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Effect of incorporated soy protein isolate and microbial transglutaminase enzyme on the rheological and quality properties of gluten-free bread based on corn flour

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ABSTRACT

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This study aimed to evaluate corn-based gluten-free bread containing soy protein isolate and microbial transglutaminase enzyme. Different concentrations of soy protein isolate (0, 3 and 6%) and microbial transglutaminase enzyme (0, 0.75 and 1.5%) were used to prepare corn-based gluten-free bread and their effects on the rheological properties of the dough (loss and storage modulus) and physical (specific volume, baking loss, porosity, shell color brightness, hardness and moisture content of the bread) with factorial experiments (2×3) was evaluated in a completely randomized design. The results showed that the loss and storage modulus of corn-based gluten-free bread enhanced with increasing angular frequency in the rheometer range (0-100 1/s). The T0SPI6 sample (6% soy protein isolate without enzyme) and the T0SPI3 sample (3% soy protein isolate without enzyme) exhibited the highest storage and loss modulus compared to the control. Moreover, incorporating 0.75% microbial transglutaminase enzyme increased the specific volume and porosity of the bread. The highest baking loss (20.79%) was achieved when the formulation's enzyme and soy protein isolate concentrations were 0.75% and 0%, respectively. The influence of soy protein isolate concentration on the L^* index revealed that maximum brightness was achieved with a 6% concentration (88.60), showing no significant difference compared to the 3% concentration (87.95). Increasing the enzyme concentration to 1.5% in the corn-based gluten-free bread formulation resulted in a 71.49% increase in the bread's hardness. The results indicate that microbial transglutaminase (0.75%) and soy protein isolate (3%) can be effectively used to produce high-quality corn-based gluten-free bread.

1- INTRODUCTION

Bread, as a staple and essential commodity, holds a prominent position in household food consumption and constitutes a primary dietary component for a large proportion of the population worldwide. Unfortunately, individuals with celiac disease are unable to consume bread and other gluten-containing products [1]. Celiac disease is a gluten-induced disorder that occurs in genetically susceptible individuals. The alcohol-soluble prolamins, including gliadin in wheat, secalin in rye, avenin in oats, and hordein in barley, are among the major causative factors associated with this disease. Following the consumption of gluten-containing foods, individuals with celiac disease develop a range of clinical manifestations resulting from impaired gluten digestion, which, over time, can lead to damage to the intestinal villi of the small intestine. Vitamin D and calcium deficiencies due to intestinal malabsorption, osteoporosis, neurological and psychiatric disorders, infertility, sexual dysfunction, and gastrointestinal disturbances are among the clinical manifestations of celiac disease [2]. The disease may also cause weight loss, diarrhea, anemia, fatigue, bloating, and folate deficiency. There is currently no definitive cure for celiac disease, and the only effective treatment is the complete elimination of gluten-containing products from the diet and lifelong adherence to a gluten-free diet. The total daily gluten intake of individuals with celiac disease should not exceed 20 mg [1–3]. The flour used for the production of gluten-free bread must be derived from sources other than wheat. Corn flour is naturally gluten-free and has a relatively high protein content, energy value, and nutritional value. This flour is obtained from the endosperm of the grain, which contains approximately 75–87% starch and 6–8% protein [4]. Gluten elimination presents major challenges to the baking industry, and many gluten-free products currently available on the market are characterized by poor quality, mouthfeel, aroma, and flavor. This issue has created a major challenge in cereal technology and has prompted extensive efforts to identify suitable alternatives to gluten in gluten-free products [5]. Various ingredients, including

modified starches, hydrocolloids, enzymes, and proteins, have been used to improve the quality of gluten-free bread [5,6]. Microbial transglutaminase is a member of the transferase enzyme family that catalyzes the polymerization and covalent cross-linking of proteins and represents a relatively novel ingredient used in the production of bakery products. Studies have shown that the use of transglutaminase in combination with egg proteins can form a more stable network within gluten-free bread systems [6]. Other studies have also demonstrated that when a protein network is formed through cross-linking between rice proteins and other proteins by transglutaminase, the resulting structure can entrap gas in a manner similar to that of gluten-containing dough [7,8]. Soybean belongs to the Fabaceae family and is relatively deficient in sulfur-containing amino acids. Its nutritional and functional properties have made soybean a valuable raw material for the development of functional foods [9,10]. Soy isoflavones have demonstrated beneficial effects on bone tissue and can therefore be incorporated into foods intended to reduce the risk of osteoporosis. In addition, soy isoflavones have been associated with a reduced risk of cardiovascular diseases, decreased oxidation of low-density lipoproteins, and prevention of breast cancer [11]. Soy flour and other soybean-derived products are used in gluten-free products to increase protein content and improve structural properties. Soy protein isolate is the purest protein form among soybean products and is particularly useful when a high protein content and specific functional properties are required [10,12]. Soy protein isolate enhances the nutritional value and elastic modulus of bread. By increasing the elastic modulus and improving gas retention within the bread matrix, it can also increase loaf volume and enhance water binding within the bread. The high protein content and neutral flavor of soy protein isolate help prevent the undesirable flavor associated with bread produced using soy flour [12].

Several researchers have investigated the production of gluten-free breads using various alternative ingredients. These studies include the work of Ordóñez-Lavín et al. [13], who incorporated jujube powder; Taleb et al. [14], who incorporated carrot pomace powder, millet, and buckwheat; Ammar et al. [15], who

incorporated carob seed, chickpea, and rice flours; and Ito and Araki [16], who incorporated **Apios americana** tuber powder. The objective of the present study was to produce corn-based gluten-free bread containing soy protein isolate and microbial transglutaminase and to investigate its physicochemical and quality characteristics. To the best of our knowledge, based on the most recent literature available in this field, no previous study has investigated this specific combination of ingredients for the production of gluten-free bread.

2- Materials and Methods

2.1. Materials

Microbial transglutaminase enzyme (Artin Shimi Co.), soy protein isolate (Isatis Someh Co., Iran), guar gum, baker's yeast (Dr. Oetker, Turkey), salt, and sugar were used for bread preparation. Ethanol, selenium oxide, sulfuric acid, hydrochloric acid, potassium sulfate, copper sulfate powder, methyl red indicator, and hexane were obtained from reputable commercial suppliers. The equipment used in this study included a laboratory sieve, laboratory oven (Memmert, Germany), digital balance (GEC Avery, UK), Kjeldahl apparatus (V50, Iran), electric dough mixer (Philips, China), baking oven (Mashhad Baking Industries, Iran), texture analyzer (Lefra, USA), and rheometer (Physica Anton Paar, Austria).

2.2. Methods

2.2.1. Dough Preparation, Bread Baking, and Sample Preparation

Three levels of soy protein isolate (0, 3, and 6%) and three levels of microbial transglutaminase enzyme (0, 0.75, and 1.5%) were used for bread preparation. According to the method described by Ataei Salehi et al. [17], all dry ingredients, except sugar, were first weighed and passed through an 80-mesh sieve to ensure thorough mixing and appropriate hydration. Subsequently, an activated yeast suspension, prepared by suspending the yeast in 20 mL of water containing half of the required sugar for 15 min, was added to the dry ingredients, followed by the addition of the remaining water. The required dough was prepared using an electric mixer, and the mixing time was determined experimentally based on the consistency of the resulting dough and was set at 3 min. The prepared doughs were then separately transferred into galvanized molds

measuring $4 \times 6.5 \times 10$ cm, whose inner walls had been greased. For fermentation, the doughs were placed in a fermentation chamber (water bath) at 30 °C and 70% relative humidity for 20 min. The breads were subsequently baked in an oven at 200 °C using the upper heating element and 190 °C using the lower heating element for 35 min. After baking, the breads were removed from the molds and cooled at room temperature for 1 h. They were then packaged in sealed polypropylene bags measuring 20×18 cm and stored at room temperature until further analyses.

2.2.2. Chemical Properties of Corn Flour

The corn flour samples were analyzed using standard AACC methods. Moisture content was determined according to method No. 16-44, ash content according to method No. 7-8, protein content according to method No. 46-01, fat content according to method No. 10-30, and pH according to method No. 52-02 [18].

2.2.3. Rheological Properties of Corn-Based Gluten-Free Dough

The effects of whey protein concentrate, sodium caseinate powder, and microbial transglutaminase on selected rheological properties of the dough were determined using a rheometer according to the methods described by Demirkesen et al. [19] and Sun et al. [20]. Following sample preparation, the viscosity of the experimental samples was measured at 25 °C using a rheometer equipped with concentric-cylinder rheometry geometry. To determine shear stress and viscosity as a function of shear rate and to characterize the flow behavior of the samples, the shear rate was increased from 12 to 1100 s^{-1} over a period of 10 min.

2.2.4. Determination of Bread Specific Volume

The volume of the resulting bread samples was determined using the rapeseed displacement method [21]. Bread specific volume was subsequently calculated by dividing the volume of each sample by its weight.

2.2.5. Determination of Bread Baking Loss

The weight of the dough pieces and the weight of the bread samples after baking and cooling for 2–3 h were measured. Bread baking loss was then calculated using Equation (1) [22].

Equation (1):

Baking loss (%) = [(Weight of dough piece – Weight of baked bread) / Weight of dough piece] × 100

2.2.6. Evaluation of Bread Porosity

The porosity of the bread samples was evaluated using an image-processing technique. Images were captured using a digital camera with a resolution of 12 megapixels and subsequently analyzed using ImageJ software [23].

2.2.7. Evaluation of Crust Lightness (L*)

Changes in the lightness of the crust of the produced breads were evaluated using an image-processing technique. Images were captured with a 12-megapixel digital camera and analyzed using Adobe Photoshop software [24].

2.2.8. Bread Texture Hardness

The firmness of the crumb of the corn bread samples was measured and evaluated on the first day after baking using a texture analyzer. The maximum force required for penetration of a cylindrical probe with a diameter of 1.38 mm into the center of the bread crumb slices at a speed of 1 mm/s was calculated as an indicator of crumb firmness. A resting time of 1.5 s was considered. The bread crumb specimens had dimensions of 2 × 2 × 2 cm³, and compression was set at 50% of the initial height of the bread specimen. The trigger force and target distance were set at 5 g and 10 mm, respectively. The test was performed at 25 °C [25].

2.2.9. Determination of Moisture Content of Bread Crumb

Table 1. Chemical properties of corn flour used for gluten-free bread.

Properties	pH	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
Amount	5.83±0.01	10.79±0.31	0.42±0.01	7.36±0.02	1.25±0.02

3.2. Evaluation of the Storage (Elastic) Modulus (G') and Loss (Viscous) Modulus (G'') of the Dough

The storage modulus (G') represents the elastic behavior of a material, whereas the loss modulus (G'') reflects its viscous or flow behavior; both moduli are time-dependent parameters. Figures 1 and 2 illustrate some of the interactive effects of soy protein isolate and

The moisture content of the bread crumb was determined according to AACC method No. 16-44 based on the difference between the initial and final sample weights [18]. For this purpose, 5 g of bread crumb was separated and placed in an aluminum dish that had previously been dried to a constant weight. The dishes were then transferred to an oven maintained at 102–105 °C and kept there until a constant weight was achieved.

2.3. Statistical Analysis

Statistical analysis of the data concerning the effects of soy protein isolate and microbial transglutaminase on the physical characteristics of corn-based gluten-free bread was conducted using a completely randomized design with a factorial experiment (3 × 3). Analysis of variance (ANOVA) was performed at a significance level of $P < 0.05$. Mean comparisons were carried out using Duncan's multiple range test, and normalization was performed using Tukey's test at $P < 0.05$. All experiments were conducted in triplicate. Statistical analyses were performed using SAS software version 9.1, and graphs were generated using Microsoft Excel 2013.

3- Results and Discussion

3.1. Evaluation of the Chemical Properties of the Flour

The mean values of the chemical properties of the corn flour used in the present study, including moisture, ash, protein, and fat contents on a dry-matter basis, as well as pH, are presented in Table 1.

microbial transglutaminase on the rheological properties of corn-based gluten-free dough. According to the results obtained, the sample containing 3% soy protein isolate exhibited the highest loss modulus, indicating that this sample had a more fluid-like behavior than the other samples. Marko and Rosell [26], who incorporated soy protein and egg albumin, and Hu et al. [27], who incorporated carboxymethyl cellulose, reported similar findings in gluten-free bread.

The addition of the enzyme decreased the loss modulus in some samples compared with the control sample. With increasing angular frequency within the range of 0–100 s^{-1} , both moduli increased. The highest values of G' and G'' , compared with the control sample, were observed for T0SPI6 (the sample without enzyme and containing 6% soy protein isolate) and T0SPI3 (the sample without enzyme and containing 3% soy protein isolate), respectively. Moreover, the higher G' value compared with G'' indicates a greater predominance of elastic behavior over viscous behavior. Crockett et al. [7] reported similar results in their study investigating the effects of

soy protein isolate and dried egg white on the physicochemical properties of gluten-free bread based on rice and cassava flours.

The results of other studies have indicated that the addition of transglutaminase increases both the storage and loss moduli. This increase suggests that the dough becomes stronger compared with the control sample. The viscoelastic properties of dough are largely dependent on the protein phase [28], while the effect of soy protein isolate is strongly influenced by its inherent characteristics and nature [29].

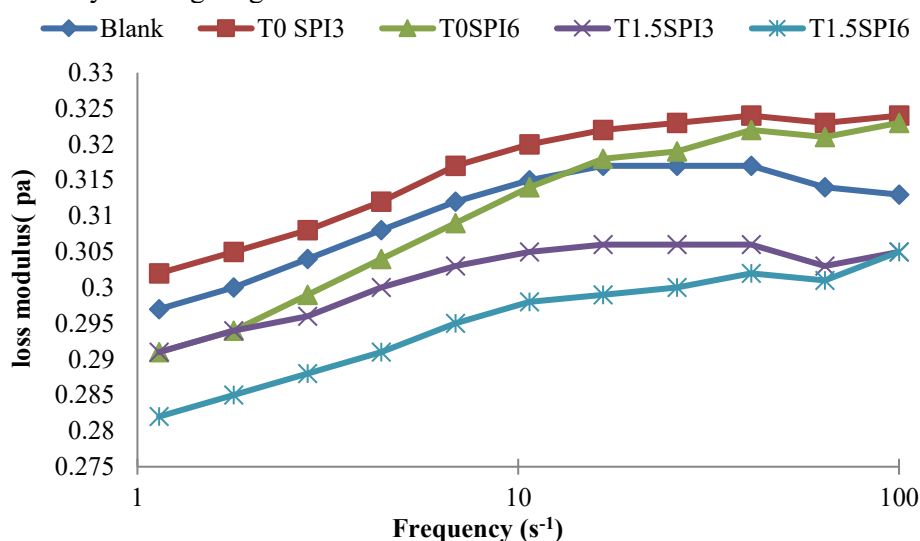


Figure 1. Effect of different concentration of the soy protein isolate and microbial transglutaminase enzyme on loss modulus of corn-based gluten free batter.

Blank: control; T0 SPI3: without enzyme + 3% soy protein isolate; T0 SPI6: without enzyme + 6% soy protein isolate; T1.5 SPI3: 1.5% enzyme + 3% soy protein isolate; T1.5 SPI6: without enzyme + 6% soy protein isolate

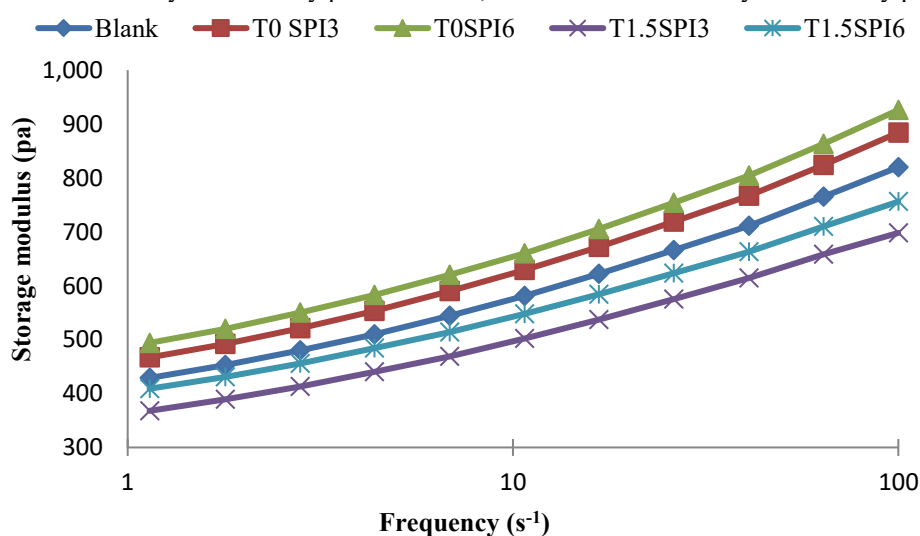


Figure 2. Effect of different concentration of the soy protein isolate and microbial transglutaminase enzyme on storage modulus of corn-based gluten free batter.

Blank: control; T0 SPI3: without enzyme + 3% soy protein isolate; T0 SPI6: without enzyme + 6% soy protein isolate; T1.5 SPI3: 1.5% enzyme + 3% soy protein isolate; T1.5 SPI6: without enzyme + 6% soy protein isolate

3.3. Effect of the Studied Variables on the Specific Volume of the Samples

According to the results obtained, all independent variables had a significant effect on the specific volume of the corn bread samples ($P < 0.05$). The addition of microbial transglutaminase up to a level of 0.75% resulted in an increase in bread specific volume, whereas a further increase in enzyme concentration

decreased the specific volume of the breads (Table 2). Consistent with these findings, Rosenthal et al. [30] reported that the addition of moderate levels of transglutaminase increased the specific volume of sorghum- and corn-based breads. Similarly, Deveux and Sadowska [31] observed an increase in specific volume in gluten-free bread containing red wheat, while Red et al. [28] reported a similar effect in gluten-free sourdough bread.

Table 2. Effect of different concentrations of the microbial transglutaminase enzyme on physical properties of gluten-free bread based on corn flour.

Transglutaminase concentration (%)	Moisture content (%)	Specific volume (ml/g)	Hardness (gN)	Porosity (%)	Baking loss (%)	L*
0	48.40 ^b	1.18 ^c	1655.9 ^c	16.56 ^b	16.92 ^{ab}	87.22 ^a
0.75	54.33 ^a	1.63 ^a	2270.3 ^b	19.35 ^a	17.68 ^a	83.74 ^b
1.5	53.20 ^a	1.43 ^b	2839.2 ^a	18.78 ^a	15.26 ^b	85.77 ^a

^{a-c} The mean in the column presented by different letters is significantly different ($P < .05$).

Other studies have also shown that the incorporation of transglutaminase at a level of 1% resulted in the highest bread volume and increased crumb softness of gluten-free rice bread compared with other enzyme levels. However, according to the study conducted by Mohammadi et al. [32], increasing the levels of microbial transglutaminase in guar-containing gluten-free bread resulted in a significant reduction in the specific volume of the resulting breads compared with the control bread. Transglutaminase promotes the formation of cross-links between glutamine and lysine residues. The resulting cross-linked network may restrict the expansion of gas cells during fermentation, thereby reducing the specific volume of the resulting breads. The findings

reported by Deveux and Sadowska [31] for red-wheat bread also support this explanation. As shown in Table 3, increasing the concentration of soy protein isolate in the bread formulation resulted in a reduction in the specific volume of the samples. This phenomenon may be attributed to the high strength of the structure formed and its greater resistance to the expansion of the gases produced during fermentation. The results of this section are consistent with the findings reported by Marko and Rosell [26] but are inconsistent with those reported by Deluzoska et al. [33]. Table 4 indicates that the maximum specific volume of the corn-based breads was obtained when the concentrations of microbial transglutaminase and soy protein isolate were 0.75% and 0%, respectively.

Table 3. Effect of different concentrations of the soy protein isolate on physical properties of gluten-free bread based on corn flour.

Soy proteins isolate concentration (%)	Moisture content (%)	Specific volume (ml/g)	Hardness (gN)	Porosity (%)	Baking loss (%)	L*
0	54.37 ^a	1.61 ^a	1466.5 ^c	18.37 ^b	17.77 ^a	84.62 ^b
3	49.22 ^c	1.39 ^b	2312.6 ^b	19.49 ^a	15.22 ^b	85.63 ^{ab}
6	52.32 ^b	1.24 ^b	2986 ^a	16.82 ^c	16.13 ^{ab}	86.50 ^a

^{a-c} The mean in the column presented by different letters is significantly different ($P < .05$).

3.4. Effect of Enzyme and Soy Protein Isolate Concentrations on Baking Loss

The results showed that increasing the enzyme concentration in the formulation initially increased the baking loss percentage, followed by a decrease at higher enzyme concentrations

(Table 2). Transglutaminase increases the ability of proteins to bind water, ultimately resulting in greater moisture retention in the bread after baking. The higher the moisture content of the bread after baking, the greater the final bread yield and, consequently, the lower the baking loss [34]. With increasing protein levels in the formulation, the baking loss percentage initially decreased, which can be

attributed to the greater moisture-retention capacity of the protein, and subsequently increased nonsignificantly (Table 3). These findings were consistent with those reported by Moore et al. [35]. According to the results presented in Table 4, the highest baking loss was obtained when the concentrations of enzyme and protein in the formulation were 0.75% and 0%, respectively.

Table 4. Interaction effects of microbial transglutaminase enzyme and soy protein isolate concentration on physical properties of gluten-free bread based on corn flour.

soy proteins isolate (%)	Transglutaminase (%)	Moisture content (%)	Specific volume (ml/g)	Hardness (gN)	Porosity (%)	Baking loss (%)	L*
0	0	54.66±0.1 ^a	1.24±0.02 ^{bc}	1699.5±3.5 ^d	16.93±0.10 ^{bc}	16.16±0.13 ^b	85.98±0.70 ^a
3	0	38.48±0.3 ^b	1.12±0.01 ^c	1138.2±4.7 ^e	16.63±0.11 ^{bc}	15.47±0.09 ^b	87.95±0.51 ^a
6	0	52.05±0.07 ^a	1.18±0.00 ^{bc}	2130.0±9.1 ^c	16.11±0.09 ^c	16.94±0.35 ^{ab}	88.60±0.80 ^a
0	0.75	54.26±0.05 ^a	2.09±0.12 ^a	1138.0±1.9 ^e	20.07±0.08 ^a	20.79±0.43 ^a	82.72±0.44 ^a
3	0.75	54.60±0.11 ^a	1.53±0.00 ^b	1580.7±8.9 ^d	21.27±0.32 ^a	15.97±0.08 ^b	84.02±0.67 ^a
6	0.75	54.13±0.50 ^a	1.28±0.01 ^{bc}	4092.3±7.3 ^a	16.72±0.16 ^{bc}	16.28±0.13 ^b	85.33±0.91 ^a
0	1.5	54.20±0.77 ^a	1.52±0.01 ^b	1562.0±2.1 ^d	18.12±0.20 ^b	16.33±0.22 ^b	85.33±0.62 ^a
3	1.5	54.60±0.23 ^a	1.51±0.01 ^b	4219.0±8.6 ^a	20.57±0.24 ^a	14.27±0.18 ^b	84.02±0.51 ^a
6	1.5	50.80±0.62 ^a	1.25±0.04 ^{bc}	2736.7±6.2 ^b	17.64±0.07 ^{bc}	15.18±0.15 ^b	87.29±0.69 ^a

^{a-c} The mean in the column presented by different letter is significantly different ($P < .05$).

3.5. Evaluation of Changes in Bread Porosity

The results showed that the addition of microbial transglutaminase up to a level of 0.75% increased bread porosity, whereas a further increase in enzyme concentration resulted in a nonsignificant decrease in porosity ($P > 0.05$) (Table 2). Based on the findings reported by Pour Esmaeil et al. [36], who investigated gluten-free bread formulations containing guar gum and microbial transglutaminase, microscopic images of the bread crumb showed that the addition of transglutaminase at a level of 1% resulted in a more uniform distribution of air bubbles and a reduction in their size compared with both the control and enzyme-free samples. However, increasing the enzyme level in these breads led to excessive compaction of the crumb as a result of the formation of a strong protein network, which restricted gas-cell expansion and produced a crumb structure with very few air cells. Consequently, bread porosity decreased.

Their findings were in complete agreement with the results obtained in the present study.

Table 3 also showed that the addition of soy protein isolate initially increased bread porosity, followed by a decrease at higher concentrations. The initial increase in porosity with increasing protein levels may be attributed to the strengthening of dough cells by the protein network, which reduces the loss of the gas produced during fermentation and consequently increases the size of the pores [37]. Deluzoska et al. [38] reported that the addition of transglutaminase to gluten-free bread at a level of 1 unit in combination with whey proteins resulted in a significant increase in bread porosity. Furthermore, increasing the enzyme level to 10 units produced more pronounced positive effects on crumb porosity compared with the 1-unit treatment.

According to previous studies, during the initial mixing stage, as the flour becomes hydrated and is transformed into a viscous dough, soluble and insoluble components migrate toward the gas bubbles. At this stage, the formation of the

bubble interface and bubble stability throughout the subsequent stages of breadmaking are affected. In addition, during dough proofing, the bubbles formed within the dough during mixing expand as a result of gas production by yeast. Therefore, bubble stability is of critical importance, with the primary cause of bubble instability being the coalescence of individual gas cells. The presence of thick layers surrounding the bubbles generates repulsive forces between them, thereby reducing the likelihood of coalescence [39,40].

The results concerning the interaction between enzyme and protein concentrations showed that the highest bread porosity was obtained when the concentrations of microbial transglutaminase and soy protein isolate were 0.75% and 3%, respectively. However, this treatment did not differ significantly in porosity from some of the other treatments (Table 4).

3.6. Effect of Enzyme and Soy Protein Isolate Concentrations on Crust Lightness (L*)

The analysis of the data showed that, among the studied variables, only enzyme concentration had a significant effect on crust lightness, whereas soy protein isolate concentration and the interaction between enzyme and soy protein isolate had no significant effects on crust lightness ($P > 0.05$). As shown in Table 2, the L* value initially decreased by 3.98% with increasing enzyme concentration up to 0.75%, whereas a further increase in enzyme concentration resulted in an increase in the L* value.

The effect of soy protein isolate concentration on the L* value showed that the highest crust lightness was obtained at a soy protein isolate concentration of 6%, although this value did not differ significantly from that obtained at 3% soy protein isolate. Bread crust color is primarily formed through the Maillard reaction, in which the reaction between nitrogen-containing compounds and reducing substances leads to browning in the surface layers. A portion of the bread crust color is also attributable to sugar caramelization. Naturally, the intensity of crust coloration depends on the concentration of the compounds involved and the conditions under which these compounds react, such as temperature and dehydration of the bread surface, both of which strongly influence the rate and intensity of color development [41].

Color measurement in bakery products is essential for evaluating product quality, determining the effects of different ingredients incorporated into the formulation, assessing the effects of changes in baking-process variables, and evaluating storage conditions [42]. Gallagher et al. [43] reported that the addition of dairy powders to gluten-free bread resulted in a reduction in the L* value compared with the control sample, which was attributed to the presence of small amounts of lactose in these ingredients. Lactose can participate in nonenzymatic browning reactions, including the Maillard reaction and caramelization.

It has also been reported that increasing whey protein levels in gluten-free rice bread decreases the L* color index while increasing the b* index (yellowness), and that the b* values of these two proteins were higher than those of sodium caseinate [44,45]. According to Zibro et al. [46], the type of protein has a significant effect on the L* value. The presence of albumin increased the L* index, whereas the incorporation of soy protein into gluten-free bread samples decreased lightness [5]. These findings were inconsistent with the results obtained in the present study.

3.7. Evaluation of Changes in Sample Hardness

The resistance of bread crumb to deformation is referred to as firmness and is considered an important indicator of bread staling. Given the role of gluten in retarding the staling phenomenon, this characteristic is more prevalent in gluten-free bread [31]. The results presented in Table 2 showed that increasing the enzyme concentration to 1.5% in the formulation of corn-based gluten-free bread increased bread hardness by 71.49%. Deluzoska et al. [33], in their investigation of the effect of microbial transglutaminase addition on gluten-free bread, reported that increasing the enzyme level resulted in a firmer bread structure. The effectiveness of microbial transglutaminase depends on the type of protein used as the substrate for the enzyme, particularly the availability of lysine and glutamine residues within the protein structure [28]. Similarly, Rosenthal et al. [30] reported that the addition of transglutaminase at a level of 10 units, compared with 1 unit, resulted in increased hardness of gluten-free sorghum bread. The results reported by Moore et al. [35]

for gluten-free bread also showed that increasing the enzyme level in samples containing skim milk powder increased crumb hardness. Other studies have likewise reported that increasing enzyme levels resulted in increased textural hardness of rice bread [44].

Table 3 showed that increasing the level of soy protein isolate in the formulation increased sample hardness, probably because of the high protein content of soy protein isolate, which forms strong interactions with water and consequently reduces moisture migration from the crumb toward the crust [43]. Regarding the interaction effect on bread crumb hardness, the incorporation of 6% soy protein isolate together with 0.75% microbial transglutaminase into gluten-free bread resulted in the highest bread hardness (Table 4). Marko and Rosell [26] also reported similar findings, demonstrating an improvement in the firmness of gluten-free bread through the use of soy protein isolate and microbial transglutaminase.

3.8. Effect of Enzyme and Soy Protein Isolate Concentrations on Bread Crumb Moisture Content

The results showed that increasing the enzyme level up to 0.75% in the bread formulations increased the moisture content of the bread crumb (Table 2). This increase can be attributed to the formation of a cross-linked network between glutamine and lysine residues as a result of transglutaminase activity. The resulting network has the ability to entrap water and, consequently, increase the water-holding capacity of the bread matrix [47,48]. On the other hand, the increase in water absorption capacity may also be attributed to the deamidation of glutamine, which occurs through the action of transglutaminase and results in its conversion to glutamic acid. This reaction reduces the hydrophobicity of the system and consequently increases water absorption [35]. The findings of this section were consistent with those reported by Pour Esmaeili et al. [36].

As shown in Table 3, increasing the protein content of the formulation initially decreased the moisture content of the bread crumb, followed by an increase at higher protein levels. According to Maleki et al. [3], the incorporation of soy flour into rice bread formulations can increase crumb moisture because of its high

fiber content and the presence of hydroxyl groups in its structure, which are capable of interacting with and binding water molecules present in the formulation [32]. These findings were highly consistent with the reports of Nasiri et al. [49] and Ordóñez-Delgado et al. [50] concerning other gluten-free cereal products. The lowest moisture content of the samples was obtained when the concentrations of enzyme and soy protein isolate in the formulation were 0% and 3%, respectively (Table 4).

4-Conclusion

In the present study, the effects of incorporating soy protein isolate and microbial transglutaminase on the rheological and physical properties of corn flour-based gluten-free bread were investigated. The results demonstrated that the incorporation of soy protein isolate at different concentrations significantly affected the physical characteristics of the bread, including specific volume, hardness, and crust color. Furthermore, the addition of microbial transglutaminase improved the rheological properties of the dough and contributed to enhanced bread quality. Increasing the concentration of this enzyme resulted in a considerable increase in bread hardness, indicating its substantial influence on the structure and texture of the bread. Given the increasing demand for gluten-free products, the findings of this study may contribute to the development of high-quality and nutritionally improved gluten-free bread and highlight the importance of addressing the dietary requirements of consumers with gluten sensitivity and other nutritional concerns. Overall, based on the findings obtained in the present study, the optimum concentrations of microbial transglutaminase and soy protein isolate in the formulation of corn-based gluten-free bread can be considered to be 0.75% and 3%, respectively.

Data Availability

The data generated and/or analyzed during the current study are available from the corresponding author upon request.

Conflict of Interest

The authors declare that they have no financial or non-financial conflicts of interest related to the publication of this article.

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Authors Contributions

Hanieh Mahalleh: Conducted the experiments, collected the data, contributed to data analysis, and prepared the initial draft of the manuscript; Zahra Mazloom: Contributed to the study design, experimental work, data collection and analysis, and scientific review of the manuscript; Mehdi Gharekhani: Conceived and supervised the study, contributed to the interpretation of the results, critically reviewed and edited the manuscript, and served as the corresponding author; Kazem Alirezalu: Contributed to the study design, analysis and interpretation of the results, and scientific review of the manuscript.

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AI tools were used in approximately 10% of the preparation of the English version, solely for translation and language editing. All scientific content and conclusions were developed and verified by the authors.

5-References

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تأثیر افزودن ایزوله پروتئین سویا و آنزیم ترانس گلوتامیناز میکروبی بر خواص رئولوژیکی و کیفی نان بدون گلوتن بر پایه آرد ذرت

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اطلاعات مقاله

چکیده

هدف از این مطالعه بررسی ویژگیهای نان بدون گلوتن بر پایه ذرت دارای ایزوله پروتئین سویا و آنزیم ترانس گلوتامیناز میکروبی بود. در این تحقیق برای تهیه نان بدون گلوتن بر پایه ذرت از سه سطح غلظت ایزوله پروتئین سویا (۰، ۳ و ۶ درصد) و سه سطح غلظت آنزیم ترانس گلوتامیناز میکروبی (۰، ۰/۷۵ و ۱/۵ درصد) استفاده شد و تأثیر آنها بر ویژگیهای رئولوژیکی خمیر (مدول افت و ذخیره) و فیزیکی (میزان حجم مخصوص، افت پخت، تخلخل، روشنایی پخته، سختی و رطوبت مغز نان) با آزمایشات فاکتوریل (۲×۳) در قالب طرح کاملاً تصادفی مورد ارزیابی قرار گرفت. نتایج نشان داد که با افزایش فرکانس زاویه‌ای در محدوده (۱۰۰^{۱/۵} - ۰) در رئومتر هردو مدول افت و ذخیره نمونههای نان بدون گلوتن بر پایه ذرت افزایش یافتند. بالاترین میزان دو مدول ذخیره و افت در مقایسه با نمونه شاهد، به ترتیب به نمونه‌ی TOSPI6 (نمونه فاقد آنزیم و ۶ درصد ایزوله پروتئین سویا) و TOSPI3 (نمونه فاقد آنزیم و ۳ درصد ایزوله پروتئین سویا) تعلق داشت. افزودن آنزیم ترانس گلوتامیناز میکروبی تا سطح ۰/۷۵ درصد، منجر به افزایش حجم مخصوص و تخلخل نان‌ها گردید و با افزایش غلظت ایزوله پروتئین سویا در فرمولاسیون، حجم مخصوص نمونه‌ها کاهش یافت. بیشترین میزان افت پخت (۲۰/۷۹٪) زمانی به دست آمد که غلظت آنزیم و پروتئین در فرمولاسیون به ترتیب ۰/۷۵ و صفر درصد بود. تأثیر غلظت ایزوله پروتئین سویا بر شاخص L* نیز نشان داد که بیشینه میزان روشنایی مربوط به غلظت ۶ درصد ایزوله پروتئین سویا (۸۸/۶۰) بود که تفاوت معنی‌داری با غلظت ۳ درصد (۸۷/۹۵) آن نداشت. افزایش غلظت آنزیم تا سطح ۱/۵ درصد در فرمولاسیون نان بدون گلوتن بر پایه ذرت، ۷۱/۴۹ درصد سختی نان را افزایش داد. با توجه به اطلاعات به دست آمده می‌توان ادعان کرد که کاربرد آنزیم ترانس گلوتامیناز میکروبی (۰/۷۵٪) و ایزوله پروتئین سویا (۳٪) میتواند در تولید نان بدون گلوتن بر پایه ذرت با کیفیت بالا مورد استفاده قرار گیرد.

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