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Original Research

Effect of microbial transglutaminase enzyme on antimicrobial and qualitative properties of gluten-free baguette bread

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ABSTRACT:

Celiac is the most common disease that will be emerged by consumption of gluten and the only way to treat it is using of gluten free diet throughout the lifetime of the patient. So, the aim of the present study was the investigation of microbial transglutaminase enzyme on the breeds and its on the breads and its effect on antimicrobial, chemical and physical specifications of bread without gluten, by mixing corn and rice flour. For this purpose, transglutaminase enzyme at the levels of 0.5, 1, 1.5 and 2 percent was added to the formulation of breads. The results showed that the enzyme transglutaminase bread samples at 2% had been significantly increased the protein, fat, ash, moisture and fiber samples. Also, this enzyme had a role in significant reducing of total amount of microorganisms and mold and yeast in bread production. In addition, results showed that the addition of transglutaminase enzymes improves the organoleptic properties and delayed staling of breads in comparison with the control sample.

Keywords:

Microbial transglutaminase enzyme, gluten-free baguette bread, anti-microbial.

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INTRODUCTION

Bread is the main food of Iranian people that provides the most part of energy, protein, mineral and vitamins B. About the 60-65% of protein, calorie and approximately 2-3 g mineral and required salt of poor people is provided from bread feeding. We know from past years that bread quality provided from wheat flour is related to the quality and quantity of Gluten protein. Gluten is the main protein of wheat flour which due to having the visco-elasticity feature requirement in paste plays the appropriate capability in maintaining the gas in bred toast performance (Moore *et al.,* 2006). Gluten usage in some people such as celiac disease leads the small intestine inflammation which consequently leads the incomplete absorption of necessity material such as ferrous, calcium and solved vitamin in fat. Celiac is a disease of mucous membrane due to the small intestine in people with gluten intolerance, and is damaged by gluten, will be emerged as a result of impaired absorption of nutrients, weight loss, diarrhea, anemia, fatigue, bloating, folat deficiency and osteoporosis (Korus *et al*., 2009). People with celiac disease, after the consumption of foods containing gluten, a series of clinical symptoms caused by impaired digestion of gluten showed that can lead to damage willy lint presented in the small intestine - in a long time. The only treat way is the complete elimination of gluten from people feeding regime (Castassia and Fasano, 2008). Now, bread is produced from flour in different shapes and textures. While considering the role of sour dough in improving bread quality, progress and development of yeast, the massive and semi-massive porous bread are produced that in the preparation of their types, ability to bake cereal, flour characteristics of the product, method of preparation and the preparation process have high important. From the most important massive bread which is produced in Iran can name the baguette, hamburger, toast, utshen, donate, shirmal and weight loss special bread (Movahed, 2012). The flour that is used for producing free-gluten bread should be the flour none of wheat flour, barely, rye and oats which we used of corn and rice flour in the study. In one hand, we used of transglutaminase enzyme in order of gluten-free in produced bread. The study indicated that transglutaminase enzyme plays a role in modulating the gliadine reaction from glutamine hydrolyze. This observation indicates that gluten processing with transglutaminase enzyme lead the decreasing in celiac allergies in people. Also it was shown that mentioned enzyme due to the anion property in chemical structure, auto bonding property to the microorganism negative loads and therefore have the anti-microbial property. So the purpose of the present study is the analysis of microbial transglutaminase enzyme on anti microbial property and baguette qualitative resulted from flour combination without gluten based on rice and corn flour in order of producing the free-gluten bread for reaching the desired quality.

MATERIALS AND METHODS

Material

Raw material required for baking the baguette bread include the corn and rice flour (provided from Bartar company), microbial transglutaminase enzyme (prepared from Spain company BDF), salt (provided from Delchasb Company), oil (provided from Tina Co), yeast (prepared from Sandikay Co), sugar (provided from Varamin Co) and poly ethylene cladding for packaging.

Physicochemical test of flour and bread samples

In the present study, chemical experiments had been carried out on corn flour, rice flour and bread samples, the percentage of protein, fat, fiber, ash, humidity (Anonymous, 2003) and more over yeast and mold test, the total amount of microorganisms (Anonymous, 2002), was carried out on gluten-free baguettes samples. Analysis had been carried out by using of a gauge baht (M350-10CT, test metric,

Rechodate, England) in the College of Agriculture and Natural Resources, Tehran University (Laboratory of Food Science and Technology Department) in this regard in order of determining the presented difference in bread core texture of samples, in the beginning one cut with 2 cm thickness from middle of bread had been provided and this slice was stamped, after saturating more slices of bread into the substance, bread crumb image on white paper was prepared and the images of treatments were compared with each other (Mazzeo *et al*., 2012).

Baguette bread production and baking method

In order of producing baguettes, the required amount of dry ingredients, was including flour, rice (50g), corn (50g), salt (5.1g), sugar (5.1g) and yeast (2g) were weighed by using a digital scale and then water (270g) and oil (6 g) was added to the above mixture and then the treatments used in the study contained the different levels of the transglutaminase germ enzyme and also observance samples without Microbial Transglutaminase enzyme (MTG) were prepared and treated separately in the tank mixer (Hobart model Germany) for 10 minutes to be prepared and mixed evenly. The initial sample rest was 20 minutes. Then the pieces of paste were executed into an approximate weight of 350 grams chin and oven (model: M80 100 Turkey) at 220°C for 15 minutes. Finally gluten-free baguette bread samples were packed after 30 minutes of cooling in polyethylene bags and until the tests were stored at room temperature were maintained (Nishita *et al*., 1976)

Produced bread platitude determination

In this method, we used of texture analyzer or Instron device (Model M350-10CT) and executed according to the AACC 09-74 standard. According to this method, bread hardness is defined based on compaction force. This test had been performed in intervals of 24, 48 and 72 hours after baking the samples were taken, so the specimens maintained separately in plastic bags at room temperature and then for evaluation by texture analyzer, the approximate dimensions of the core slices 2 cm× 2 cm were separated. Amount of force (the force that must be applied on upper jaw of the device) was considered equal to 40% of the thickness of the bread, so that the sample is compressed about 8 mm. Also the speed of movement of the upper jaw in downward adjustment was equal to 30 mm per minute. In this test the probe plate was used (Anonymous, 2003).

Bread organoleptic property determination

In order of evaluating the organoleptic characteristics of bread, cakes properties analysis by using of five sensors had been executed. practical standard, expert opinion and personal desire and trained experts about the product had performed according to the standard evaluation forms available in Tehran University, in this study, samples after cooling were trimmed, encoded and analyzed by several trained analyzed. Evaluators ranked accordance with the characteristics of form bread (0-10), features Shell (0-15), crumb color (0-10), bread crumb (0-15), aroma (0-15), taste tasting (0-20) and chewed the latest (0-15) gave the bread rating.

Statistical analysis method

A completely randomized design with three replications was used to analyze experimental data. Means were compared by Duncan's multiple-range test ($\alpha = 5\%$) in SPSS 16.

RESULTS AND DISCUSSION

Table 1, shows the rice and corn flour properties used in baguette bread production.

Table 1. Chemical properties of corn and rice flour

S. No	Material	Moisture %	Ash %	Protein %	Fiber %	Fat %
1	Rice flour	6.43	0.77	8.43	0.46	2.54
2	Corn flour	9.38	0.43	5.19	0.99	3.08

Table 2. Result of baguette bread samples physicochemical properties

S. No	Treatment	Protein %	Fat %	Ash %	Moisture%	Fiber %
1	C	5.74±0.3^{b}	3.23±0.06^{a}	1.98±0.12^{a}	40.09±0.34^{c}	0.3±0.05^{c}
2	T_1	6.50±0.6^{ab}	3.23±0.05^{a}	2.01±0.01^{a}	42.10±0.32^{b}	1.1±0.09^{b}
3	T_2	6.61±0.07^{a}	2.95±0.07^{b}	2.02±0.01^{a}	42.40±0.28^{b}	1.17±0.5^{ab}
4	T_3	6.65±0.04^{a}	2.84±0.2^{bc}	2.03±0.09^{a}	43.40±0.28^{a}	1.29±0.09^{ab}
5	T_4	6.70±0.09^{a}	2.64±0.03^{c}	2.07±0.06^{a}	44.12±0.32^{a}	1.35±0.03^{a}

In each column, means with at least one common letter have no significant difference ($p < 0.01$).
C- Control, T_1 code - 0.5% enzyme concentration, T_2 code - 1% enzyme, T_3 code - 1.5% enzyme, T_4 code - 2% enzyme

Bread microbiological and physicochemical properties

According to Table 2, adding MTG, increases the protein content of baguette bread samples compared to observance. Based on the results, the amount of protein in the diet supplemented with 2% enzymes had the highest and the lowest was in observance, Moreover, significant differences were observed in all treatments ($p \leq 0.01$).

The reason for this is that by increasing the amount of MTG, at first, increasing the formation of crosslinking between amino acids, resulting in the creation of new structures among the structures formed and led to marginal increases in protein bread (Kuraishi *et al*., 2001). The results of the Kuraishi *et al*. (2001) study, matching the increasing of the enzyme with transglutaminase, indicated the formation of cross-links between the amino acids increasing and this leads to an increasing in the new structure and protein quantity. The results of comparing the average amount of fat in free-gluten baguette bread samples with different percentages of MTG enzymes in Table 2 show that the amount of fat in the diet supplemented with 2% enzyme had the least and in observance treatment was maximum, while significant difference at all the treatments were observed in the treatment T1 ($p \leq 0.01$). The results of the Nuernberg *et al*. (2012) showed that as they add an enzyme transglutaminase can replace appropriate fats in low-fat ice cream. The results of comparing the average moisture content of gluten-free baguette bread samples containing different percentages of MTG are shown in Table 2. Based on the results, the amount of moisture in samples containing 2% enzymes had the highest and the lowest amount was in observance, while no significant difference was observed in all treatments ($p \leq 0.01$). Due to slightly increasing in moisture in bread samples containing MTG enzyme, the formation of glutamine amino acids and lysine crosslinking network between the enzymes has the ability of entrapping water which is the feature that will increase the water holding capacity. The water absorption capacity can be increased due to deregulation of the glutamine into glutamic acid amide which decreases the hydrophobic environment and increases the water absorption.

Table 3. Microbiological properties of baguette bread

S. No	Treatment	Microorganism total count	Mold and yeast
1	C	6.57±0.01^{a}	6.98±0.01^{a}
2	T_1	5.32±0.12^{b}	5.88±0.01^{b}
3	T_2	5.28±0.13^{b}	5.83±0.03^{c}
4	T_3	5.02±0.02^{c}	5.81±0.03^{c}
5	T_4	5.02±0.02^{c}	5.37±0.01^{d}

In each column, means with at least one common letter have no significant difference ($p < 0.01$).
C- Control, T_1 code - 0.5% enzyme concentration, T_2 code - 1% enzyme, T_3 code - 1.5% enzyme, T_4 code - 2% enzyme

Table 4. Colorimetric determination results of baguette bread samples

S. No	Treatment	Color rank L*	Color rank a*	Color rank b*	ΔE
1	C	60.44±0.5^{c}	8.25±0.5^{a}	34.95±0.4^{a}	37.45±0.1^{a}
2	T_1	64.16±1.8^{b}	7.25±0.3^{a}	34.46±0.8^{a}	36.01±0.8^{a}
3	T_2	65.63±0.5^{b}	5.34±0.5^{b}	33.45±1.3^{ab}	34.89±3.7^{ab}
4	T_3	66.96±0.5^{a}	4.26±0.3^{b}	33.07±1.7^{ab}	33.31±0.2^{ab}
5	T_4	67.31±0.2^{a}	2.13±0.5^{c}	32.03±0.9^{b}	30.57±1.3^{b}

In each column, means with at least one common letter have no significant difference ($p < 0.01$).
C- Control, T_1 code - 0.5% enzyme concentration, T_2 code - 1% enzyme, T_3 code - 1.5% enzyme, T_4 code - 2% enzyme

The gluten-free fiber baguette bred samples including different percentage of MTG are shown in Table 2. Based on the results, the amount of moisture in samples including 2% enzymes had the highest and the lowest was in observance, while no significant difference was observed with all treatments ($p \leq 0.01$). The reason for this is due to the presence of substances with fibrous polysaccharide component properties has been used in enzyme (Motokia and Segurob, 1997). In other words, polysaccharide compounds smoothly increases the fiber bread samples compared to observance impaction. Motokia and Segurob, 1997, compared the results with a study that suggested the transglutaminase enzyme has the polysaccharide compounds, fiber and protein. By addition of transglutaminase enzyme, bacterial and yeast and mold growth in the total count of microorganisms in the food including the enzyme was decreased compared with observance ($p \leq 0.01$) with enzyme supplementation increased the acidity that prevent the mold and yeast growth to be active.

There is also a positive electric charges in the structure of MTG has the ability bonding to have negative charges. Such features create a strong connection decreasing the mold and yeast growth smoothly (Table 3).

Colorimetric test results

Regarding to the Table 4, it was indicated that mentioned enzyme amount in 0.5, 1.5 and 2% level lead the meaningful increasing of L* color component and meaningful decreasing of a* and b* components in MTG enzyme bread rather than observance sample.

So the observance sample (no MTG enzyme), had the lowest rating colored L*. There was a significant difference in other treatments ($p \geq 0.5$) but a* and b* color components in the control group (without MTG enzymes) has the highest obtained

Table 5. Results of average comparison resulted from organoleptic evaluation of baguette bread samples

S. No	Treatment	Smell	Flavor	Color	Texture
1	C	8±0.02^{b}	15.2±0.2^{a}	6±0.6^{c}	9±0.2^{c}
2	T_1	13±0.2^{a}	16±0.2^{b}	8±0.4^{b}	12±0.2^{b}
3	T_2	13.1±0.2^{a}	17.7±0.1^{a}	8.1±0.7^{b}	13.7±3.2^{a}
4	T_3	13.2±0.2^{a}	17.8±0.2^{a}	9.3±0.7^{a}	14±0.1^{a}
5	T_4	13.9±0.2^{a}	17.9±0.2^{a}	9.5±0.8^{a}	14.1±2^{a}

In each column, means with at least one common letter have no significant difference ($p < 0.01$).
C- Control, T_1 code - 0.5% enzyme concentration, T_2 code - 1% enzyme, T_3 code - 1.5% enzyme, T_4 code - 2% enzyme

value compared with other treatments, while significant difference with other treatments ($p \geq 0.01$). In other words, the bread including the transglutaminase enzyme with greater values, index L* was more, a* and b* were less than with the core. This is due to the formation of protein network between glutamine and lysine by enzyme transglutaminase that it would decrease the acidity and decrease the amount of lysine, which results in decreasing the amount of reaction Maylard and whiter color of the crumb (Moore *et al*., 2006).

Also regarding to the comparison results of evaluation average of color difference test in free-gluten baguette bread including the different MTG enzyme percent in Table 4 was shown that MTG enzyme addition in 0.5, 1, 1.5 and 2% levels will meaningfully decrease the color difference in bread rather than the control. So the control (no MTG enzyme) and treated with T_4 had the highest and lowest points of color differences significantly with each other ($p \geq 0.01$), but no difference was observed between the observance and other treatments. Meanwhile, T_4 and T_3, T_2 and T_1 treatments, respectively, had the lowest difference colors. The research results matched with Moore *et al.* (2006) reported that by adding the MTG, Maylard reactive will be decreased, so will result in a whiter and more desirable product.

Organoleptic test results

In Table 5, organoleptic evaluation results of baguette bread samples in each column, are the average in one word common, according to the Duncan test probability level have 1% meaningful difference. With regard to the smell, flavor, texture, form and shape, chewing capability, color viewpoints, T_4 has the highest rank and observance treat had the least rank.

CONCLUSION

In the present study, transglutaminase enzyme effect on anti microbial property and free-gluten bread qualitative had been analyzed. For analyzing the data we used of data comparison with Duncan multi domain test. With result analysis we observed that by adding the microbial transglutaminase addition, protein, humidity and fiber amount in treats including the enzyme had been increased and the most effect in T_4 treatment including 2% enzyme had been observed. The reason of this affair is that by increasing the MTG enzyme, transversal bonding formation between glutamine and lysine amino acid is increased which the result is resented in formed structures which lead the quantitative increasing in protein in tested bread and also by forming these bonding, water molecules had been entrapped and the result is the increasing of humidity which lead the platitude and hardness decreasing of samples. Also MTG, is operated similar to a thickener which leads, fat decrease in the mentioned samples. Mentioned enzyme is with polysaccharide combination in its structure, leads the quantitative increasing of fiber in samples including enzyme. Moreover, adding enzyme in different levels have effective effect on mold and paste growth decreasing and also decreasing in micro organism abundance. The reason can be known due to the acidity decreasing of environment and also positive electrical loads the presence in transglutaminase enzyme structure which created the bonding capability between protein in plant and animal combination of anti-microbial combination and leads the decreasing in microbial load in final product. Regarding to the obtained results from executed test on samples we can conclude that free-gluten baguette bread with desired quality and can be produced by using of MTG at 2% level.

REFERENCES

Anonymous. (2002). Iranian National Standard Test Method, No. 1810. Institute of Standards and Industrial Research of Iran. Microbiology of food and animal feed – method of finding Salmonella in food.

Anonymous. (2003). Approved Methods of American

Association of Cereal Chemists.(10th ed), St Paul, Minn, USA.

Castassia C and Fasano A. (2008). Celiac Disease. P.1-22. In: Arendt EK and Dal Balleo F(eds), gluten-free cereal product and beverages. Academic Press. Technol 217, 125-127 p.

Kuraishi C, Yamazaki K and Susa Y. (2001). Transglutaminase: its utilization in the food industry. *Food Reviews International*, 17(2): 221-246.

Korus J, Ziobro R, Witczak M and Juszczak L. (2009). The impact of resistant starch on the characteristics of gluten free dough and bread. *Food Hydrocolloids*, 23(3): 988-995.

Motokia M and Segurob K. (1997). Transglutaminase and its use for food processing. *Trends in Food Science and Technology*, 9(5): 204-210.

Moore MM, Heinbockel M, Dockery P, Ulmer HM and Arendt EK. (2006). Network formation in gluten free bread with application of transglutaminase. *Cereal Chemistry,* 83(1): 28-36.

Mazzeo MF, Bonanavita R, Maurano F, Bergamo P, Siciliano RA and Rossi M. (2012). Biochemical modification of gliadins induced by microbial transglutaminase. *Biochimica et Biophysica Acta,* 1830 (11): 1214-1218.

Movahed S. (2012). Science of Bread. Marze Danesh Press. Tehran, Iran. 188 p. (In Farsi).

Nishita KD, Roberts RL, Bean MM and Kennedy BM. (1976). Development of a yeast leavened rice-bread formula. *Cereal Chemistry*, 53(5):185-189.

Priscilla Nuernberg R, Vivian MB and Bordignon-Luiz MT. (2012). Effect of microbial transglutaminase on functional and rheological properties of ice cream with different fat contents. *LWT-Food Science and Technology*, 48(2): 224-230.