



## Production of low-calorie sponge cake using microbial transglutaminase enzyme and checking some of its thermometric, thermogravimetric and rheological properties

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### ABSTRACT

Microbial transglutaminase is an enzyme from the group of transferases, which is widely used to modify the functional characteristics of proteins in various foods. The covalent bonds created by this enzyme have unique effects on gel formation capacity, thermal stability and water retention capacity in proteins. The purpose of this research was to investigate the effect of different amounts of microbial transglutaminase enzyme as a fat substitute on the thermometric, thermogravimetric and rheological characteristics of low-calorie sponge cake. For this purpose, seven different treatments were produced with 0, 25, 50 and 100% reduced fat, 10 ppm and 20 ppm transglutaminase enzyme. Based on the results, the maximum amount of protein (8.43%), apparent density (0.77 g/cm<sup>3</sup>), solid density (1.19 g/cm<sup>3</sup>), hardness (4033 g), adhesion (0.61 MJ), cohesion (0.73), gumminess (2943 g), second enthalpy and chewability (204.62 MJ) were observed in the treatment with 20 ppm enzyme and 100% reduced fat. The lowest free water was observed in the treatment containing 10 ppm enzyme with 25% reduced fat. The treatment with 10 ppm of enzyme and 50% reduced fat had the best taste, smell and color according to the evaluators. The amount of protein increased due to the presence of transglutaminase enzyme in the samples and its tissue characteristics improved. But reducing 100 percent of fat had a negative effect on the sensory properties of the produced product. Therefore, complete removal of fat is not recommended for the production of this product, but by using transglutaminase enzyme, fat can be reduced to a significant extent.

## 1- Introduction

There are numerous concerns regarding dietary fat as a source of excess calories, saturated fatty acids, and cholesterol, as well as its association with cardiovascular diseases, cancer, and obesity. Fat provides the most calories (9 kcal/g) compared to protein and carbohydrates (4 kcal/g). Excessive fat intake is one of the most important factors contributing to overweight and obesity, which themselves are risk factors for cardiovascular diseases, high blood pressure, and diabetes. According to international organizations, total fat intake should be less than 30% of daily energy, saturated fats less than 10%, and monounsaturated and polyunsaturated fats at least 2/3 of daily energy [1]. One method to reduce fat consumption is the use of low-fat products. However, consumers demand low-fat products with quality similar to full-fat products [2]. Proteins are suitable compounds for simulating the properties of fat in food products [3]. Transglutaminase, also known as EC 2.3.2.13, is a transferase enzyme widely found in nature. Transglutaminase is a protein with a molecular weight of 37,368 Daltons, containing 331 amino acids. This enzyme can form bonds between glutamine from one protein and lysine from another protein. Notably, this enzyme has no adverse effects on the bioavailability of lysine and does not alter the nutritional value of the resulting protein [4]. In this regard, several studies have been conducted, some of which are mentioned below. Raza et al. (2012) added microbial transglutaminase to ice creams with 4%, 6%, and 8% fat content and evaluated their rheological properties, overrun, melting behavior, and texture. Ice creams containing microbial transglutaminase had a higher overrun compared to samples without the enzyme. Additionally, microbial transglutaminase increased the flow behavior index and pseudoplastic properties compared to samples without the enzyme. Ice creams with 4% and 6% fat content containing microbial transglutaminase exhibited behavior similar to ice cream samples without the enzyme but with 8% fat content [5]. Depiro et al. (2010) observed that transglutaminase could increase cheese yield by retaining moisture in the curd [6]. Gaiuchi et al. (2009) evaluated the effect of transglutaminase on the physical properties of yogurt made from a mixture of milk and whey and concluded that enzymatic treatment increased yogurt firmness. According to textural and

rheological results, the enzyme compensated for the physical changes caused by adding whey to yogurt [7]. Rodriguez et al. (2012) used inulin as a fat replacer in sponge cake. In this study, the effect of using different levels of inulin in the cake formulation on its physicochemical and microstructural properties was investigated [8]. Lee et al. (2005) also used beta-glucanotrimamylodextrins as a shortening replacer in cake. They observed that as the replacement level increased from 20% to 60%, the specific gravity of the batter increased, while viscosity and the number of air bubbles decreased, which was consistent with the changes in cake volume [9]. Gomez et al. (2006) studied the performance of various hydrocolloids, such as sodium alginate, carrageenan, pectin, hydroxypropyl methylcellulose, locust bean gum, guar gum, and xanthan gum, on the quality of yellow layer cake. According to the results, the overall acceptance of layer cakes improved with the addition of hydrocolloids, except when pectin was used [10]. Arab Shirazi et al. (2012) studied the replacement of egg with xanthan gum and hydroxypropyl methylcellulose in sponge cake. According to the results, gum replacement improved water absorption, dough development time, and dough resistance time. Additionally, the area under the resistance curve and the dough resistance coefficient were higher in samples containing gums compared to the control. Moreover, moisture increased in all treatments compared to the control, while protein decreased. Notably, sponge cakes containing gums had better sensory quality [11]. To date, no research has been conducted on the use of microbial transglutaminase in low-fat sponge cake. Therefore, this study aimed to produce low-fat sponge cake using microbial transglutaminase and investigate its calorimetric, thermogravimetric, and rheological properties.

## 2- Materials and Methods

### 2-1-Raw Materials

White wheat flour for cake preparation was obtained from Aras Mehr Flour Factory in Tabriz. Ground sugar, oil, vanilla, baking powder, powdered milk, and eggs were purchased from local grocery stores. Whey powder was obtained from Pegah Fars Company, and microbial transglutaminase with an activity of 80 units per gram was purchased from

Saman Trading Company in Tehran. The characteristics of the cake flour used in this study are shown in Table 1.

**Table 1- Characteristics of flour used for cake preparation**

Properties*	Amount
Moisture (percentage)	13.14 + 0.01
Protein (percentage)**	7.5 + 0.15
Moist gluten (percentage)	20.2 + 0.76
Gluten index	87 + 1.14
Ash (percentage)	0.508 + 0.02
Zolani number (cc)	14 + 0.13

\* The results obtained were the result of three repetitions.

\*\*Analysis results are reported based on 14% flour moisture.

## 2-2- Cake Production Method

The dough was prepared using the sugar-dough method according to the instructions in Table 2. After preparing 1500 g of cake batter, 40 g of batter

was poured into galvanized molds of specific dimensions ( $4 \times 5 \times 8$  cm) and baked in an oven at 180–190°C for 40–45 minutes. After baking, the cakes were cooled at room temperature for 30–45 minutes, then packaged in lightweight polyethylene bags with heat sealing and stored at room temperature until further testing.

**Table 2- The steps of preparing cake dough using the sugar-dough method**

Material	weight (gr)	Percentage	Steps to do
oil	17.50	266	Heating was done for 10 minutes until the light color of the cream was produced.
sugar	22	330	
egg	22	330	Eggs were added in 3 to 5 equal parts.
flour	28.40	425.60	All the powdered ingredients were sieved together and added to form a paste. It came out half straight
Baking powder	0.50	7.50	
milk powder	0.61	9.20	
vanilla	0.15	2.30	
whey powder	1.23	18.40	
water	7.61	11.40	After adding water, the dough became smooth.

**Table 3- Amount of fat and transglutaminase enzyme used in different samples of sponge cake**

Treatment	Transglutaminase enzyme treatment (ppm)	Reduced fat (%)
A (Control)	0	0
B	10	25
C	20	25
D	10	50
E	20	50
F	10	100
G	20	100

## 2-3- Cake Batter Tests

### 2-3-1- Batter Consistency

The batter was poured into a funnel with a wide internal diameter of 10 cm and a narrow internal diameter of 1.6 cm. The funnel was completely filled with batter, and the weight of the batter exiting the funnel in 15 seconds was measured. Batter consistency was reported in grams per second [13]. Higher recorded numbers indicate lower batter consistency.

### 2-3-2- Specific Gravity

The specific gravity of the batter was calculated by measuring the ratio of the weight of 240 mL of batter to 240 mL of water [14].

## 2-4- Cake Tests

### 2-4-1-Moisture Measurement

Cake moisture was measured using the AACC 11-44 method. Two to three grams of the sample were weighed with an accuracy of 0.01 g in pre-weighed plates. The plates were placed in an oven at 103°C for 60 minutes. After reaching a constant weight, the plates were weighed in a desiccator, and moisture content was calculated using the following formula [15]:

$$\text{Moisture (\%)} = (\text{Weight loss in grams} / \text{Sample weight}) \times 100$$

### 2-4-2- Ash Measurement

Ash measurement is based on burning all organic matter and weighing the remaining mineral compounds in the flour. Cake ash was measured according to the AACC 01-08 method [15]. Three to five grams of the sample were weighed with an accuracy of 0.0001 g in a pre-weighed crucible. The crucible was placed in a furnace at 550°C for 4–6 hours until reaching a light gray ash or constant weight. The crucible was then cooled in a desiccator and weighed. Ash percentage was calculated using the following formula:

$$\text{Ash (\%)} = (\text{Weight of residue} / \text{Sample weight}) \times 100$$

### 2-4-3- Fat Measurement

Cake fat was measured according to Iranian National Standard No. 2862 [16].

### 2-4-4- Protein Measurement

Protein was measured according to the AACC 12-46 method [17]. Total nitrogen was measured, and protein was calculated using the cereal protein factor (5.7). One gram of the ground sample was weighed in a digestion flask, and 25 mL of 98% sulfuric acid was added for digestion. Complete digestion continued until the solution turned clear green. After complete digestion, nitrogen distillation was performed. For this purpose, 250–300 mL of water and 50 mL of NaOH were added to the digested solution along with pearls to prevent explosion. A flask containing 50 mL of boric acid and a few drops of methyl red indicator was placed at the end of the distillation apparatus so that the end of the apparatus was completely immersed in the acid. Distillation continued until a minimum volume of 250 mL was reached. The resulting solution was titrated with 0.1 N sulfuric acid until it turned pink, and total protein percentage was calculated using the following formula:

$$\text{Protein (\%)} = (\text{Protein factor} \times \text{Normality of sulfuric acid} \times \text{mL of acid consumed}) / \text{Sample weight}$$

### 2-4-5- Cake Volume Measurement

Cake volume was measured using the rapeseed displacement method [14].

### 2-4-6- Specific Volume Measurement

Specific volume was calculated as the ratio of volume to weight of the cake [18].

### 2-4-7- Apparent Density Measurement

Apparent density was calculated as the ratio of weight to volume of the cake [19].

### 2-4-8- Bulk Density Measurement-

Bulk density was measured using the pycnometer method [19].

### 2-4-9- Porosity Measurement

Cake porosity was calculated using the following formula [19]:

$$\text{Porosity} = 1 - (\text{Apparent density} / \text{True density})$$

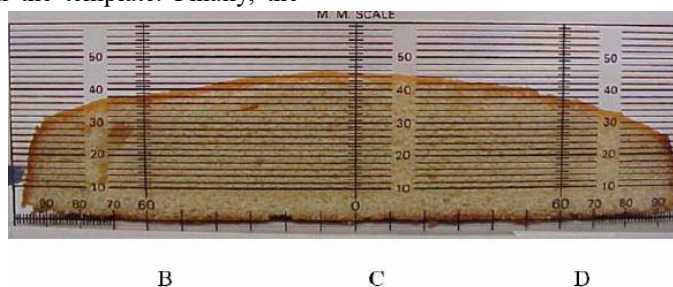
#### 2-4-10- Cake Symmetry and Uniformity Measurement

Cake symmetry and uniformity were measured using the AACC 91-10 method [15]. A transparent ruler was prepared for measuring symmetry and uniformity. A longitudinal section of the cake was prepared and placed on the template. Finally, the

height of the cake at points C, B, and D was measured, and cake symmetry and uniformity were calculated using the following formulas:

$$\text{Symmetry} = C - (B + D) / 2$$

$$\text{Uniformity} = B - D$$



**Figure 1- Transparent ruler used to measure cake symmetry and uniformity**

#### 2-4-11- Calorimetry (DSC)

To measure enthalpy ( $\Delta H$ ), 10 mg of the sample was placed in small stainless steel containers. The containers were sealed and heated from 25 to 100°C at a rate of 10°C per minute and held at 100°C for five minutes. To accelerate amylopectin retrogradation, the samples were also held at 4°C for 15 minutes [20].

#### 2-4-12- Calorimetry (DSC) and Thermogravimetry (TGA)\*\*

Ten milligrams of the sample were placed in the sample holder of the device, and the temperature was increased from 25 to 180°C at a rate of 10°C per minute. Free and bound water were measured using the device's graphs, and enthalpy was measured using DSC [20].

#### 2-4-13- Texture Measurement

The textural properties of the enriched cakes were measured one day after preparation using a texture analyzer. A cylindrical probe with a diameter of 6 mm was selected for the penetration test to evaluate the textural properties of the prepared cakes. The probe speed was set at 1 mm/s, and the penetration depth was 10 mm. The probe's forward and return speed was 2 mm/s [21].

#### 2-4-14- Sensory Evaluation

Fifteen trained evaluators were used to assess the sensory properties (aroma, taste, and color) of the sponge cakes one day after preparation. A 9-point hedonic scale was used for sensory evaluation.

#### 2-5- Statistical Analysis

All experiments were conducted in a completely randomized design with three replications. Statistical analysis of the results, including analysis of variance and comparison of means using Duncan's method at a 5% significance level, was performed using SPSS software.

### 3- Results and Discussion

#### 3-1- Specific Gravity

Table 4 shows the specific gravity of cake batter samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There was a significant difference in specific gravity between treatments ( $p \leq 0.05$ ). Treatment F, with 10 ppm transglutaminase and 100% reduced fat, had the highest specific gravity ( $1.24 \pm 0.002 \text{ g/cm}^3$ ) compared to other treatments. The control treatment had the lowest specific gravity ( $1.08 \pm 0.002 \text{ g/cm}^3$ ). It seems that with increasing enzyme levels, the elastic properties of the batter improve, and the strength of the gas-retaining cell walls increases, leading to better gas retention. As a result, the gas bubbles in the batter have a greater ability to expand, and the baked product has a larger volume. Therefore, as volume increases, specific gravity decreases. According to the results of Suran et al. (2003), increasing cake volume significantly reduces relative density [22].

#### 3-2-Consistency

Table 4 shows the consistency of cake batter samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There was a significant difference in consistency between treatments ( $p \leq 0.05$ ). Higher recorded numbers indicate lower batter consistency. Treatment E, with 20 ppm transglutaminase and 50% reduced fat, had the lowest consistency ( $40.42 \pm 0.01$  g/s) compared to other treatments. Treatment

B, with 10 ppm transglutaminase and 25% reduced fat, had the highest consistency ( $1.21 \pm 0.01$  g/s). Nourmohammadi et al. (2012) reported a consistency of 0.38 g/s for sponge cake batter samples without transglutaminase [23]. Therefore, it can be concluded that fat reduction significantly reduces cake batter consistency [24].

**Table 4- The results of comparison of average relative density and consistency in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Specific gravity (gr/cm <sup>3</sup> )	Consistency (gr/s)
A (Control)	$1.08 \pm 0.002^c$	$3.03 \pm 0.01^d$
B	$1.09 \pm 0.001^c$	$1.21 \pm 0.01^d$
C	$1.12 \pm 0.007^b$	$37.62 \pm 0.02^a$
D	$1.14 \pm 0.004^b$	$11.25 \pm 0.04^c$
E	$1.15 \pm 0.05^b$	$40.42 \pm 0.01^a$
F	$1.24 \pm 0.002^a$	$25.39 \pm 0.02^b$
G	$1.13 \pm 0.02^b$	$31.32 \pm 0.06^b$

All numbers are mean  $\pm$  standard deviation, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat.

increased the protein content of the samples compared to the control [25].

### 3-3- Protein

Table 5 shows the protein content of different sponge cake treatments based on different levels of reduced fat and enzyme at a significance level of  $p \leq 0.05$ . There was a significant difference in protein content between treatments. The control treatment had the lowest protein content ( $7.16 \pm 0.02\%$ ). Treatment G, with 20 ppm transglutaminase and 100% reduced fat, and Treatment F, with 10 ppm transglutaminase and 100% reduced fat, had the highest protein content at  $8.43 \pm 0.07\%$  and  $8.34 \pm 0.02\%$ , respectively. The increase in protein content in the treatments is due to the use of transglutaminase, which is itself a protein. The final protein content of the cakes was not excessively high, which reduced baking time and resulted in a desirable final texture. A similar result was reported by Mohammadi Gharamani et al. (2015), who studied the effect of Persian gum and carboxymethyl cellulose on the physicochemical and sensory properties of sponge cake. The results showed that adding Persian gum and carboxymethyl cellulose

### 3-4- Fat

Table 5 shows the fat content of different sponge cake treatments based on different levels of reduced fat and enzyme at a significance level of  $p \leq 0.05$ . There was a significant difference in fat content between treatments. Treatment B, with 10 ppm transglutaminase and 25% reduced fat, had the highest final fat content ( $43.81 \pm 0.01\%$ ). Other treatments had lower fat content compared to the control. Treatment F, with 10 ppm transglutaminase and 100% reduced fat, had the lowest final fat content ( $12.69 \pm 0.08\%$ ). As the enzyme level increases, the fat content decreases further. Seghal et al. (2011) reported a fat content of 7% in fish cakes made from Indian carp. In the present study, a similar result was observed due to the addition of the protein enzyme transglutaminase [26].

### 3-5-Moisture

Table 5 shows the moisture content of different treatments of sponge cake based on various levels of

reduced fat and enzyme at a significance level of  $p \geq 0.05$ . There is a significant difference in moisture content among the treatments. Treatment F, with 10 ppm of transglutaminase enzyme and 100% reduced fat, had the highest final moisture content ( $31.40 \pm 0.05\%$ ) compared to other treatments. Following that, Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, also had a high moisture content, which can be attributed to the low fat level and the better performance of the transglutaminase enzyme. Pourasmail et al. ( ) in their study on the addition of transglutaminase enzyme to bread reported an increase in moisture content with higher enzyme levels in the formulation, which aligns with our findings [27].

### 3-6-Ash

Table 5 also shows the ash content of different treatments of sponge cake based on various levels of reduced fat and enzyme at a significance level of  $p \geq 0.05$ . There is a significant difference in ash content among the treatments. Treatment F, with 10 ppm of transglutaminase enzyme and 100% reduced fat, and Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the highest final ash content ( $5.38 \pm 0.04\%$  and  $2.70 \pm 0.06\%$ , respectively). Treatment B, with 10 ppm of transglutaminase enzyme and 25% reduced fat, had the lowest final ash content ( $0.14 \pm 0.04\%$ ). Nourmohammadi et al. (2012) reported the ash content of sponge cake as 0.49%, which is slightly lower than the ash content in the present study. This difference can be attributed to the use of different types and percentages of ingredients in the cake formulation [23].

**Table 5- The results of comparing the average amount of protein, fat, moisture and cake ash in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Protein (%)	fat (%)	Moisture (%)	Ash (%)
A (Control)	$7.16 \pm 0.02^c$	$34.35 \pm 0.01^b$	$15.60 \pm 0.30^c$	$0.57 \pm 0.04^{c,d}$
B	$7.60 \pm 0.04^b$	$43.81 \pm 0.01^a$	$19.29 \pm 0.20^b$	$0.14 \pm 0.04^d$
C	$7.58 \pm 0.05^{b,c}$	$29.69 \pm 0.02^c$	$18.47 \pm 0.06^b$	$0.60 \pm 0.03^c$
D	$7.76 \pm 0.03^b$	$14.03 \pm 0.02^d$	$21.43 \pm 0.20^b$	$0.92 \pm 0.05^c$
E	$7.75 \pm 0.05^b$	$12.95 \pm 0.01^d$	$23.08 \pm 0.03^b$	$0.85 \pm 0.07^c$
F	$8.34 \pm 0.02^a$	$12.69 \pm 0.08^d$	$31.40 \pm 0.05^a$	$5.38 \pm 0.04^a$
G	$8.43 \pm 0.07^a$	$13.60 \pm 0.15^d$	$28.01 \pm 0.01^a$	$2.70 \pm 0.06^b$

All numbers are mean  $\pm$  standard deviation, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat.

### 3-7-Volume

Table 6 shows the volume of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There is a significant difference in volume among the treatments. In most treatments, the volume decreased slightly compared to the control treatment ( $125.00 \pm 1.15 \text{ cm}^3$ ), which can be attributed to the formation of cross-links due to the presence of the transglutaminase enzyme. Only in Treatment C, with 20 ppm of transglutaminase enzyme and 25% reduced fat, was the final cake volume slightly higher than the control. Ronda et al. (2005) reported in their study that during normal baking, the volume of the cake increases, but if binding agents are used in the formulation, such as transglutaminase enzyme, they can reduce the final volume of the product. Another reason for the

volume reduction could be the decreased gas retention capacity of the dough. There is an inverse relationship between the specific weight of the dough and the volume of the cake; the lower the specific weight, the higher the volume, and vice versa [28].

### 3-8-Weight

Table 6 also shows the weight of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There is a significant difference in weight among the treatments ( $p \geq 0.05$ ). The weight of the cake decreased slightly only in Treatment F, with 10 ppm of transglutaminase enzyme and 100% reduced fat, compared to the control. In other treatments, the weight increased significantly compared to the control ( $p \geq 0.05$ ). The transglutaminase enzyme, due to its protein structure, retained more moisture in the samples,

resulting in increased weight. A similar result was reported by Russell et al. (2001) in their study on various hydrocolloids, where the presence of hydroxyl groups in the fiber structure increased water absorption, leading to increased sample weight [29].

### 3-9-Specific Volume

Table 6 also shows the specific volume of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There is a significant difference in specific volume among the treatments ( $p \geq 0.05$ ). The specific volume of the cake decreased in all treatments compared to the control. Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the lowest specific volume ( $1.28 \pm 0.03 \text{ cm}^3/\text{g}$ ). Mehran Shandi et al. (2013) used transglutaminase enzyme in sponge cake formulations at different levels and concluded that the use of this enzyme reduces the specific volume of the cake, which aligns with the results of the present study [30].

### 3-10-Symmetry

Table 6 also shows the symmetry of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There is a significant difference in symmetry among the treatments ( $p \geq 0.05$ ). Treatments B and C, with 10 ppm of transglutaminase enzyme and 25% reduced fat, and 10 ppm of transglutaminase enzyme with 50% reduced fat, respectively, had the highest final symmetry ( $26.00 \pm 1.15 \text{ mm}$  and  $24.00 \pm 0.57 \text{ mm}$ ). The increased gas retention is due to the presence of protein (transglutaminase enzyme). Additionally, the distribution of fine particles on the protein affects gelatinization and results in better shaping in the oven. The better symmetry and uniformity of these treatments can be attributed to the lower amount of transglutaminase enzyme and a less resistant network to the entry of air bubbles into the product. These bubbles can act as initial nuclei for the distribution of gas from chemical leavening agents. Uniform distribution of air bubbles improves the symmetry and uniformity of the cake. The sugar-dough mixing method also directly affects symmetry. A similar result was reported by Peighambardoust (2009) [12].

**Table 6- Comparison results of average volume, weight, specific volume, and cake symmetry in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Volume (cm <sup>3</sup> )	Weight (gr)	Specific volume (cm <sup>3</sup> /gr)	Cake symmetry (mm)
A (Control)	$125.00 \pm 1.15^a$	$65.80 \pm 0.23^b$	$1.89 \pm 0.01^a$	$18.00 \pm 0.57^b$
B	$110.00 \pm 2.30^b$	$66.80 \pm 0.28^a$	$1.64 \pm 0.04^b$	$1/15 \pm 26/00^a$
C	$125.00 \pm 0.57^a$	$67.20 \pm 0.11^a$	$1.86 \pm 0.05^a$	$12.00 \pm 1.15^c$
D	$100.00 \pm 1.15^b$	$66.90 \pm 0.11^a$	$1.49 \pm 0.01^b$	$24.00 \pm 0.57^a$
E	$105.00 \pm 1.73^b$	$66.90 \pm 0.11^a$	$1.56 \pm 0.02^b$	$17.00 \pm 0.57^b$
F	$95.00 \pm 1.73^c$	$65.70 \pm 0.05^b$	$1.44 \pm 0.02^{b,c}$	$19.00 \pm 0.57^{a,b}$
G	$85.00 \pm 2.30^c$	$66.00 \pm 0.11^a$	$1.28 \pm 0.03^c$	$10.00 \pm 1.15^c$

All numbers are mean  $\pm$  standard deviation, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat

### 3-11-Apparent Density

Table 7 shows the apparent density of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. In all treatments, the apparent density of the cake increased compared to the control ( $p \geq 0.05$ ). Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the highest apparent density ( $0.77 \pm 0.01 \text{ g/cm}^3$ ). According to the results of Desrochers et al. (2004),

apparent density has an inverse relationship with cake volume; as the volume increases, the apparent density significantly decreases [31]. Therefore, Treatment G, which had the lowest volume, had the highest apparent density.

### 3-12-Solid Density

Table 7 also shows the solid density of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. In most treatments, the



average solid density of the cake decreased slightly compared to the control, but the difference was not significant ( $p \geq 0.05$ ). The average solid density of the control treatment was  $1.20 \pm 0.001$  g/cm<sup>3</sup>. The solid density of a food product depends on the ingredients used in the formulation. Since the ingredients in the formulation did not differ significantly in this study, the solid density did not change much, which aligns with the results of Pour Safari et al. (2012) [32].

### 3-13-Porosity

Table 7 also shows the porosity of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared

to the control treatment. In all treatments, the porosity of the cake decreased significantly compared to the control ( $p \geq 0.05$ ). Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the lowest porosity ( $34.94 \pm 1.60\%$ ). The low porosity of the treated samples is due to the strong network that prevents the entry of air bubbles during mixing. Zybrow et al. (2012) used different levels of transglutaminase enzyme in bread formulations and concluded that the enzyme can slightly increase the porosity of the samples, which does not align with the results of the present study. This discrepancy can be attributed to differences in raw materials and the amount of enzyme used in the formulation [33].

**Table 7- Comparison results of the average amount of apparent density, Solid density and porosity in sponge cake samples in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Apparent density (gr/cm <sup>3</sup> )	Solid density (gr/cm <sup>3</sup> )	Porosity (%)
A (Control)	$0.52 \pm 0.003^c$	$1.20 \pm 0.001^a$	$56.23 \pm 0.33^a$
B	$0.60 \pm 0.01^b$	$1.19 \pm 0.001^a$	$48.97 \pm 1.28^b$
C	$0.53 \pm 0.001^c$	$1.20 \pm 0.005^a$	$55.18 \pm 0.14^a$
D	$0.66 \pm 0.008^b$	$1.19 \pm 0.002^a$	$43.75 \pm 0.69^b$
E	$0.63 \pm 0.009^b$	$1.19 \pm 0.003^a$	$46.66 \pm 0.82^b$
F	$0.69 \pm 0.01^b$	$1.19 \pm 0.001^a$	$42.26 \pm 1.04^b$
G	$0.77 \pm 0.01^a$	$1.19 \pm 0.01^a$	$34.94 \pm 1.60^c$

All numbers are mean  $\pm$  standard deviation, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat.

### 3-14-Texture Properties

Table 8 shows the average texture characteristics of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There is a significant difference in all six factors among the treatments ( $p \leq 0.05$ ). Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the highest average hardness ( $4033.00 \pm 2.30$  g). Treatment C, with 20 ppm of transglutaminase enzyme and 25% reduced fat, had the lowest hardness ( $1810.00 \pm 1.15$  g). Kappelman and De Jong (2002) concluded in their study that the use of transglutaminase enzyme can create cross-links in the gluten in flour, leading to high molecular weight polymers. The formation of these polymers can strengthen the gluten network and thus create desirable physical-chemical and rheological properties, which partially aligns with the results of

the present study. Generally, hardness is a combination of the resistance of the crumb and crust to compression. On the other hand, the strength of the cake is directly related to density and inversely related to volume; as density increases, the strength and resistance of the cake increase, and thus hardness also increases. Retrogradation of bakery products occurs immediately after baking and during cooling. This process depends on the product formulation, production conditions, and storage. The effects of staling in bakery products include changes in texture, crust, crumb, loss of moisture, and flavor. During staling, starch molecules strongly bind together, expelling water from the network. The increase in hardness can be attributed to starch retrogradation during storage, where increased staling leads to increased hardness. Additionally, with an increase in the dry components of the dough, a denser and thicker product is produced [34].

In most treatments, adhesiveness increased compared to the control. Treatment with 20 ppm of transglutaminase enzyme and 25% reduced fat had the lowest adhesiveness ( $0.28 \pm 0.01$ ). Treatment with 20 ppm of transglutaminase enzyme and 100% reduced fat had the highest final adhesiveness ( $0.61 \pm 0.02$ ).

In treatments containing 10 ppm of transglutaminase enzyme with 50% reduced fat, 20 ppm of transglutaminase enzyme with 25% reduced fat, and 20 ppm of transglutaminase enzyme with 100% reduced fat, cohesiveness increased compared to the control. In other treatments, cohesiveness decreased compared to the control. Renzetti et al. (2007) reported in their study that the use of microbial transglutaminase in food products improves cohesiveness, which partially aligns with the results of this study [35].

The control treatment had the highest average springiness ( $7.26 \pm 0.02$  mm) compared to other

treatments. Treatment C, with 20 ppm of transglutaminase enzyme and 25% reduced fat, had the lowest springiness ( $6.30 \pm 0.01$  mm). Increased elasticity, cohesiveness, and flexibility can reflect increased specific volume and better aeration. High elasticity indicates freshness, aeration, and good elastic properties of the product [34, 35].

In treatments containing 10 ppm and 20 ppm of enzyme with 100% reduced fat, gumminess increased compared to the control. In other treatments, gumminess decreased compared to the control.

In treatments containing 10 ppm and 20 ppm of enzyme with 100% reduced fat, chewiness increased compared to the control. In other treatments, chewiness decreased compared to the control. Treatment with 20 ppm of enzyme and 25% reduced fat had the lowest chewiness ( $79.75 \pm 1.18$  J).

**Table 8- Comparison results of the average amount of texture characteristics in sponge cake samples in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Hardness (gr)	adhesiveness (mj)	Cohesiveness	Springiness (mm)	Gumminess (gr)	Chewiness (j)
A (Control)	$2778.50 \pm 4/04^b$	$0.45 \pm 0.01^b$	$0.67 \pm 0.005^a$	$7.26 \pm 0.02^a$	$1872.80 \pm 1.67^b$	$133.34 \pm 1.17^b$
B	$2559.00 \pm 2/88^b$	$0.32 \pm 0.01^c$	$0.65 \pm 0.005^b$	$7.15 \pm 0.02^a$	$1651.15 \pm 0.51^b$	$115.77 \pm 0.61^b$
C	$1810.00 \pm 1/15^c$	$0.28 \pm 0.01^{c,d}$	$0.71 \pm 0.01^a$	$6.30 \pm 0.01^c$	$1288.70 \pm 1.68^c$	$79.75 \pm 1.18^c$
D	$2448.50 \pm 2.30^b$	$0.50 \pm 0.02^b$	$0.68 \pm 0.005^a$	$6.75 \pm 0.01^b$	$1672.10 \pm 1.15^b$	$110.68 \pm 1.17^b$
E	$2440.00 \pm 1.73^b$	$0.38 \pm 0.01^c$	$0.65 \pm 0.01^b$	$6.72 \pm 0.01^b$	$1587.40 \pm 1.21^b$	$104.61 \pm 1.21^b$
F	$3925.00 \pm 2/88^a$	$0.46 \pm 0.02^b$	$0.65 \pm 0.01^b$	$6.80 \pm 0.01^b$	$2540.00 \pm 1.15^a$	$16.1 \pm 12.171^a$
G	$4033.00 \pm 2.30^a$	$0.61 \pm 0.02^a$	$0.73 \pm 0.01^a$	$7.09 \pm 0.02^a$	$2943.00 \pm 1.73^a$	$204.62 \pm 1.74^a$

All numbers are mean  $\pm$  standard deviation, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat.

### 3-15-Calorimetry and Thermogravimetry

Table 9 (Figure 2) shows the changes in initial and final enthalpy, free water, and bound water in different treatments of sponge cake based on various levels of fat and enzyme at a significance level of  $p \leq 0.05$ . Enthalpy is a measure of the quantity and quality of crystals and the loss of molecular order within the granules. Based on the data in this table, Treatment with 10 ppm of enzyme and 50% reduced fat had the lowest initial enthalpy ( $-106.89 \pm 1.00$  J). Treatments with 20 ppm of enzyme and 25% reduced fat had the highest initial enthalpy ( $-66.18 \pm 1.00$  J). Treatment with 10 ppm of enzyme and 100%

reduced fat had the lowest final enthalpy ( $-2814.39 \pm 0.71$  J). Treatments with 20 ppm of enzyme and 100% reduced fat had the highest final enthalpy ( $-1933.57 \pm 1.00$  J). The final enthalpy in all treatments was negative, which reduced baking time and created a desirable final texture. Low enthalpy indicates less structural order and stability of the crystals. Based on the results, the transglutaminase enzyme increased structural flexibility [36].

Treatment with 10 ppm of enzyme and 25% reduced fat had the lowest free water ( $22.21 \pm 0.00\%$ ). Treatments with 20 ppm of enzyme and 25% reduced fat had the highest free water ( $26.62 \pm 0.00\%$ ). Treatment with 10 ppm of enzyme and 50%

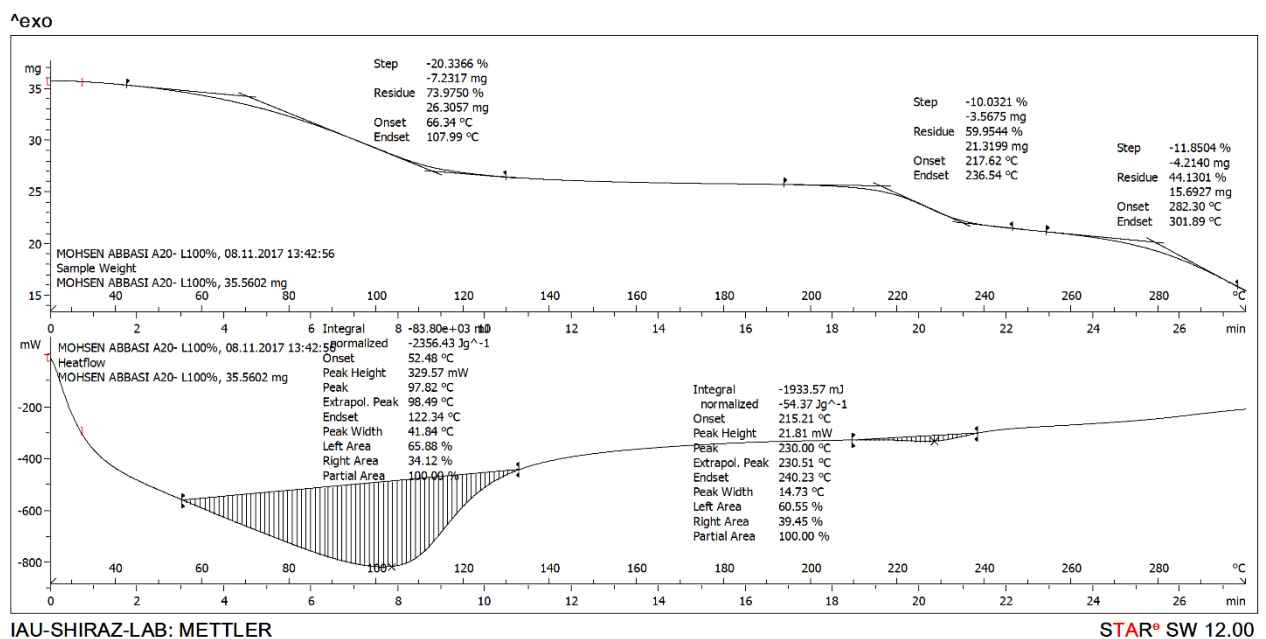
reduced fat and treatments with 20 ppm of enzyme and 25% and 50% reduced fat had the highest bound water ( $29.00 \pm 1.00\%$ ). Treatment with 10 ppm of enzyme and 25% reduced fat had the lowest bound water ( $24.21 \pm 1.00\%$ ). In 2002, Pietrasik conducted

a study on the effect of transglutaminase enzyme on the texture properties of meat burgers and concluded that the enzyme reduces free water and increases product shelf life, which aligns with the results of the present study [37].

**Table 9- The results of comparing the average amount of enthalpy, free and bound water in sponge cake samples in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Enthalpy 1 (j)	Enthalpy 2 (j)	free water (%)	Bound water (%)
A (Control)	-67.00 $\pm$ 1.00 <sup>a</sup>	-2491.00 $\pm$ 0.50 <sup>b</sup>	24.03 $\pm$ 0.50 <sup>a</sup>	27.00 $\pm$ 1.00 <sup>a</sup>
B	-78.27 $\pm$ 2.00 <sup>a</sup>	-2243.27 $\pm$ 0.94 <sup>b</sup>	22.21 $\pm$ 0.00 <sup>b</sup>	24.21 $\pm$ 1.00 <sup>b</sup>
C	-66.18 $\pm$ 1.00 <sup>a</sup>	-2754.18 $\pm$ 0.81 <sup>c</sup>	26.62 $\pm$ 0.00 <sup>a</sup>	29.00 $\pm$ 1/00 <sup>a</sup>
D	-106.89 $\pm$ 1.00 <sup>b</sup>	-2537.89 $\pm$ 0.83 <sup>b</sup>	25.25 $\pm$ 0.50 <sup>a</sup>	29.00 $\pm$ 1/00 <sup>a</sup>
E	-73.42 $\pm$ 1.00 <sup>a</sup>	-2726.42 $\pm$ 0.26 <sup>c</sup>	25.42 $\pm$ 0.00 <sup>a</sup>	29.00 $\pm$ 1/00 <sup>a</sup>
F	-76.39 $\pm$ 0.65 <sup>a</sup>	-2814.39 $\pm$ 0.71 <sup>c</sup>	22.39 $\pm$ 0.00 <sup>b</sup>	26.00 $\pm$ 1.00 <sup>b</sup>
G	-83.80 $\pm$ 1.00 <sup>a</sup>	-1933.57 $\pm$ 1.00 <sup>a</sup>	22.32 $\pm$ 0.60 <sup>b</sup>	25.50 $\pm$ 1.00 <sup>b</sup>

All numbers are mean  $\pm$  standard deviation, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat.



**Figure 2- An example of differential calorimetry in a sample of sponge cake produced with dough containing microbial transglutaminase enzyme and reduced fat (Treatment G: 20 ppm transglutaminase and 100% reduced fat).**

### 3-16-Sensory Evaluation

Table 10 shows the average sensory evaluation scores for aroma, taste, and color of sponge cake samples containing different levels of transglutaminase enzyme and reduced fat compared to the control treatment. There is a significant

difference in all three factors among the treatments ( $p \leq 0.05$ ). Treatment D, with 10 ppm of transglutaminase enzyme and 50% reduced fat, had the highest average score for aroma (7.37) compared to other treatments. Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the lowest average score for aroma (2.67). The significantly lower sensory scores for aroma in Treatments F and G compared to the control can be

attributed to the 100% reduction in fat. Treatment D, with 10 ppm of transglutaminase enzyme and 50% reduced fat, had the highest average score for taste (8.47) compared to other treatments. Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the lowest average score for taste (2.60). Generally, fat plays an important role in creating a desirable mouthfeel and taste; reducing fat from 100% to 0% relatively decreased consumer satisfaction with the cake's taste. Treatment D, with 10 ppm of transglutaminase enzyme and 50% reduced fat, had the highest average score for color

(8.83) compared to other treatments. Treatment G, with 20 ppm of transglutaminase enzyme and 100% reduced fat, had the lowest average score for color (1.13). Based on the research of Idora et al. (2007), the darker color of the cake in Treatments F with 10 ppm of transglutaminase enzyme and 100% reduced fat, Treatment E with 20 ppm of transglutaminase enzyme and 50% reduced fat, and Treatment G with 20 ppm of transglutaminase enzyme and 100% reduced fat can be attributed to the intensification of the Maillard reaction and caramelization during production and thermal processing [38].

**Table 10- The results of comparing the average amount of Sensory evaluation in sponge cake samples in sponge cake samples produced with dough containing microbial transglutaminase enzyme and reduced fat**

Treatment	Smell	Ttaste	Color
A (Control)	6.20 <sup>a, b</sup>	6.87 <sup>b</sup>	6.40 <sup>b</sup>
B	6.27 <sup>a</sup>	6.37 <sup>b</sup>	7.27 <sup>a</sup>
C	5.00 <sup>b</sup>	6.87 <sup>b</sup>	6.57 <sup>b</sup>
D	7.37 <sup>a</sup>	8.47 <sup>a</sup>	8.83 <sup>a</sup>
E	5.77 <sup>b</sup>	2.47 <sup>d</sup>	4.40 <sup>c</sup>
F	3.73 <sup>c</sup>	4.37 <sup>c</sup>	2.40 <sup>d</sup>
G	2.67 <sup>c</sup>	2.60 <sup>d</sup>	1.13 <sup>d</sup>

All numbers are mean, different letters indicate significance at  $P \leq 0.05$  level. A: control, B: 10 ppm transglutaminase and 25% reduced fat, C: 20 ppm transglutaminase and 25% reduced fat, D: 10 ppm transglutaminase and 50% reduced fat, E: 20 ppm transglutaminase and 50% reduced fat, F: 10 ppm transglutaminase and 100% reduced fat, G: 20 ppm transglutaminase and 100% reduced fat.

### 3-17-Conclusion

Producing low-fat cereal products is a goal for most food industries, and this phenomenon has led food industry researchers to propose solutions to reduce fat in food products while maintaining the functional properties of fat in the food system. One of the solutions to address the quality issues resulting from fat removal in food products is the use of various fat substitutes. In the treatment with 10 ppm of transglutaminase enzyme and reduced fat, the mechanical and rheological properties of the dough and the organoleptic properties of the cake improved favorably due to the protein structure of the enzyme and the formation of beneficial bonds in the cake structure compared to the control and other treatments. However, complete fat removal is not recommended.

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جایگزین چربی،

کم کالری

ترانس گلوتامیناز میکروبی یک آنزیم از گروه ترانسفرازها بوده، که به صورت گسترده به منظور اصلاح ویژگی های عملکردی پروتئین ها در مواد غذایی مختلف مورد استفاد قرار می گیرد. پیوندهای کووالانسی ایجاد شده توسط این آنزیم اثرات منحصر به فردی روی ظرفیت تشکیل ژل، پایداری حرارتی و ظرفیت نگهداری آب در پروتئین ها دارد. هدف از این پژوهش بررسی تاثیر مقادیر مختلف آنزیم ترانس گلوتامیناز میکروبی به عنوان جایگزین چربی بر ویژگی های گرماسنجی، گرماوزن سنجی و رئولوژیکی کیک اسفنجی کم کالری بوده است. برای این منظور هفت تیمار مختلف با ۰، ۲۵، ۵۰ و ۱۰۰ درصد چربی کاهش یافته ۱۰ پی پی ام و ۲۰ پی پی ام آنزیم ترانس گلوتامیناز تولید شد. براساس نتایج حاصل حداکثر میزان پروتئین (۸.۴۳٪)، دانسیته ظاهری (۰/۷۷ گرم بر سانتی متر مکعب)، دانسیته جسمی (۱/۱۹ گرم بر سانتی متر مکعب)، سختی (۴۰۳۳ گرم)، چسبندگی (۰/۶۱ مگاژول)، پیوستگی (۰/۷۳)، صمغیت (۲۹۴۳ گرم)، آنتالپی دوم و قابلیت جویدن (۲۰۴/۶۲ مگاژول) در تیمار با ۲۰ پی پی ام آنزیم و ۱۰۰ درصد چربی کاهش یافته مشاهده شد. کمترین آب آزاد در تیمار حاوی ۱۰ پی پی ام آنزیم با ۲۵ درصد چربی کاهش یافته مشاهده شد. تیمار با ۱۰ پی پی ام آنزیم و ۵۰ درصد چربی کاهش یافته بهترین طعم، بو و رنگ را از نظر ارزیاب ها داشته است. مقدار پروتئین به دلیل حضور آنزیم ترانس گلوتامیناز در نمونه ها افزایش داشت و خصوصیات بافتی آن بهبود یافت. ولی کاهش ۱۰۰ درصد مقدار چربی تاثیر منفی بر خصوصیات حسی محصول تولید شده داشت. بنابراین حذف کامل چربی، برای تولید این محصول پیشنهاد نمی شود ولی با استفاده از آنزیم ترانس گلوتامیناز، می توان چربی را تا حد قابل توجهی کاهش داد.

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