage can serve as an efficient alternative to soy protein isolate and carboxymethyl cellulose in gluten-free bakery formulations.

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Conflict of Interest

The authors have no conflicts of interest to report.

Funding Statement

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مقاله علمی_پژوهشی

بهبود ویژگی های کیفی نان بدون گلوتن با استفاده از ایزوله پروتئین باقلا و موسیلاژ دانه بالنگوی شیرازی

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چکیده

نان به عنوان یکی از مهم‌ترین فرآورده‌های غذایی در بسیاری از کشورها به شمار می‌رود. وجود پروتئین گلوتن در گندم عامل اصلی ایجاد ساختار مطلوب خمیر و بهبود ویژگی‌های نان است. با این حال، حذف گلوتن از فرمولاسیون نان، به دلیل ضرورت‌های تغذیه‌ای یا ملاحظات سلامت برای بیماران مبتلا به سلیاک، چالش کاهش کیفیت نان را به همراه دارد. به همین دلیل، تحقیقات گسترده‌ای بر استفاده از جایگزین‌های هیدروکلوئیدی جهت جبران نقش عملکردی گلوتن متمرکز شده است. از این رو هدف از پژوهش حاضر، بهبود ویژگی‌های کیفی نان بدون گلوتن با استفاده از ایزوله پروتئین باقلا و موسیلاژ دانه بالنگوی شیرازی است. به منظور تعیین تیمارهای آزمایشی، از چهار متغیر مستقل شامل مقادیر مختلف ایزوله پروتئین سویا، ایزوله پروتئین باقلا، کربوکسی متیل سلولز و موسیلاژ دانه بالنگوی شیرازی استفاده شد. پس از افزودن مواد مذکور به فرمولاسیون و تهیه نمونه‌ها، آزمون‌های فیزیکوشیمیایی، بافتی و ارزیابی حسی انجام شد. نتایج آزمون ویژگی‌های بافتی خمیرآبه نشان داد که افزایش سهم ایزوله‌های پروتئین و موسیلاژ دانه بالنگوی شیرازی منجر به افزایش سفتی خمیرآبه می‌شود. به طوری که بیشترین و کمترین میزان سفتی به ترتیب در نمونه ۵ (۲ درصد ایزوله پروتئین سویا ، ۲ درصد ایزوله پروتئین باقلا، ۰/۲۵ درصد کربوکسی متیل سلولز، ۰/۲۵ درصد بالنگو) ۵۰۸ گرم و نمونه ۹ (٤/۷۵ درصد ایزوله پروتئین سویا و ۰/۲۵ درصد کربوکسی متیل سلولز) ۱۹ گرم مشاهده شد. استفاده از ایزوله پروتئین باقلا در مقایسه با ایزوله پروتئین سویا موجب کاهش افت پخت (کمترین میزان افت پخت مربوط به نمونه ۳ حاوی ٤ درصد ایزوله پروتئین سویا، ۰/۵ درصد کربوکسی متیل سلولز، ۰/۵ درصد موسیلاژ بالنگوی شیرازی به مقدار ۲۱/۵٦ درصد) و افزایش تخلخل (بیشترین میزان تخلخل مربوط به نمونه ۷ حاوی ٤/۵ درصد ایزوله پروتئین باقلا و ۰/۵ درصد موسیلاژ بالنگوی شیرازی به مقدار ۳۱/٦ درصد) گردید. به علاوه استفاده از کربوکسی متیل سلولز در مقایسه با موسیلاژ دانه بالنگوی شیرازی موجب کاهش بیشتر افت پخت شد. اما در رابطه با پارامتر تخلخل، هنگام استفاده هم‌زمان از ایزوله پروتئین سویا و موسیلاژدانه بالنگوی شیرازی افزایش قابل ملاحظه در پارامتر مذکور مشاهده شد. استفاده از ایزوله پروتئین سویا موجب افزایش سفتی بافت نان (بیشترین میزان سفتی مربوط به نمونه ۱۱، حاوی ٤/۷۵ درصد ایزوله پروتئین سویا و ۰/۲۵ درصد کربوکسی متیل سلولز به مقدار ۱۳۲۸/۲۷ گرم) و بالعکس ایزوله پروتئین باقلا موجب کاهش سفتی (کمترین میزان سفتی مربوط به نمونه ۱۲، حاوی ۵ درصد ایزوله پروتئین باقلا به مقدار ۱٤۰/۱۷ گرم) نمونه‌ها شد. استفاده هم‌زمان از ایزوله پروتئین سویا و موسیلاژدانه بالنگوی شیرازی موجب بهبود انسجام بافت نمونه‌های نان بدون گلوتن شد. به لحاظ شاخص قهوه‌ای شدن پوسته، تأثیر ایزوله پروتئین سویا در مقایسه با ایزوله پروتئین باقلا بیشتر است. در نهایت به منظور تعیین فرمولاسیون بهینه نان بدون گلوتن، مطابق با بخش بهینه یابی نرم افزار، مقادیر ۰/۹۸۱ درصد ایزوله پروتئین سویا، ۳/۵۹ ایزوله پروتئین باقلا و ۰/٤۲۲ درصد موسیلاژ دانه بالنگوی شیرازی بهترین فرمولاسیون جهت تهیه نان بدون گلوتن در این پژوهش است.