Identification of the effect of transglutaminase enzyme on physicochemical properties of Milkfish (*Chanos chanos*) surimi gel

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Abstract. Surimi is a myofibril protein that is stabilized from the fish flesh, which is mechanically removed, washed with water and mixed with cryoprotectant. The yield of milkfish surimi is quite high at 58.72%, but the strength value of the gel is still not good, while the important quality attribute of a surimi product is its elastic texture in terms of the strength of the surimi gel. One technique to increase the formation of surimi gel is by adding the transglutaminase enzyme. The purpose of this study was to determine the effect of enzyme addition on the physicochemical properties of surimi. The research design consisted of two treatments, namely without enzymes and with the addition of enzymes 0.1 units/g surimi. The research method consisted of two stages, namely preparation of making surimi and surimi gel processing. Observation parameters included gel strength, whiteness, moisture content, and pH. The best research results obtained were the addition of enzymes with physicochemical properties of gel strength (3795 g.cm), whiteness (78.90), moisture content (76.70%), and pH (6.35). The treatment of adding enzymes significantly affected the strength of the gel and the moisture content but did not significantly influenced the whiteness and pH.

1. Introduction

Surimi is a myofibril protein that is stabilized from the flesh of fish, which is mechanically removed, washed with water and mixed with cryoprotectant. In general, surimi is processed through cutting, washing, mixing with cryoprotectant, and freezing [1]. In making surimi the washing process is a critical stage. Large amounts of water are used to remove sarcoplasmic proteins, blood, fat and other nitrogen components which can affect the quality of surimi [2]. Washing is known to increase the concentration of myofibril protein so that it can increase the strength of surimi gel [3]. In addition to the washing process, the addition of salt to surimi has also been shown to increase the strength of the gel in surimi [2].

One type of fish that can be used as raw material for making surimi is milkfish (*Chanos chanos*). According to the USDA National Nutrient Database For Standard Reference (2013), milkfish contain 20.53% protein and 6.73% fat. So they are classified as high-protein and medium-fat fish [4]. The special nutritional value of fresh milkfish that is the content of omega-3 is 19.56%; omega-6 at 7.47%; and omega-9 at 19.24% [5].

The yield of milkfish surimi, according to the study of Sarie *et al.* (2018) was 58.72% [6]. The highest yield was obtained for milkfish surimi among three other types of fish, namely tilapia, belida, and...
and jackfruit seeds. So, milkfish is one type of fish that has the potential to be used as a raw material of surimi. However, based on the research results of [7], it was found that the quality of surimi powder from milkfish is still not good, because the strength value of the resulting gel is still below SNI Surimi standard [7]. The strength value of milkfish surimi powdered gel ranges from 407.41 to 574.26 g/cm², while the SNI strength of surimi gel is at least 600 g/cm².

An important quality attribute of surimi products is their elastic texture in terms of the strength of surimi gel. To increase the strength of the resulting surimi gel can be added by ingredients that have the ability to form a gel so that it can improve the texture of surimi. The additional material that can be given is the transglutaminase enzyme [8]. The benefits of using transglutaminase enzyme include to reduce syneresis (water loss) in yogurt, improve rheological properties, encapsulation of fatty and soluble fat ingredients, and improve gel formation and gel properties [9].

The addition of the microbial transglutaminase enzyme (MTGase) is expected to produce better gel characteristics from surimi. MTGase has been widely used to improve the texture of protein-based products, including surimi [10]. The work mechanism of transglutaminase enzyme is that the transglutaminase catalyzes the reaction between the lysine and the glutamine amino acid residues and forms the ε-(γ-glutamyl) lysine isopeptide that results in the combining of inter or intramolecular Bond of amino acid residues with the food protein [11]. Essential amino acids make up 49.49% of the total amino acids of milkfish. Lysine is one type of essential amino acid that is dominant in milkfish as much as 7.3%, while glutamic acid is a dominant type of essential amino acid that is as much as 16.2% [12]. The content of lysine and glutamic acid in milkfish is quite high, so that it allows good enzymes to work in the process of forming surimi gel. However, it is not yet known the effect of adding enzymes to the milkfish surimi gel produced. So in this study, researchers tried to compare physicochemical characteristics of surimi gel between treatments without the addition of enzymes and with the addition of enzymes.

2. Method

2.1. Material

Milkfish, as the main ingredient in this study, was purchased fresh from the Makassar central fish market (Makassar, Indonesia). Other ingredients used include sucrose, the MTGase was supplied by Shaanxi Fuheng Biotechnology Co. Ltd (Shaanxi, China), NaCl, water, and ice. The tools used consist of production tools and tools for analysis. Production equipment includes a digital scale, analytical scale, stainless container, knife, blender, mixer, freezer, and refrigerator. While the tools for analysis include a texture analyzer, chromameter, oven, desiccator, and pH meter.

2.2. Surimi Processing

The first stage of the research procedure was the removal of bone from the fish flesh. After that, the fish flesh was milled and then washed using cold water at 10°C for three times. A comparison of washing water and fish meat was 3:1 (v/b). After that, filtering and pressing were conducted and then mixing with 4% sucrose. The next step was packaging and storage at -18°C to get frozen surimi.

2.3. Surimi Gel Preparation

The procedure for making surimi gel was the thawing of frozen surimi to a temperature of 5°C, then adding 2.5% NaCl. Then it was homogenized with ice until the surimi water level reached 80%, and then surimi paste was obtained. Next, it was divided into two treatments, namely without enzymes and with the addition of enzymes. The addition of the MTGase enzyme (0.1 units/g surimi) using a mixer at a temperature of 5°C for 5 minutes, then tightly packed in a jar and given two stages of heat treatment using a water bath, the first stage was an enzymatic process at 45°C for 30 minutes and the second step was the enzyme inactivation process at 90°C for 15 minutes. After that, cooled down at room temperature and stored for 24 hours at 4°C.
2.4. **Analysis of Gel Strength**

The strength of surimi gel was analyzed with a texture analyzer (Texture Type TA-XT2i Stable Micro Systems, Surrey, United Kingdom). Samples with a length of 2.5 cm are placed under the Y diameter of the probe with a measurement speed of 10 mm/sec. Then suppress the sample with the cylinder probe. The strength value of the gel was obtained by multiplying the compressive power (g) of the tool by the distance (cm) produced until the kamaboko piece is broken. Gel strength was calculated using the following formula [13]:

\[ \text{Gel strength (g.cm)} = \text{breaking force (g) x distance to rupture (cm)} \]

2.5. **Analysis of Whiteness**

The color analysis was carried out with Chromameter (type CR 200, Minolta Corp, Osaka, Japan). Analysis of the sample was carried out with three replications. The instrument was calibrated with a white marking card (CR-A43) until the monitor showed the values of L*, a* and b* according to the values indicated on the standard white color. Next, the sample is placed in a tube with a lens overlaid and the reflectance value (L*, a* and b*) are read on the measuring device [14].

\[ \text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^*^2 + b^*^2} \] (1)

2.6. **Oven Moisture Analysis Method**

A sample of 3 grams was inserted into a dried cup, and its weight was known. The sample was then dried in an oven at 105°C for 6 hours, then cooled in a desiccator 15 minutes and weighed. The drying and weighing process was carried out until it reached a constant weight. Moisture content was calculated according to the formula [15]:

\[ \text{Moisture content (\%)} = \frac{\text{initial weight (g)} - \text{final weight (g)}}{\text{initial weight (g)}} \times 100\% \] (2)

2.7. **pH measurement**

Sample preparation for pH determination (extraction method): weigh exactly 1 gram of sample, add 20 mL of water, then shake with a stirrer until it was completely homogeneous, left the sample for about 15 minutes, and measure the pH.

The steps to determine the pH of the sample were as follows (pH-meter has been calibrated): measure the sample temperature, set the pH meter at the measured temperature, turn on the pH-meter, leave it stable (15 - 30 minutes), rinse the electrode with distilled water, dry it electrodes with tissue paper, dip the electrode in the sample solution, set the pH measurement, let the electrode dip in for a while until a stable reading was obtained, note the pH of the sample.

2.8. **Statistical analysis**

All the analyses were done in triplicate. The data were subjected to ANOVA by statistical software, SPSS (Statistical Product and Service Solution) version 26. In all cases, the criterion for statistical significance was set at P <0.05.

3. **Result and Discussion**

3.1. **Effect of MTGase on gel strength of surimi gels**

The strength gel test was intended to determine the effect of adding the MTGase enzyme to the value of the strength gel on the surimi gel produced. The gel strength of surimi gels from milkfish without and with addition of MTGase (0.1 units/g surimi) is shown in Table 1.
Based on the results of the study in Table 1, it can be seen that the addition of the enzyme significantly affected the strength value of the resulting surimi gel. The value of gel strength in treatments without enzymes was 3279.81 g.cm and treatment with the addition of enzymes was 3795.36 g.cm. The results of both curves can be seen that the gel strength value has increased after the addition of the MTGase enzyme. The increase in gel strength is caused by the transglutaminase catalyzes the reaction between the lysine and the glutamine amino acid residues and forms the ε- (γ-glutamyl) lysine isopeptide that results in the combining of inter or intramolecular Bond of amino acid residues with the food protein [11]. This was consistent with the statement of Chanarat and Benjakul[16], that the formation of non-disulfide covalent bonds in proteins, specifically the ε- (γ-glutamyl) lysine crosslinking, was catalyzed by MTGase through the transfer of acyl between the γ-amide groups from the glutamine residue and the lysine ε-amino group from lysine residues, contributed to the improvement of gel quality in surimi.

### 3.2. Effect of MTGase on the whiteness of surimi gels

The value of whiteness in surimi in two treatments, without and with the addition of enzymes, showed the non-significant effect (Table 1). The value for the treatment without enzymes was 79.52, while those with the addition of enzymes was 78.90. The treatment without enzymes has a higher whiteness value than the treatment with the addition of enzymes, but the values obtained from the two treatments in the study did not have a significant effect. This was similar to the study that reported that there was no difference in the whiteness of surimi gels added with increasing levels of MTGase at all fish gelatin levels used [17].

### 3.3. Effect of MTGase on the moisture content of surimi gels

Moisture content analysis was intended to determine the moisture content contained in the surimi gel for both treatments. Based on statistical tests, it was found that treatment without and with the addition of enzymes in surimi significantly affected of milkfish surimi gel moisture content. The moisture content in treatment without enzymes was lower than the treatment with the addition of enzymes (Table 1). The moisture content of the treatment without enzymes was 75.84%, while the treatment with the addition of enzymes was 76.70%. Surimi moisture content with the addition of enzymes was higher because the addition of MTGase could increase the ability of gel in water holding. The addition of MTGase could enhance the crosslinking of proteins to some degree, resulting in the formation of a stronger network with greater water holding capacity [16]. The moisture content of the two treatments produced complied with SNI. This was in accordance with SNI 2694: 2013, those surimi moisture content requirements were a maximum of 80%.

### 3.4. The pH values of the surimi gel samples

Table 1 showed the pH value of surimi gels without and with the addition of MTGase. Surimi gel pH value in two treatments obtained statistical test results that have no significant effect. A pH value of the treatment without enzyme was 6.32, while for the treatment with the addition of enzymes was 6.35 was obtained. Both treatments in the study did not have a significant effect on the pH value obtained because the addition of the transglutaminase enzyme in the manufacture of surimi gel did not include

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**Table 1.** Characteristic of milkfish with different processing

| Treatments     | Gel strength (g.cm) | Whiteness | Moisture content (%) | pH    |
|----------------|---------------------|-----------|----------------------|-------|
| Without MTGase | 3279.81<sup>b</sup> | 79.52<sup>a</sup> | 75.84<sup>a</sup> | 6.32<sup>a</sup> |
| With MTGase    | 3795.36<sup>a</sup> | 78.90<sup>a</sup> | 76.70<sup>b</sup> | 6.35<sup>a</sup> |

Description: Values with different letters in the same column show significantly different (p<0.05)

With MTGase; 0.1 units/g surimi
the factors that influenced changes in pH values, both decreasing and increasing the pH value as stated [18], that MTGase does not alter the pH, color or flavor of food.

4. Conclusion
The addition of the transglutaminase enzyme was able to improve the physical quality of milkfish surimi by increasing the strength of the resulting gel. However, it did not have a significant effect on the whiteness and pH value.

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