The effect of transglutaminase on gluten-free soy bread baked using ohmic heating

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Abstract. This study aims to compare the quality of gluten-free bread made from black soybean flour using and without transglutaminase (TGase) baked using ohmic heating technology. Parameters of bread quality observed were specific volume, moisture content, texture profile analysis, porosity, and color. To understand the changes in the ohmic heating process parameters during baking, an evaluation of temperature, electrical conductivity, current intensity, electrical power parameters was carried out. To analyze the data, the independent sample t-test method was used. The results obtained indicate that the addition of TGase affects the characteristics of the bread and process parameters during baking with ohmic heating. Bread with the addition of TGase has an increase in volume, texture, moisture content, and porosity of the bread that is better than without the addition of TGase. Based on the parameter process, the rate of increase in temperature, electrical conductivity, current, and power intensity is highly affected by the addition TGase. Electrical conductivity increases linearly with increasing temperature.

1. Introduction

Generally, gluten-free bread can be used as a major alternative food for the individual who suffers from celiac disease and autism. In recent years, various studies have been conducted on gluten-free bread development, due to the increase in gluten-intolerant customers’ demand [1]–[4]. Besides that, the increasing demand for gluten-free products was also due to the healthy trend lifestyle that developed in public. So far, commercially and experimentally-produced gluten-free bread still has some drawbacks and limitations, such as the bread’s volume development that did not live up to expectations, weak structure, crumb, and organoleptic aspect which much different from wheat-based products [5]. Gluten is the main component in the wheat which is value important in determining the quality of the bread. Gluten affects the interaction between protein and starch which is related to the formation of gas cells, including the stabilization and retention of gas in cells during the baking process. Gluten-free bread is bread made from ingredients without gluten. The absence of gluten in gluten bread dough has prompted researchers to look for alternatives that can mimic the performance of gluten in forming a protein matrix in the dough.

Transglutaminase (TGase) catalyzes the acyl transfer reaction between the carboxamide of a protein-bound peptide or glutamine and a primary amine. When TGase acts on a protein molecule, a lysine -(γ-glutamyl) cross-link is formed [6]. The use of TGase in the manufacture of gluten-free bread
is known to improve the rheological aspects of the dough and improve the quality of the resulting bread [7]–[9]. However, the use of TGase is highly dependent on the addition level and protein content in the ingredients. In this research, gluten-free bread will be made using 100% soy flour. The high protein content of soybean flour is a good source of the substrate to increase enzyme activity in the formation of matrix protein in the batter. This will affect the crumb structure in entrapping gas during development so as to produce maximum volume. Other than that, the use of soy flour in products will increase the nutritional value of the resulting bread.

In the manufacture of gluten-free bread, the development is completely controlled by the protein and starch matrix in the crumb so that baking has a large impact on the characteristics of the resulting bread[4], [10]. Conventional roasting methods are usually done to achieve the goal. The main problems in conventional baking are high temperatures that can reduce the strength of protein in maintaining the crumb structure, decrease in nutrients, the occurrence of maillard reactions, and non-uniform heating[11]. Ohmic heating is a heating technique based on passing an electric current through a material. The potential for ohmic heating which has a high heating rate and is volumetric, and can prevent overheating (temperatures below 100°C) is expected to reduce unwanted reactions such as protein denaturation, so as to maintain the crumb structure and optimize TGase performance during the baking process. This study aims to identify the impact of adding TGase on gluten-free bread baked using the ohmic heating method. Then the comparison of the characteristics of bread and changes in process parameters was evaluated during the process of making gluten-free bread.

2. Material and methods

2.1 Material

The materials that used in the making of gluten-free bread are soy flour (100 gr), dry yeast (3 %), sugar (25 %), egg (10 %), salt (2 %), konjac glucomannan gel (5 % with a concentration of 0.2%), emulsifier (1.5 %), margarine (5 %), water (180 %), and enzymes transglutaminase (TGase) (1 %).

2.2 Methods

The making of gluten-free bread batter was done using some modification of Bender et al. method [11]. At first, dry yeast was dissolved in 100 ml of water (25°C) and placed into the proofer for 3 minutes, and also the enzyme was dissolved in 80 ml of water. All of the dry ingredients were mixed by mixer (KLAZ Inc., Indonesia) for 2 minutes with 84 rpm. After that, the suspension of yeast and dissolved enzyme are added into the mixer, followed by increasing the mixer’s velocity to 126 rpm for 5 minutes until the ingredients are perfectly mixed. The batter is then placed into the baking chamber that was made of acrylic which was covered with margarine before and then wrapped with plastic wrap. After that, the batter was placed into the proofer (GETRA, Japan) with a temperature of 35°C for 40 minutes. For the next step, the baking process was conducted by the ohmic heating method for 30 minutes. The bread which has been baked then gets separated from its baking chamber and cooled for 2 hours at room temperature for further analysis. All of the measurements were done in 3 replications.

2.3 Ohmic heating design

Ohmic tool system was consisting of a slide regulator (Takayoshi 260 Volt. Inc. Japan) as the electrical current power supply, a baking chamber of batter made of Acrylic Polymethyl Methacrylate (PMMA) with a thickness of 5 mm, a pair of electrodes made of stainless steel (8.5 x 9 cm) with a thickness of 2.5 mm each placed 12 cm apart on both sides of the baking chamber, and a data logger (Arduino) equipped with a current sensor and 3 pieces thermocouples (T1, T2, and T3).
Figure 1. Shows a sketch of baking equipment using the ohmic heating method which includes (1) current, (2) chamber, (3) electrodes, (4) power supply, (5) data logger, (6) thermocouple, (7) computer.

The bread was baked using an alternating current source with 90 V (gradient potential 7.5 V/cm) for 30 minutes with a current frequency of 50 Hz. All recorded data are voltage, current intensity, and temperature change as a function of time. The data of three thermocouples were recorded data loggers at different positions to monitor temperature distribution during process baking. The sample temperature was based on the result data logger of the thermocouple were placed 2.5 cm from side each electrode (T1, T2) and in the center of the batter (T3). In the ohmic heating process by passing an electric current source, including “Joule's law”, the heat generated per unit time and volume in the material is:

$$q = \sigma (E)^2$$  \hspace{1cm} (1)

Where, $q$ is the rate of internal heat generated per volume (W/m$^3$), $E$ is the electric field (V/m), and $\sigma$ is the electrical conductivity (S/m)

The determination of electrical conductivity was decided based on the Gally et al. method [12] using the formula below:

$$\sigma = \frac{L \times I}{A \times X}$$  \hspace{1cm} (2)

Where, $\sigma$ is the electrical conductivity (S/m), $L$ is the distance between electrodes (cm), $A$ is the area of electrode (m$^2$), $I$ is the current intensity (A), and $V$ is the voltage (V). Electrical conductivity of batter vs core temperature as a function of the addition TGase were presented. The experimental data were implemented in the model (linear interpolation between the points).

For the determination of current intensity, the calculation was done using the formula below:

$$I = \frac{V}{R}$$  \hspace{1cm} (3)

Where $I$ is the current intensity (A), $V$ is the voltage (V), while $R$ is the resistance (Ohm).

In determining the electrical power is determined by the formula [13]:

$$P = \sum V I \Delta t$$  \hspace{1cm} (4)

Where $P$ is the electrical power (J) and $t$ is the time (s).
2.4 Determination of bread quality

The specific volume was calculated using the rapeseeds displacement method which was approved by AACC 55-50.01 [14]. The specific volume (cm³/g) was calculated as the volume change ratio with bread mass. Water content was determined using the AOAC method [15].

Texture parameter was determined using Texture Profile Analyzer (TPA) (CT3 Texture Analyser Brookfield Engineering Lab, Ins.) with probe type. For the analysis, the bread was cut using a slicer with a thickness of 20 mm. The analysis was done by two compression tests using cylinder probe 20 mm, velocity 1 mm/s, trigger load 6.8 g, and compression distance 10 mm. Rheology parameters analyzed are hardness, springiness, cohesiveness, resilience and chewiness. The analysis was done 2 hours after the baking process.

Crumb porosity was determined using the Bender et al. method [11]. For each formulation, the bread was cut into half and the crumbs on the sides of the slices were photographed using a digital camera (DSLR Canon EOS 600D). The crumbs photo was cropped into 20×20 mm in actual dimension size and converted into 16-bit grayscale. Then, the analysis was done using the Otsu algorithm assumption as a threshold method to differentiate gas-cell and non-cell. This software uses the contrast between two phases (porous and solid side) to estimate the porosity. The mean size of pore diameter, area of the pore (the percentage of pore total area with the total area of bread) and pores (total of pores) were determined using the software.

Color test determination was done by using the Olojede et al. method [16]. Color lightness parameter (L*), redness (+a*), and yellowness (+b*) were done based on CIELAB system color (CIE, 1986) using Chroma meter CM-5 Minolta (Konica Minolta, Inc., Japan) which was equipped with a xenon pulsed lamp and D65 illuminant. Crumb colors were measured in 3 different zones on the top surface of the bread.

2.5 Statistical analysis

To determine the effect of treatment factors (addition of TGase and without the addition of TGase), a comparison of variance was carried out on the sample using the independent sample t-test method on the average sample using MiniTab 17 software. The level of confidence used was 95%. All samples were analyzed in 3 replications. The process parameters are shown in the graph as a function per unit.

3. Results and discussion

3.1 The effect of addition of TGase dan without TGase on quality gluten-free bread.

The differences in the characteristics of the bread produced with the addition of TGase and without the addition of TGase can be seen in Table 1. From the results of the volume specific measurement, bread with the addition of TGase gave a better effect than without the addition of TGase. Bread with the addition of TGase had a higher volume of expansion than without the addition of TGase (Figure 2). This is due to the effect of TGase which can produce a cross-linking reaction between glutamic acid and lysine contained in soybean flour, thereby increasing the formation of a protein matrix. In the dough, the formation of a protein matrix will have an impact on the process of trapping CO2 gas produced by yeast during proofing, thereby increasing the volume of development. [12] [13]. The increase in specific volume was also reflected in an increase in cell density and the area and number of pores (Table 1). Bread with the addition of TGase showed a higher cell density than without the addition of TGase. Higher pore count, area, and porosity were obtained in bread with the addition of TGase and were strongly correlated with the smaller average pore size found in bread with the addition of TGase. The results under the research of Dłuzewska et al., reports that the addition of TGase caused a significant increase in the specific volume and porosity of gluten-free bread [9].

Analysis of the texture properties of bread was carried out using a texture analysis profile. The results of Table 1 show that lower hardness values were produced in bread with the addition of TGase. The finding of hardness value in this bread was highly correlated with volume expansion, as well as the higher cohesiveness value which was found to be higher in the control. These results showed the
same result as in the research done by Dłuzewska et al [9] who reports that the resilience, spring, and chewing values were found to be higher in bread produced with the addition of TGase. Chewiness is a parameter that is closely related to how easily a food is chewed in the mouth and its value was determined by the calculation of some texture parameters including hardness, springiness, and cohesiveness. The color of the crumb on bread without the addition of TGase had a higher L (brightness) and b* (yellow) on bread without the addition of TGase. Because the ohmic heating process can only reach a temperature of 100°C, a maillard reaction can’t occur [19]. So the color change is thought to occur due to the impact of the starch gelatinization reaction, and the degradation of monomeric anthocyanin during the baking process. Different results could occur in various food materials because of the different reaction and oxidation of the medium during the ohmic heating process [20].

Table 1. Comparison of characteristic gluten-free bread with the addition TGase and without addition TGase.

| Parameter test                      | Without enzymes | With enzymes | p-value |
|-------------------------------------|-----------------|--------------|---------|
| Specific volume (cm³/g)             | 1.64 ± 0.28     | 3.25 ± 0.06  | 0.007   |
| Moisture crumb (%)                  | 48.06 ± 0.41    | 43.301 ± 0.77| 0.002   |
| Texture Properties                  |                 |              |         |
| (a) Hardness (N)                    | 42.97 ± 0.025   | 41.80 ± 0.17 | 0.005   |
| (b) Cohesiveness                    | 0.64 ± 0.01     | 0.72 ± 0.01  | 0.007   |
| (c) Resilience                      | 0.25 ± 0.02     | 0.34 ± 0.01  | 0.006   |
| (d) Springiness (mm)                | 8.42 ± 0.38     | 9.36 ± 0.05  | 0.054   |
| (e) Chewiness                       | 42.90 ± 5.23    | 47.13 ± 3.88 | 0.408   |
| Pore properties                     |                 |              |         |
| (a) Count                           | 33.00 ± 3.58    | 36.00 ± 0.54 | 0.026   |
| (b) Average cell area               | 3.10 ± 0.16     | 3.89 ± 0.14  | 0.010   |
| (d) Porosity (%)                    | 26.40 ± 4.02    | 28.06 ± 0.32 | 0.50    |
| Color Crumb                         |                 |              |         |
| (a) L*                              | 64.20 ± 1.01    | 60.40 ± 1.04 | 0.011   |
| (b) a*                              | 0.26 ± 0.02     | 0.40 ± 0.26  | 0.48    |
| (c) b*                              | 26.77 ± 0.25    | 24.17 ± 0.12 | 0.03    |

Figure 2. The appearance of the batter before and after proofing, and the intersection of the resulting gluten-free bread with the addition TGase and without addition TGase.
3.2 The effect addition of TGase dan without TGase on parameter process

Figure 3 Core temperature changes of gluten-free bread during the baking process using ohmic heating that measured by three thermocouples (a) without TGase (b) with TGase.

Figure 3 shows the differences in heat distribution on three thermocouples placed between the electrodes during the baking process using ohmic heating. The graphic of temperature-time gives the convenience of evaluating changes in temperature and starch gelatinization temperature during the baking process. Overall, the addition of the transglutaminase enzyme can greatly affect the baking temperature during the process. This is indicated by the uniformity of temperature rise and a longer duration of the high temperature compared to the bread without the addition of the TGase enzyme. A high temperature during the baking process will cause the gelatinization reaction to occur completely so that the bread produced will perfectly be cooked. It is estimated that TGase is able to change the rheological properties of batter, which the porosity properties of the bread can increase the rate of electrical conductivity which has an impact on the heating temperature [12]. The temperature that occurs is not constant and there is a decrease. This is due to a decrease in the concentration of the material so that it affects the rate of electrical conductivity. Several parameters that affect the heating rate in the ohmic heating method are the current parameter, size of the material, and the properties of materials. TGase can modify the proteins by combining amines and altering the intermolecular crosslinking or deamidation, which causes the basic changes in their molecular structure [21], [22].

Figure 4 Current intensity changes during the baking process using ohmic heating without TGase and with TGase.

Figure 5 Electrical power changes during the baking process using ohmic heating without TGase and with TGase.
Electrical conductivity has a great role in the baking process using ohmic heating, which is linearly correlated with starch gelatinization temperature. The increase in electrical conductivity in the baking process of gluten-free bread batter increases with the increment in the temperature (Figure 7). Electrical conductivity increase with temperature, as is expected and consisted with literature data ([12], [20], [23], [24]. The value of electrical conductivity in the addition of TGase (ranges from 0.00267-0.0056 S/m) is smaller than without the addition of TGase (ranges from 0.000338-0.0058 S/m). This is happening due to the increase in concentration of liquid batter. The decrease in the resistance value of the material can occur due to the higher concentration of ions in the material so that the batter becomes a more conductive solution. TGase with its capability in catalyzing crosslinking reaction can alter the rheological properties of material or compounds in the batter. On the other hand, during the baking process using ohmic heating, the size of particles is decreased so that the resistance value of the material (batter) also decreasing and it will enhance material conductivity as long as the increase of temperature and voltage treatment given. The velocity of the current stream in the material is way more complicated due to material density so that it will increase the energy in the material that will affect the temperature enhancement. The resistance of these foodstuffs in passing the generated electric current is the cause of the heat in the food, so that energy is converted into heat energy. This result is in accordance with the research that conducted by Icier et al. [23] who report that the temperature enhancement is in line with the enhancement of electrical conductivity during the baking process using ohmic heating.

In the baking process, the power current requirement during the process is very important for energy, times, and cost-efficiency. The change in electrical conductivity is linear with the increase of current intensity and the electrical power used (Figure 4 and 5). The value of current intensity in the addition of TGase (ranges from 1.54-3.20A) is smaller than without the addition of TGase (ranges from 1.96-3.41). The value of electrical power in the addition of TGase (ranges from 139-287.10 J/s) is smaller than without the addition of TGase (ranges from 174-310.05 J/s). According to cier et al. [23] this is caused by energy consumption which is directly proportional to the incoming electric current and the voltage used. Where in the baking test, it is known that the value of electric current consumption continues to increase to heat baking batter gluten-free bread, so the power required will increase during the process.

4. Conclusions
Overall, the addition of TGase has a significant effect on the baking process of soy-based gluten-free bread using ohmic heating. It is shown that soy-based gluten-free bread produced with the addition of TGase has quality (in the parameter of specific volume, texture, and color) better than without the
addition of TGase. To understand the changes in the ohmic heating process parameters during baking, an evaluation of electrical conductivity, current intensity, power electrical, and temperature parameters was carried out. The rate of increase in temperature, electrical conductivity, current, and power intensity is highly affected by the addition TGase. TGase with its capability in catalyzing crosslinking reaction is able to alter the rheological properties of gluten-free bread batter. The electrical conductivity of soy gluten-free bread is highly temperature-dependent. The process parameters can be used as a reference in designing other baking ohmic-based instruments for other gluten-free bread baking.

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