

## The Effect of Sodium Caseinate and Microbial Transglutaminase Enzyme on Rheological, Physical and Sensorial Properties of Corn-based Gluten Free Bread

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### Abstract

Celiac disease is a common autoimmune disorder which is triggered by receiving gluten. In this study, corn flour was used as gluten-free flour. Therefore, in order to simulate the properties of gluten, the use of enzymes with cross-linking ability such as microbial transglutaminase at levels of (0, 0.75 and 1.5%), with sodium caseinate at levels of (0, 3 and 6%) were used as a substitute for gluten and their effect on rheological properties of dough, physical and sensory properties of corn gluten-free bread was evaluated. The results indicated that with increasing angular frequency, both the storage and drop modules increased. The specific volume of breads produced from corn was obtained when the specific volume concentration of the transglutaminase enzyme and sodium caseinate were in the highest amount ( $1.460^{\pm 0.02}$ ). The addition of enzyme and sodium caseinate to the bread formulation reduced the baking loss in comparison to the sample without these materials. The addition of the microbial transglutaminase enzyme to 0.75% resulted in a significant decrease in porosity and then the porosity increased by increasing the enzyme concentration. The addition of 3% of sodium caseinate along with 0.75% of the microbial transglutaminase enzyme to gluten-free bread resulted in the highest bread stiffness. The final acceptance of the produced samples decreased by increasing the enzyme and protein, so that the samples lacking these two compounds obtained the highest final acceptance score. Finally, according to the results, it can be concluded that using 0.75% of the microbial transglutaminase enzyme and 3% of sodium caseinate can be helpful for the production of gluten-free corn bread.

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### Keywords

Corn Flour  
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### Introduction

Bread is basic components of the diet in many countries, due to the regional, cultural, economic and social characteristics of the bread and provides the main part of daily energy and

protein. In addition, the amount of vitamin, iron and calcium intake from bread is also significant (Rajabzade, 1993; Aminpour, 2006). Celiac patients cannot tolerate the gluten fraction of wheat in bread. Celiac disease is a

gastrointestinal disease, which gluten consumption causes inflammation and swelling of the small intestine and result in indigestion and lack of absorption of micronutrients and vitamins (Tayebi *et al.*, 2014). The only way to overcome this problem is to follow a gluten-free diet (Deora *et al.*, 2014). Several studies in the literature demonstrate the high quality flour is rich in protein, diet fiber, vitamins, magnesium, micro element and antioxidant produce bread (Badiu *et al.*, 2014).

Since gluten is an essential component of bread, the main technological problem in this context is the removal of gluten and its replacement by other components. Gluten is the main constitutive protein of dough structure that is composed of gliadin and glutenin. The absence of gluten often leads to the production of relatively liquid dough and can result in the production of bread with crisp texture, poor color, low volume, and other quality defects. In recent years, research and developments are significantly increased to produce gluten-free breads that include the use of flour or a combination of gluten-free flours with hydrocolloids, enzymes, and dairy elements as gluten substitutes to improve the structure and durability (Rostamian *et al.*, 2014).

The use of proteins with different sources in gluten-free breads formula leads to improved nutritional and functional properties. Moreover, proteins due to the formation of network structure react to gluten-free cereal proteins like wheat gluten network and can maintain the dough structure during fermentation. Also, proteins due to increased elastic modulus (through creating cross links), improved quality and taste (through increased millard browning reaction), improved structure and facilitation of foam formation in

dough are added to gluten-free bread. Since the dough used in gluten-free bread should not be prepared from wheat, corn flour is a good alternative for this purpose (Sciarini *et al.*, 2010; Rostamian *et al.*, 2014). The nutritional value of corn is significantly lower than wheat and its protein lacks gluten; therefore, it cannot be used to produce high-quality bread alone (Payan, 2004). For this reason, components that have the ability to mimic gluten properties will be used in corn flour dough to improve bread properties (Matos *et al.*, 2013; Moore, 2006).

Among enzymes that are used in food industry, transglutaminase, due to modification and promotion of protein network in various food systems, has a special place (Renzetti *et al.*, 2008; Deora *et al.*, 2014). Microbial transglutaminase (MTG) enzyme produced from *Streptovorticulum* spp. The microbial transglutaminase enzyme catalyzes the acyl group transfer reaction of lysine glutamine bond in food proteins such as egg, milk, soya and wheat (Tseng & Lai, 2002). This molecular bond is such as other dipeptide bond and has non-effect on nutrition value (Pourmohammadi *et al.*, 2012). Studies showed that whey protein and transglutaminase had good effect on quality of gluteen-free bread (Dłużewska *et al.*, 2015).

Sodium caseinate is a surfactant agent due to the fact that amphiphilic nature of the protein is applied as an emulsifier, thickener and foaming agent and increases water absorption in flour (Kenny *et al.*, 2000).

Gallagher *et al.* (2003) studied the effect of adding rice starch and isolating milk protein on gluten-free-bread. They found this ingredient increased volume and improved appearance and this product was like wheat (Gallagher *et al.*, 2003). Brites *et al.* (2010)

investigated the effect of different types of corn (i.e. regional and hybrid), milling process, formulation, and processing variables on the quality, sensitive and functional properties (i.e. special volume, texture, and color) of gluten-free bread. They stated that a significant difference exists between two types of corns in terms of protein, amylose, and minimum, maximum and final viscosity. Also, low speed of water milling compared with electric mill created a flour with less ash and higher minimum, maximum, and final viscosity. According to corn starch gelatinization, flour bleaching increases consistency, viscosity, and elasticity of dough (Brites *et al.*, 2010).

Nunes *et al.* (2009), have studied the effect of low lactose dairy powder, sodium caseinate, milk protein isolate, isolate and whey protein concentrate (WPC) on the properties of rice-based gluten-free bread. This investigation showed that the reduction of hardness and increasing of specific volume over time by adding low lactose dairy powder, milk protein isolate and whey protein concentrate (WPC). Sodium caseinate had the opposite effect and hardness increased specific volume reduced (Nunes *et al.*, 2009).

According to recent investigatin and importance of celiac food, the objective of this study was to assess the impact of caseinate sodium and microbial transglutaminase enzyme on quality properties of corn-based gluten-free bread.

## Material and methods

### Materials

In this study, for the preparation of gluten-free bulk bread microbial transglutaminase enzyme (Artin Chemical, Iran), sodium caseinate (Iran Caseinate, Iran), guar gum (Sigma, Iran) and bakery yeast (Dr. Oetker, Turkey)

have been used. Salt, sugar and corn flour were bought from local markets.

### Dough preparation and baking bread

In this research sodium caseinate was added to the flour at 3 levels (0, 3 and 6%) and microbial transglutaminase enzyme was also added at 3 levels on (0, 0.75 and 1.5%) percent flour basis. This bread was produced using a method described by Ataye Salehi *et al.* (2012). Each sample was sieved and screened of 80 mesh (except sugar). Active yeast suspension was prepared by 20 ml water contain sugar at fermentation time 15 min, and then was added to the mixture. All components have been mixed in mixer for 3 min (in order to achieve the desired consistency of dough). The dough was placed in galvanized molds 4×6.5×10 cm that where the process of fermentation was continued in oven at 30 °C, relative humidity 70% for 20 min. Baking was proceeded in the oven in temperature 200 °C (high flame) and 190 °C min (low flame) for 35 min. After backing, the breads were cooled and packed in polypropylene bags in 18×20 cm and were stored in room temperature (Ataye Salehi *et al.*, 2012).

### Corn-dough chemical properties

The sample of corn flour was tested using methods of AACC (2000), moisture content with the number 16-44, the percentage of ash in the method of 7-8, the protein percentage by the method of 1-46, the percentage of fat by the method of 10-30 and the pH was evaluated according to the 52-02 method (AACC, 2000).

### Rheological properties of corn based gluten free dough

The effects of sodium caseinate powder and microbial transglutaminase enzyme on some rheological properties of the

dough were evaluated with the rheometer following the method of Demirkesen *et al.* (2010). The loss modulus and the storage modulus measurements were conducted using a Physica Anton Paar rheometer (MCR 301, Austria) equipped with a parallel plate geometry. Frequency sweep test was determined in the range from 1 to 100 S<sup>-1</sup>. All measurements were performed at 25 °C. In the case of the dynamic oscillatory experiments, first linear viscoelastic region was determined in range of 1 Hz (Demirkesen *et al.*, 2010).

### Hardness

The hardness of bread samples was measured with a texture Analyzer at 25 °C. The slices with a thickness of 2×2×2 cm<sup>3</sup> were cut from the center of the loaves, and then the slices were relaxed for 1.5 s. the bread slice was compressed 50% of first height. Start and final points respectively were 5 g and 10 mm (Lazaridou *et al.*, 2007).

Measurements were done at the speed of movement of the head 1 mm/s with a cylinder of a diameter of 38.1 mm.

### Bread specific volume

The specific volume of the breads was determined by rapeseed grape displacement method (Gujral *et al.*, 2003).

### Weight loss

The weight of the dough and the weight of the bread samples were measured after baking and cooling for 2-3 h weight loss ratio was calculated according Eq. (1), (Soleimanifard *et al.*, 2013).

$$\text{Weight loss percentage} = A-B/A \times 100 \quad (1)$$

A: dough weight

B: bread weight after baking

### Porosity

To evaluate the porosity of the samples, the image processing method was measured with a digital camera (12 megapixel, Canon, Japan) and Image J software (Shahidi *et al.*, 2011). Vertical slice of bread was prepared placed in a wooden box of imaging chamber. The angle between lens axis of the camera and the light source was fixed in 45 degrees angle. The images were analyzed by Image J software. This test was performed after baking in three replications (Fani Sadrabadi *et al.*, 2013).

### Sensory evaluation

The sensory analysis was conducted with a group of 10 panelists. Breads were submitted for an acceptance test to determine the overall acceptance by using Eq. (2) panelists were asked to assess the breads acceptability by evaluation coefficients 1, 2, 2, 4, 3, 3, and 3, respectively for of color, springiness, porosity, color, stiffness, flavor and chewing properties, and to rate samples from 1 to 5 (1 unacceptable and 5 very acceptable) (Katina *et al.*, 2006).

$$Q = \frac{\sum(P \times G)}{\sum P} \quad (2)$$

Q: Overall acceptance (Bread Quality Number), P: coefficient of characteristics rating and G: coefficient of characteristics Evaluation.

### Statistical Analysis

The effect of sodium caseinate powder and microbial transglutaminase enzyme on the physical and sensory properties of corn-free gluten bread was carried out in a completely randomized design with factorial experiment (2×3). If significant difference was found out, Duncan's multiple comparison test was

used ( $P \leq 0.05$ ). Statistical analyses were performed using SAS software version 9.1 and drawing charts with Microsoft Excel (2013), 3 replications were used for the measurement of bread samples.

**Table 1.** Chemical properties of corn flour used in this research

Properties	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	pH
Content	10.79±0.11	0.42±0.1	7.365±0.05	1.25±0.08	5.83±0.01

### Viscoelastic properties

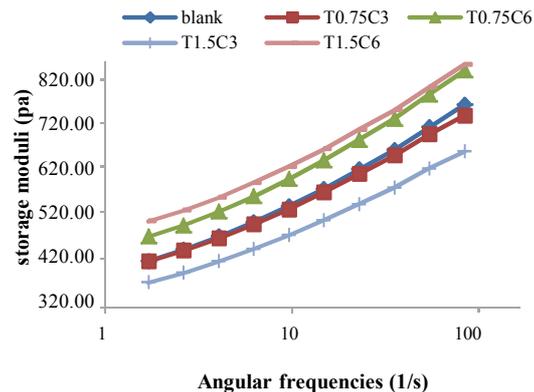
Elastic modulus and loss modulus demonstrate flow behavior of material and both of these parameters are time-dependent. Figure (1) and (2), show effect of sodium caseinate and microbial transglutaminase enzyme on rheological properties of free-gluten dough.

The result showed that elastic moduli ( $G'$ ) and loss moduli ( $G''$ ) increased in frequency sweep (0-100  $s^{-1}$ ) and T1.5C6 (1.5% enzyme+ 6% sodium caseinate) had low  $G'$  and  $G''$  compared with T1.5C3 (1.5% enzyme+ 3% sodium caseinate) and controlled sample. Also, higher value of  $G'$  than  $G''$  shows its elastic behaviour. Matos *et al.*, (2014) reported  $G'$  and  $G''$  increased with the addition of caseinate sodium in rice-based gluten-free bread (Matos *et al.*, 2014). Several researches showed WPI and WPC reduce both  $G'$  and  $G''$  moduli while the sodium caseinate reduce  $G'$  and  $G''$  moduli. Addition of caseinate increased  $G''$  moduli more considerably than whey protein isolate. Also, WPC decreased  $G'$  moduli of frozen dough (Kenny *et al.*, 2000). Gujral *et al.* (2003) found microbial transglutaminase enzyme increased both  $G'$  and  $G''$  modulus and this increment was more prominent at higher concentration of enzyme. Protein had a significant effect on viscoelastic properties of the dough (Gujral *et al.*, 2003).

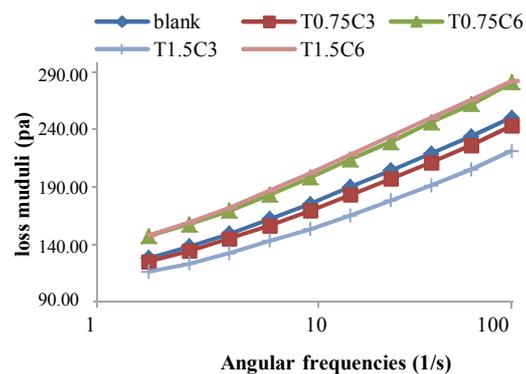
## Result and discussion

### Chemical properties of corn flour

Chemical properties such as moisture, ash, protein and fat (dried based) and pH showed in Table (1).



**Figure 1.** The effect of sodium caseinate powder and microbial trans-glutaminase enzyme on gluten-free paste based on corn flour (T0.75C3: 0.75% transglutaminase +3% sodium caseinate; T0.75C6: 0.75% transglutaminase +6% of sodium caseinate; T1.5C3: 1.5% transglutaminase +3% of caseinate sodium and T1.5C6: 1.5% transglutaminase +6% sodium caseinate)



**Figure 2.** The effect of sodium caseinate powder and microbial transglutaminase enzyme on moduli of corn based gluten free flour (T0.75C3: 0.75% transglutaminase + 3% of caseinate; T0.75C6: 0.75% transglutaminase +6% sodium caseinate; T1.5C3: 1.5% transglutaminase +3% of sodium caseinate and T1.5C6: 1.5% transglutaminase +6% sodium caseinate)

### Special volume

Bread volume depended on the ability of the protein network to maintain the carbon dioxide gas during fermentation (Smerdel *et al.*, 2013; Katina *et al.*, 2006). The results showed all independent variables had a significant

effect on the specific volume ( $P \leq 0.01$ ). Addition of 0.75% transglutaminase on the surface resulted in a significant increase in the specific volume of bread, while further increase in the enzyme had negative impact (Table 2).

**Table 2.** Effect of Content of microbial transglutaminase enzyme on measured parameters

Content of microbial transglutaminase enzyme (%)	Specific volume (ml/g)	bread weight loss (%)	Tissue porosity (%)	Stiffness (g)	Overall score
0	1.239 <sup>b</sup>	20.59 <sup>a</sup>	21.83 <sup>a</sup>	1809 <sup>b</sup>	4.06 <sup>a</sup>
0.75	1.352 <sup>a</sup>	14.42 <sup>c</sup>	17.90 <sup>c</sup>	2159 <sup>a</sup>	4.03 <sup>b</sup>
1.5	1.329 <sup>a</sup>	15.23 <sup>b</sup>	20.63 <sup>b</sup>	2155 <sup>a</sup>	3.66 <sup>c</sup>

Means followed by different letters within a column are significantly different ( $P \leq 0.05$ ).

According to findings Renzetti *et al.* (2008), addition of the transglutaminase enzyme in sorghum and corn bread resulted in an increase in specific volume. Also, according to other studies, addition of 1% transglutaminase resulted in the highest volume and increased in softness of rice-based gluten free bread to compare with other samples (Renzetti *et al.*, 2008).

According to studies by Mohammadi *et al.* (2014), with increasing in level of microbial transglutaminase to guar-based gluten free bread had a significant effect and reduced the specific volume of breads compared with control sample. The transglutaminase enzyme created a cross-link bound between the glutamine and lysine amino acids that can prevent the growth of gas cells during fermentation and reduce the specific volume of the breads.

The results of Pourmohammadi *et al.* (2012) showed that adding transglutamines to wheat and barley flour-based breads, decreased the volume of breads, which can be attributed to the formation of cross-links between proteins.

In another study, the effect of adding the microbial transglutaminase on rice bread was tested and the results showed that increased enzyme concentration

had a negative effect on the specific volume of bread, which is due to the high ability of transglutaminase in the conversion of weak gluten to strong gluten structure.

At high concentrations, transglutaminase has excessive cross-linking, and then dough becomes stronger. This explanation can also be justified in the case of corn meal dough when using 0.75% level compared to 1.5% (Gujral & Rosell, 2004). Table (3) shows that increasing in the concentration of sodium caseinate, the volume of bread reduce at first, which is in agreement with the findings of Nunes *et al.* (2009). The researchers reported the addition of sodium caseinate led to a reduction in specific volume. Several studies reported that sodium caseinate reduced specific volume. Caseinate Sodium is a highly soluble composition that can easily be dispersed in an aqueous and homogenized mixture containing of oil or fat. On the other hand, breads containing sodium caseinate are affected by the starch phase and protein network did not form in the bread structure. Casein is resistant to heat so it prevents the accumulation of casein to create a protein network during cooking.

**Table 3.** Effect of sodium caseinate content on measured specimens

Sodium Caseinate Content (%)	Specific Volume (ml/g)	Bread Weight Loss (%)	Tissue Porosity (%)	Stiffness (g)	Overall Score
0	1.343 <sup>a</sup>	21.93 <sup>a</sup>	22.83 <sup>a</sup>	1864 <sup>b</sup>	4.29 <sup>a</sup>
3	1.224 <sup>b</sup>	14.75 <sup>b</sup>	18.60 <sup>b</sup>	2537 <sup>a</sup>	3.87 <sup>b</sup>
6	1.353 <sup>a</sup>	13.57 <sup>c</sup>	18.93 <sup>b</sup>	1722 <sup>c</sup>	3.59 <sup>c</sup>

Means followed by different letters within a column are significantly different ( $P \leq 0.05$ ).

The dominance of the starch phase on casein led to the formation of a weak gel that reduced bread volume. Therefore, the difference in the thermal stability of whey protein and casein can play a major role in the final structure of bread. The use of 6% protein levels in comparison with other levels resulted in a significant increase in specific volume in corn gluten-free breads.

Sahraeian *et al.* (2013), in studying

the effect of water-based powder on gluten-free sorghum bread, showed that the addition of whey protein powder at a level of 6% compared to the control sample resulted in a significant increase in the volume. Table (4) showed the maximum specific volume of breads derived from corn were obtained when the levels of the enzyme transglutaminase and sodium caseinate were in their highest concentration.

**Table 4.** Interaction of the concentration of microbial transglutaminase and sodium caseinate in the measured parameters

Sodium Caseinate Content (%)	Content of microbial transglutaminase enzyme (%)	Specific Volume (ml/g)	Bread Weight Loss (%)	Tissue Porosity (%)	Stiffness (g)	Overall Score
0	0	1.28±0.02 <sup>bc</sup>	32.00±0.45 <sup>a</sup>	22.70±0.50 <sup>b</sup>	1567±2.33 <sup>h</sup>	4.59±0.01 <sup>a</sup>
3	0	1.22±0.04 <sup>c</sup>	15.74±0.36 <sup>c</sup>	20.70±0.31 <sup>c</sup>	2071±0.99 <sup>d</sup>	4.01±0.01 <sup>c</sup>
6	0	1.21±0.01 <sup>c</sup>	14.04±0.21 <sup>ef</sup>	22.10±0.18 <sup>b</sup>	1789±2.10 <sup>f</sup>	3.49±0.01 <sup>g</sup>
0	0.75	1.45±0.07 <sup>a</sup>	15.26±0.13 <sup>cd</sup>	19.00±0.03 <sup>d</sup>	1448±1.61 <sup>i</sup>	4.40±0.01 <sup>b</sup>
3	0.75	1.22±0.00 <sup>c</sup>	14.54±0.22 <sup>de</sup>	17.30±0.61 <sup>e</sup>	3451±7.80 <sup>a</sup>	4.00±0.01 <sup>d</sup>
6	0.75	1.38±0.07 <sup>a</sup>	13.47±0.50 <sup>f</sup>	17.40±0.11 <sup>e</sup>	1580±2.22 <sup>g</sup>	3.79±0.03 <sup>f</sup>
0	1.5	1.30±0.01 <sup>bc</sup>	18.53±0.70 <sup>b</sup>	26.80±0.81 <sup>a</sup>	2576±3.13 <sup>b</sup>	3.89±0.01 <sup>e</sup>
3	1.5	1.23±0.00 <sup>c</sup>	13.98±0.01 <sup>ef</sup>	17.80±0.20 <sup>de</sup>	2091±1.55 <sup>c</sup>	3.60±0.01 <sup>g</sup>
6	1.5	1.46±0.02 <sup>a</sup>	13.19±0.09 <sup>f</sup>	17.30±0.61 <sup>e</sup>	1799±4.25 <sup>c</sup>	3.50±0.02 <sup>h</sup>

Means followed by different letters within a column are significantly different ( $P \leq 0.05$ ).

### Bread weight loss

The results showed that by increasing the enzyme levels in the formulation, a significant decrease in the percentage of bread weight loss was initially observed (Table 2). As the protein levels increased, the percentage of bread weight loss decreased due to their ability to maintain moisture (Table 3).

Fani Sadrabadi *et al.* (2013) stated that the addition of 4% of caseinate sodium, in comparison with 2% of this protein, produced the lowest amount of bread weight loss. By increasing sodium caseinate, moisture content in bread increased and because of its high ability to maintain moisture, the percentage of

bread weight loss decreased.

Bread weight loss indicates weight loss due to baking or evaporation in bread, which is economically important because of the final weight loss of the bread (Soleimanifard *et al.*, 2013). Table (4) also showed that the lowest amount of bread weight loss was obtained, with the highest concentrations of enzyme and protein used in the formulation.

### Texture porosity

Porosity is one of the most important parameters for determining bread quality, especially bread crumb. (Armero & Collar, 1996). The results

showed (Table 2) that the addition of the enzyme transglutaminase microbial to 0.75% significantly decreased in porosity, and then the porosity increased with increasing enzyme concentration. Table (3) also showed that the addition of caseinate sodium initially decreased and then increased porosity. Fani Sadrabadi *et al.* (2013) observed that sodium caseinate in comparison with other dairy powders, had the lowest porosity which was measured in gluten-free bread samples. Increasing the porosity with the increase in protein content can be attributed to the strengthening of the dough cells by the protein network, which leads to a decrease in the loss of gas produced and increase in the level of the cavities (Fani sadrabadi *et al.*, 2013).

Dłużewska *et al.* (2015) who detected addition of microbial transglutaminase enzyme to gluten-free bread at 1 unit with whey proteins resulted in significant increase of porosity in bread crumb and the addition of the enzyme at 10 units compared to unit 1 resulted in a greater positive effect on the porosity of gluten-free bread crumb.

According to previous studies, during the first mixing when the flour becomes hydrated and converted to a viscous paste, the soluble and insoluble compounds placed on top of the bubble so the formation of the bubble in the whole process and the stability of the bubbles were impressed by them.

In addition, during the course of the dough, the bubbles that are produced during the mixing in the dough are expanded by yeast production. Therefore, the stability of bubbles is the most important issue, the main reason for their instability being the interconnection of single-gas cells the presence of thick layers on bubbles causes repulsion between them and the chance of interconnection decreases (Ozkoc *et al.*, 2009). Also, the results of interaction of enzyme and protein concentration showed that the highest

porosity of bread was obtained when the concentration of enzyme and caseinate sodium was 1.5 and zero% respectively (Table 4).

### Hardness

Hardness is the resistance of the bread crumb to deformation, which is considered as an important indicator in staling. Due to the role of gluten in preventing the staling, this phenomenon is more common in gluten-free bread (Furlan *et al.*, 2013). The results of Table (2) showed that addition of 0.75% of the enzyme in comparison to 1.5% resulted in a higher increase in the hardness of bread tissue, and the addition of 3% of caseinate sodium also produced the highest degree of hardness (Table 3).

About the interaction effect on the hardness of bread tissue, adding 3% of sodium caseinate and 0.75% of microbial transglutaminase enzyme to gluten-free bread increased in hardness of the bread (Table 4).

In studies done whey protein powder on gluten-free products resulted in 6% protein had a lower hardness compared with control sample at a time interval of 2 h of baking (Sahraeian *et al.*, 2013). Also, according to other researchers, some proteins such as whey protein had reduced the amount of hardness in gluten-free bread (Deora *et al.*, 2014).

Gallagher *et al.* (2003) who studied the addition of caseinate sodium (high protein content) briefly increased the hardness of gluten-free bread, this fact was attributed to the high protein content of sodium caseinate which has strong cross-linking with water and reduction in the migration of moisture from the brain to the crust (Gallagher *et al.*, 2003).

Dluzewska *et al.* (2015) reported that the effect of adding the glycolytic transglutaminase enzyme on gluten-free breads showed that the structure of the tissue became more effective,

depending on the type of protein used as a substrate for the enzyme of action and specifically the availability of protein and lysine and glutamine chains (Dluzewska *et al.*, 2015).

Renzetti *et al.* (2008) studied the 10 units transglutaminase to compare with 1 unit resulted in an increase in the hardness of gluten-free sorghum bread. The results of Moore *et al.* (2006) on gluten-free bread showed that the increase in the level of the enzyme in the sample containing the skim-milk powder increased in bread hardness. Other reports also indicated more content of the enzyme resulted in increasing the hardness of rice-based bread (Gujral & Rosell, 2004; Sahraeiyan *et al.*, 2013)

Specific volume has an effect on firmness of the structure, decreasing the specific volume bread increased bread firmness (Gujral & Rosell, 2004). In this study, the concentration of 1.5% enzyme had the lowest specific volume and highest texture stiffness.

### Evaluation of Bread Properties

The results showed different levels of enzyme and protein concentration and their interactions had a significant effect ( $P \leq 0.05$ ) on the final acceptance of breads. The final acceptance rate of the samples reduced by increasing the enzyme and protein concentration (Tables 2 and 3), so that samples

without these two compounds obtained the highest final acceptance scores. According to Mohammadi *et al.* (2015), the addition of high levels of microbial transglutaminase had significant reduction in the volume and density of the bread compared to the control sample and enzyme which had the lowest overall score (Mohammadi *et al.*, 2015). In another research Mahalleh (2016), the effect of isolated soy protein, egg white powder and transglutaminase enzyme on the sensory properties of corn-free gluten bread was studied and obtained similar results.

### Conclusion

Rheological characterization (frequency sweep) showed a significantly increased in  $G'$  and  $G''$  values and corn flour had an elastic behaviour. Microbial transglutaminase had a negative impact on hardness. The results showed that the addition of this enzyme at first had an increase in specific volume, and then this parameter reduced, porosity and weight loss showed opposite trend. Sensory evaluation suggested low protein and non-enzyme samples had more acceptability. Milk protein isolate has a high nutrition value, water absorption and water holding capacity of dough. Therefore, it can be recommended to be used with free-gluten dough.

### References

- AACC. (2000). *Approved methods of the American association of cereal chemists*. (Ed. 10<sup>th</sup>). The Association: St. Paul. MN.
- Aminpour, A. (2006). The nutritional value of bread and The pattern in most people's needs. In *Proceedings of Special Meeting of bread. Nutrition and Food Research Institute, a state Press*.
- Armero, E., & Collar, C. (1996). Antistaling additives, flour type and sourdough process effects on functionality of wheat doughs. *Journal of Food Science*, 61(2), 299-303. doi: <https://doi.org/10.1111/j.1365-2621.1996.tb14180.x>
- Ataye Salehi, E., Rostamian, M., & Milani, J. (2012). Textural and thermal analysis of staling in gluten-free bread prepared from maize and chickpea flours. *Journal of Innovation in Food Science and Technology*, 3(4-10), 35-40. (in Persian)

- Badiu, E., Aprodu, I., & Banu, I. (2014). Trends in the development of gluten-free bakery products. *The Annals of the University Dunarea de Jos of Galati Fascicle VI-Food Technology*, 38(1), 21-36.
- Brites, C., Trigo, M.J., Santos, C., Collar, C., & Roseel, C.M. (2010). Maize-based gluten-free bread: influence of processing parameters on sensory and instrumental quality. *Food and Bioprocess Technology*, 3(5), 707-715. doi: <https://doi.org/10.1007/s11947-008-0108-4>
- Demirkesen, I., Mert, B., Sumnu, G., & Sahin, S. (2010). Rheological properties of gluten-free bread formulations. *Journal of Food Engineering*, 96(2), 295-303. doi: <https://doi.org/10.1016/j.jfoodeng.2009.08.004>
- Deora, N.S., Deswal, A., & Mishra, H.N. (2014). Functionality of alternative protein in gluten-free product development. *Food Science and Technology International*, 21(5), 364-379. doi: <https://doi.org/10.1177/1082013214538984>
- Dłuzewska, E., Marciniak-Lukasiak, K., & Kurek, N. (2015). Effect of transglutaminase additive on the quality of gluten-free bread. *CyTA-Journal of Food*, 13(1), 80-86. doi: <https://doi.org/10.1080/19476337.2014.917336>
- Fani Sadrabadi, F., Yasini Ardakani, S.A., & Azizi, M.H. (2013). The effect of dairy powders on the gluten-free voluminous breads. *International Journal of Current Microbiology and Applied Sciences*, 2(12), 551-559.
- Gallagher, E., Gormley, T.R., & Arendt, E.K. (2003). Crust and crumb characteristics of gluten free breads. *Journal of Food Engineering*, 56(2-3), 153-161. doi: [https://doi.org/10.1016/S0260-8774\(02\)00244-3](https://doi.org/10.1016/S0260-8774(02)00244-3)
- Gujral, H.S., & Rosell, C.M. (2004). Functionality of rice flour modified with a microbial transglutaminase. *Journal of Cereal Science*, 39(2), 225-230. doi: <https://doi.org/10.1016/j.jcs.2003.10.004>
- Gujral, H.S., Guardiola, I., Carbonell, J.V., & Rosell, C.M. (2003). Effect of cyclodextrinase on dough rheology and bread quality from rice flour. *Journal of Agriculture and Food Chemistry*, 51(13), 3814-3818. doi: <https://doi.org/10.1021/jf034112w>
- Katina, K., Heinio, R.-L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT-Food Science and Technology*, 39(10), 1189-1202. doi: <https://doi.org/10.1016/j.lwt.2005.08.001>
- Kenny, S., Wehrle, K., Stanton, C., & Arendt, E.K. (2000). Incorporation of dairy ingredients into wheat bread: effects on dough rheology and bread quality. *European Food Research and Technology*, 210(6), 391-396. doi: <https://doi.org/10.1007/s002170050569>
- Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C.G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*, 79(3), 1033-1047. doi: <https://doi.org/10.1016/j.jfoodeng.2006.03.032>
- Mahalleh, H. (2017). The effect of protein isolate, egg white powder and transglutaminase enzyme on the physicochemical and sensory properties of corn-based gluten-free bread. (Unpublished master's thesis) (pp. 141): Department of Food Science and Engineering, Islamic Azad University, Tabriz Branch. (in Persian)
- Matos, M.E., Sanz, T., & Rosell, C.M. (2014). Establishing the function of proteins on the rheological and quality properties of rice based gluten free muffins. *Food Hydrocolloids*, 35, 150-158. doi: <https://doi.org/10.1016/j.foodhyd.2013.05.007>
- Mohammadi, M., Azizi, M.H., Neyestani, T.R., Hosseini, H., & Mortazavian, A.M. (2015). Development of gluten-free bread using guar gum and transglutaminase. *Journal of Industrial and Engineering Chemistry*, 21, 1398-1402. doi: <https://doi.org/10.1016/j.jiec.2014.06.013>
- Moore, M.M., Heinbockel, M., Dockery, P., Ulmer, H.M., & Arendt, E.K. (2006). Network formation in gluten-free bread with application of transglutaminase. *Cereal Chemistry*, 83(1), 28-36. doi: <https://doi.org/10.1094/CC-83-0028>

- Nunes, M.H.B., Ryan, L.A.M., & Arendt, E.K. (2009). Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. *European Food Research and Technology*, 229(1), 31-41. doi: <https://doi.org/10.1007/s00217-009-1023-2>
- Ozkoc, S.O., Sumnu, G., & Sahin, S. (2009). The effects of gums on macro and micro-structure of breads baked in different ovens. *Food hydrocolloids*, 23(8), 2182-2189. doi: <https://doi.org/10.1016/j.foodhyd.2009.04.003>
- Payan, R. (2005). Introduction to technology of cereal products. Tehran Ayyiizh Publication, (in Persian)
- Pourmohammadi, K., Aalami, M., Shahedi, M., & Sadeghi Mahoonak, A.R. (2012). Effects of microbial transglutaminase on the quality of wheat bread supplemented with hull-less barley flour. *Electronic Journal of Food Processing and Preservation*, 2(2), 81-98. (in Persian)
- Rajabzade, N. (1993). *Bread Technology*. Tehran University Press. (in Persian)
- Renzetti, S., Bello, F.D., & Arendt, E.K. (2008). Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. *Journal of Cereal Science*, 48(1), 33-45. doi: <https://doi.org/10.1016/j.jcs.2007.07.011>
- Furlan, L.T.R., Padilla, A.P., & Campderros, M. (2013). Improvement of gluten-free bread properties by the incorporation of bovine plasma proteins and different saccharides into the matrix. *Food Chemistry*, 170, 257-264. doi: <https://doi.org/10.1016/j.foodchem.2014.08.033>
- Rostamian, M., Milani, J.M., & Maleki, G. (2014). Physical properties of gluten-free bread made of corn and chickpea flour. *International Journal of Food Engineering*, 10(3), 467-472. doi: <https://doi.org/10.1515/ijfe-2013-0004>
- Sahraeiyan, B., Habibi Najafi, M.B., Karim, M., Haddad Khodaparast, M.H., Ghiafeh Davoodi, M., & Naghipour, F. (2013, October). *The effect of the cheese powder on the quantitative and qualitative features of gluten-free sorghum bread*. Paper presented at the 21<sup>st</sup> National Congress of Food Science and Technology, Shiraz. (in Persian)
- Sciarini, L.S., Ribotta, P.D., Leon, A.E., & Perez, G.T. (2010). Influence of gluten-free flours and their mixtures on batter properties and bread quality. *Food and Bioprocess Technology*, 3(4), 577-585. doi: <https://doi.org/10.1007/s11947-008-0098-2>
- Shahidi, F., Mohebbi, M., & Ehtiai, A. (2011). Image analysis of crumb digital images in barbary bread enriched with soy flour. *Iranian Food Science and Technology Reserch Journal*, 6(4), 247-253. (in Persian)
- Smerdel, B., Pollak, L., Novotni, D., Cukelj, N., Benkovic, M., Lusic, D., & Curic, D. (2013). Improvement of gluten-free bread quality using transglutaminase, various extruded flours and protein isolates. *Journal of Food and Nutrition Research*, 51(4), 242-253.
- Soleimanifard, M., Aalami, M., Khodaeian Chegini, F., Najafian, G., Sadeghi Mahoonak, A.R., & Khomeyri, M. (2013). Investigation on the effects of kefir on bulky bread quality and its shelf life. *Journal of Innovation in Food Science and Technology*, 5(4-18), 53-66. (in Persian)
- Tayebi, M., Shahedi, M., Milani, j., & Ghorbanpour, A. (2014, August). *Gluten-free bread formulation*. Paper presented at the 22<sup>nd</sup> National Congress of Food Science and Technology, Gorgan. (in Persian)
- Tseng, C.-S., & Lai, H.-M. (2002). Physicochemical properties of wheat flour dough modified by microbial transglutaminase. *Journal of Food Science*, 67(2), 750-755. doi: <https://doi.org/10.1111/j.1365-2621.2002.tb10671.x>

## تأثیر افزودن کازئینات سدیم و آنزیم ترانس گلوتامیناز میکروبی بر خواص رئولوژیکی، فیزیکی و حسی نان بدون گلوتن بر پایه آرد ذرت

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### چکیده

بیماری سلیاک یک اختلال شایع خودایمنی است که با دریافت گلوتن برانگیخته می‌شود. در این مطالعه آرد ذرت به‌عنوان آرد فاقد گلوتن مورد استفاده قرار گرفت لذا به‌منظور تقلید ویژگی‌های گلوتن، استفاده از آنزیم‌هایی با قابلیت تشکیل پیوند عرضی مانند ترانس گلوتامیناز میکروبی در سطوح (۰، ۰/۷۵ و ۱/۵ درصد) همراه با کازئینات سدیم در سطوح (۰، ۳ و ۶ درصد) به‌عنوان جایگزین گلوتن مورد استفاده قرار گرفت و تأثیر آنها بر ویژگی‌های رئولوژیکی خمیر، فیزیکی و حسی نان فاقد گلوتن ذرت مورد ارزیابی قرار گرفت. نتایج نشان داد که با افزایش فرکانس زاویه‌ای هردو مدول ذخیره و آفت افزایش یافت. بیشینه حجم مخصوص نان‌های تولیدی از ذرت ( $1/460 \pm 0/02$ ) زمانی به‌دست آمد که میزان غلظت آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش آفت پخت نسبت به نمونه فاقد این مواد گردید. افزودن آنزیم ترانس گلوتامیناز میکروبی تا سطح ۰/۷۵ درصد منجر به کاهش شدید میزان تخلخل گردید و سپس با افزایش غلظت آنزیم، میزان تخلخل نیز افزایش یافت. افزودن ۳ درصد کازئینات سدیم به همراه ۰/۷۵ درصد آنزیم ترانس گلوتامیناز میکروبی به نان بدون گلوتن منجر به ایجاد بیشترین سختی نان گردید. میزان پذیرش نهایی نمونه‌های تولیدی با افزایش غلظت آنزیم و پروتئین کاهش یافت به‌نحوی که نمونه‌های فاقد این دو ترکیب، بالاترین امتیاز پذیرش نهایی را به‌دست آوردند. در نهایت باتوجه به نتایج به‌دست آمده می‌توان بیان داشت که استفاده از ۰/۷۵ درصد آنزیم ترانس گلوتامیناز میکروبی و ۳ درصد کازئینات سدیم برای تولید نان ذرت بدون گلوتن می‌تواند مفید واقع شود.

**واژه‌های کلیدی:** آرد ذرت، ترانس گلوتامیناز، پردازش تصویر، کازئینات سدیم