Injuruty: Interdiciplinary Journal and Humanity
Volume 2, Number 4, April 2023
e-ISSN: 2963-4113 and p-ISSN: 2963-3397

EFFECT OF ADDITIONAL TRANSGLUTAMINASE ENZYMES ON PHYSICAL AND CHEMICAL PROPERTIES OF KAMABOKO LONG JAWED MACKEREL FISH (Rastralliger kanagurta)

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Abstract
Long jawed mackerel is an economically important type of fish that is widely caught and consumed in Indonesia. The diversity of processed mackerel products in Indonesia is still relatively low. One of the innovations in fish-based food processing that can be done is the use of mackerel to make kamaboko. One of the enzymes that can improve the functional properties of mackerel meat protein is the transglutaminase enzyme. This enzyme has the potential to be applied to mackerel due to the content of the amino acid lysine found in myosin and glutamine in the transglutaminase enzyme so as to produce peptide bonds that form the gel kamaboko (ashi). The purpose of this study was to determine the effect of the transglutaminase enzyme on physical and chemical properties and to determine the ideal concentration of the use of the transglutaminase enzyme in the manufacture of mackerel kamaboko in compliance with SNI Kamaboko. The method used in this research is an experimental method. Researchers used a completely randomized design (CRD) with four treatments and five replications. The treatment used was the addition of transglutaminase enzyme concentrations E0 (0%), E1 (0.1%), E2 (0.5%), E3 (1%). Kamaboko mackerel is then tested for physical properties which include appearance, folding test, bite test, and gel strength, while testing for chemical properties includes moisture, fat, and protein content. The results showed that the addition of transglutaminase enzymes to mackerel kamaboko had an effect on the texture of kamaboko. Kamaboko mackerel treated E2 and E3 is kamaboko in accordance with SNI kamaboko.

Keywords: enzymes on physica; chemical properties; kamaboko long jawed

INTRODUCTION

The potential for mackerel processing in Indonesia is very large. According to the Ministry of Maritime Muawanah et al., (2018) the number of mackerel catches in Indonesia reached 214,387-291,863 tons. Mackerel or known as mackarel fish is an economically important fish and its catch potential increases every year. This fish has a pretty good and savory taste so much loved by the public. According to the DIY Provincial Food Security Agency (2017), the nutritional composition of mackerel is quite high, namely every 100 grams of mackerel meat contains 76% water, 22 g protein, 1 g fat, 20 mg calcium, 200 mg phosphorus, 1 g iron, vitamin A 30 SI, and vitamin B1 0.05 mg. According to Le Fol et al.,(2017) mackerel has a protein content of 19.14% while fat is 8.19%. Eating mackerel is beneficial for health because it contains high protein and contains higher omega-3 fatty acids than other sources. These omega-3 fatty acids are useful in reducing the risk of heart disease.

In general, mackerel is processed only by frying, it is still rare to find other processed products made from mackerel. The diversity of processed mackerel products in Indonesia is also still relatively low, so efforts are needed to increase the diversification of processed mackerel products that can be accepted by the public (Wati, 2018). One of the fish-based processed food innovations that can be done is the use of mackerel into kamaboko.

Fish before being processed into kamaboko, the meat will be processed first into semi-finished ingredients commonly called surimi. Surimi is an elastic myofibril concentrate made from ground fish meat as the main ingredient or crushed fish meat that has been thoroughly washed, purified unwanted substances and stabilized with stabilizer compounds. Surimi is also defined as fish meat that has been ground and separated from bones, skin, and intestines and washed to remove fat and water-soluble compounds (As et al., 2015). Surimi has the characteristics of being able to bind water and fat, and other functional properties. One form of processed surimi or products that utilize the functional properties of fish protein is kamaboko (Radityo & Darmanto, 2014).

Enzymes are polymeric molecules composed of a series of amino acids in a composition and arrangement of chains arranged regularly and fixedly. Enzymes play an important role in various proteins, enzymes are produced and used by living cells to catalyze reactions including energy conversion and cell defense metabolism (Richana & Lestina, 2002). Practically, enzymes are widely used in various fields of activity. Enzymes are widely used in industry, especially the biotechnology industry. Judging from the sources and benefits, enzymes are used in the food industry because enzymes are an ideal tool used to manipulate biological materials. Some of the advantages of using enzymes in food processing are that they are safe for health because they are natural ingredients, catalyze very specific reactions without side effects, are active at low concentrations, and can be used as indicators of the suitability of the processing process. One of the enzymes that is often used in food processing in the field of fisheries, especially surimi products, is the transglutaminase enzyme.

The transglutaminase enzyme catalyzes a crosslinking reaction between glutamine amino acid residues and lysine amino acid residues. In this case, the transglutaminase enzyme has the potential to be applied to mackerel because of the high content of amino acids lysine (1,886 g / 100g) and glutamine (3,065 g / 100g) Yongsawatdigul et al., (2002). Transglutaminase (TGase) is able to form bonds between the amino acids lysine and glutamine to form protein polymers that give meat texture and produce ammonia in the carboxyamide group of glutamine groups in protein molecules. This mechanism will make fish protein have high elastic properties and are able to trap water quite a lot, so that the elasticity of surimi gel can increase (Laksono et al., 2019). According to Kikuchi et al., (2014) transglutaminase enzymes can work on specific substrates, three of which are gluten in flour, myosin, and casein in surimi so that the addition of transglutaminase enzyme in mackerel-based kamaboko in this study is thought to improve and improve the quality and elasticity of kamaboko gel.

The purpose of this study was to determine the effect of transglutaminase enzyme on physical and chemical properties and determine the concentration of the ideal use of transglutaminase enzyme in making mackerel kamaboko in meeting Kamaboko SNI Kamaboko. The benefit of this research is that it is expected to increase insight or knowledge about the effect of transglutaminase enzymes on the characteristics of mackerel-based kamaboko.

METHOD RESEARCH

This study used experimental research methods. It consists of research design, work procedures, research parameters, and data analysis. The experimental design used in this study was a one-factor Complete Randomized Design (RAL), namely the administration of different concentrations of transglutaminase enzymes in surimi which will be processed into kamaboko. The treatment given is 4 treatments that will be repeated 5 times. Based on the research of (Ardiansyah & Ratnawili, 2021)with modifications determined the treatment plan given to this study includes:

E0 : Addition of 0% transglutaminase enzyme concentration
E1 : Increased concentration of transglutaminase enzyme 0.1%
E2 : Addition of transglutaminase enzyme concentration 0.5%
E3 : Increased concentration of transglutaminase enzyme 1%
This research work procedure was carried out in several stages starting with the preparation of mackerel meat, making surimi, adding the concentration of transglutaminase enzymes, the process of making kamaboko, and testing the physical and chemical properties of kamaboko

RESULT AND DISCUSSION

The results of mackerel kamaboko research with the addition of transglutaminase enzymes include sensory testing, chemical properties, and physical properties of mackerel kamaboko. Kamaboko sensory testing consists of an appearance test, a folding test, and a bite test. The chemical properties of kamaboko consist of testing the water content, fat content, and protein content of fish meat, surimi, to kamaboko. Testing of the physical properties of kamaboko includes a mackerel kamaboko gel strength test. Sensory testing which includes folding tests and bite tests is also included in testing the physical properties of mackerel kamaboko.

Mackerel Kamaboko Appearance Test Results

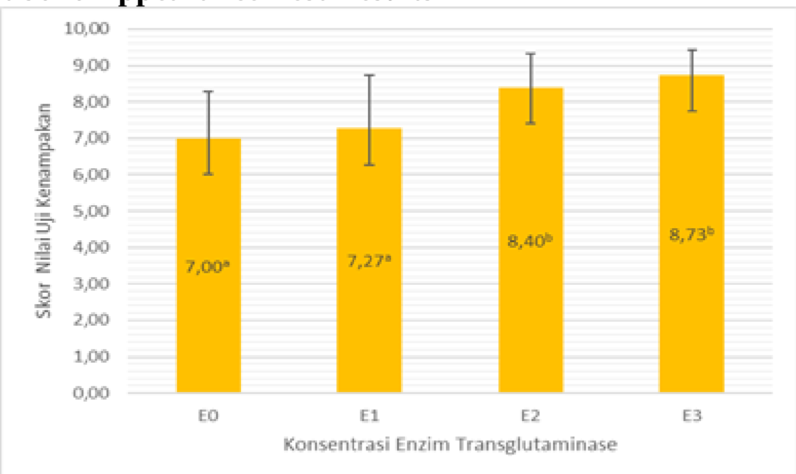

Figure 1 Mackerel Kamaboko Appearance Test Chart with Addition of Transglutaminase Enzyme

Based on these results, it is explained that kamaboko E0 and E1 have the appearance of fleshy kamaboko, little fiber, and no foreign objects. The highest score was found at E3 with an average score of 8.73. The result is not much different from the E2 kamaboko with a score of 8.4. The score shows that kamaboko E2 and E3 have the appearance of kamaboko fleshy, without fiber, and without foreign bodies.

The results of sensory testing of mackerel kamaboko on appearance specifications were carried out further tests with Mann Whitney ($p < 0.05$) found that kamaboko E1 addition of 0.1% transglutaminse enzyme had a significant effect on kamaboko E2 addition of 0.5% transglutaminase enzyme.

Mackerel Kamaboko Water Content Test Results

Water content analysis is carried out to determine the water content contained in kamaboko mackerel. Water content is an important factor that can affect the quality of the gel from kamaboko. In connection with this, it is necessary to know the water content contained in mackerel kamaboko. Details of moisture content test results can be seen in Figure 2

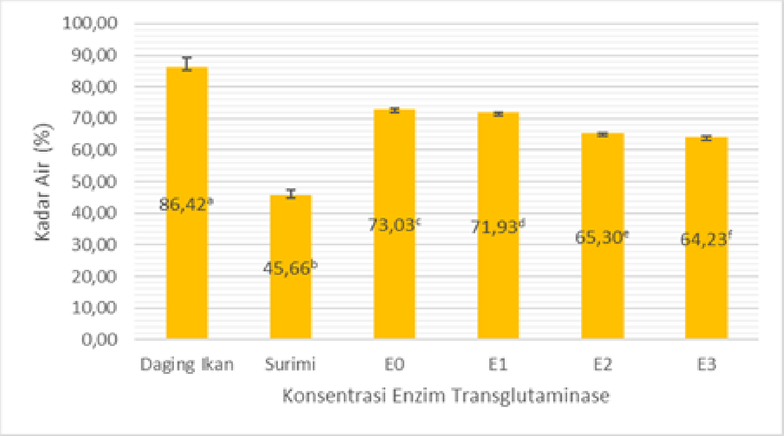

Figure 2 Graph of Water Content of Fish Meat, Surimi, and Mackerel Kamaboko with the Addition of Transglutaminase Enzyme

The results of testing the water content of mackerel meat were 86.42% and after being processed into surimi by going through a washing process 3 times the water content of surimi, it became 45.66%. Surimi is then processed into kamaboko with the addition of transglutaminase enzyme to increase water content. Kamaboko E0 has a moisture content of 73.03%. Kamaboko E1 with the addition of 0.1% transglutaminase enzyme decreased to 71.93%. Kamaboko E2 and E3 also continued to decrease in water content to 65.30% and 64.23% respectively. Based on the results of further DMRT (Duncan Multiple Range Test) ($p < 0.05$) that the water content of fish meat has a significant influence on the water content of surimi. These results were also obtained in each treatment of mackerel kamaboko E0, E1, E2, and E2 that the concentration of transglutaminase enzymes had a noticeable effect.

Mackerel Kamaboko Fat Content Test Results

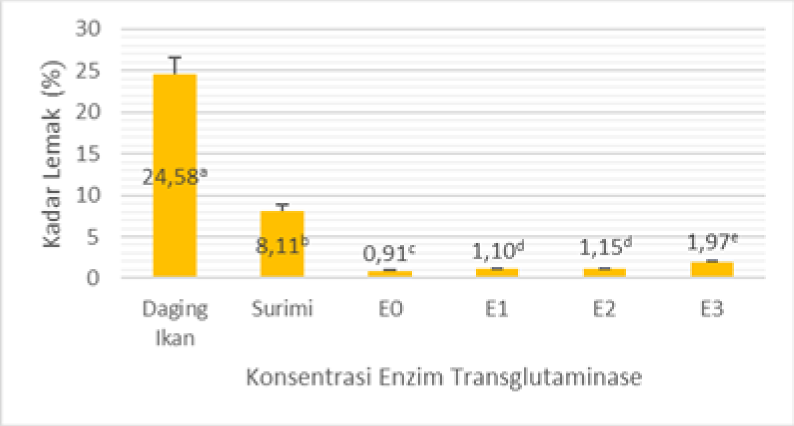

Figure 3 Graph of Fat Content of Fish Meat, Surimi, and Mackerel Kamaboko with the Addition of Transglutaminase Enzyme

Based on the graph of fat content test results, mackerel meat has a high fat content of 24.58%. Mackerel meat is then processed into surimi and obtained a fat content of 8.11%. Mackerel kamaboko with E0 treatment has a fat content of 0.91%. This result was the lowest fat content while the E1 treatment experienced an increase in fat content by 1.10% and continued to increase in E2 and E3 kamaboko had fat content results of 1.15% and 1.97% respectively. Kamaboko with the highest fat content results are found in kamaboko E3 by 1.97%.

Based on the results of further DMRT (Duncan Multiple Range Test) ($p < 0.05$) that the fat content of fish meat has a significant influence on the fat content of surimi. The results were also obtained in each treatment of mackerel kamaboko E0 against E1 that the concentration of transglutaminase enzyme had a noticeable effect. E1 treatment of E2 has no noticeable effect on fat test results. The results of E2 fat content have a significant influence on mackerel campaboko E3

Mackerel Kamaboko Protein Test Results

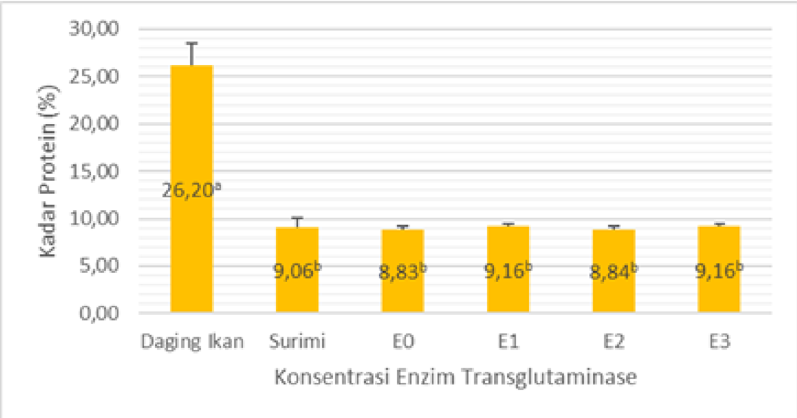

Figure 4 Graph of mackerel kamaboko protein levels with addition of transglutaminase enzyme

The protein content of kamaboko E0 and E2 had almost the same results of 8.83% each, and 8.84% there were no significant protein content results in kamaboko E1 and E3 both kamaboko had protein levels of 9.16%. Based on the results of further DMRT (Duncan Multiple Range Test) ($p < 0.05$) that fish meat protein levels have a significant influence on surimi protein levels. Protein levels in kamaboko E0, E1, E2, and E3 had no significant effect on each treatment

Mackerel Kamaboko Folding Test Results

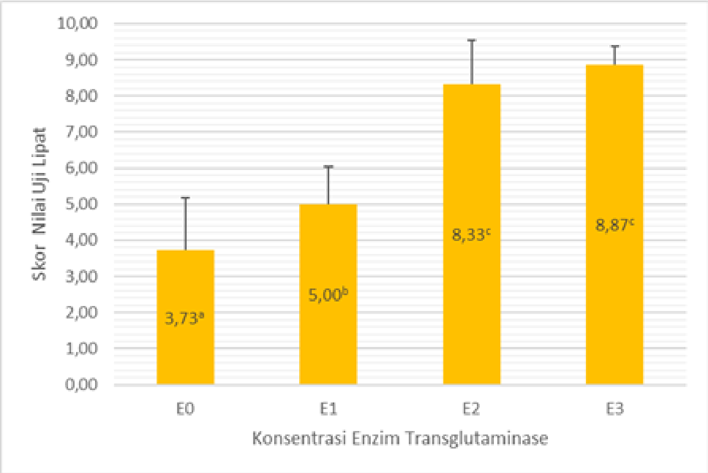

Figure 5 Mackerel Kamaboko folding test graph with addition of transglutaminase enzyme

Based on sensory testing on the folding test specifications, the best results were obtained at kamaboko E3 with a score of 8.87. Kamaboko with the lowest folding test sensory score was obtained at kamaboko E0 with an average value of 3.73. The addition of the enzyme transglutaminase 0.5% increased the folding test score from 5.00 to 8.83. It was explained that a score of 5.00 on the folding test showed that after folding kamaboko into a semicircle there were slight cracks in mackerel kamaboko, while with the addition of 0.5% transglutaminase enzyme and 1% kamaboko folding test scores increased to 8.33 and 8.87 respectively. This shows that kamaboko does not crack after folded folded 4. The results of testing the physical properties of mackerel kamaboko on the sensory test of folding test specifications carried out further tests with Mann Whitney ($p < 0.05$) found that E0 treatment had a real effect on E1. Kamaboko E1 also obtained results that have a real effect on Kamaboko E2. These results are different from the results obtained by kamaboko E2 which has no real effect on kamaboko E3.

Mackerel Kamaboko Bite Test Results

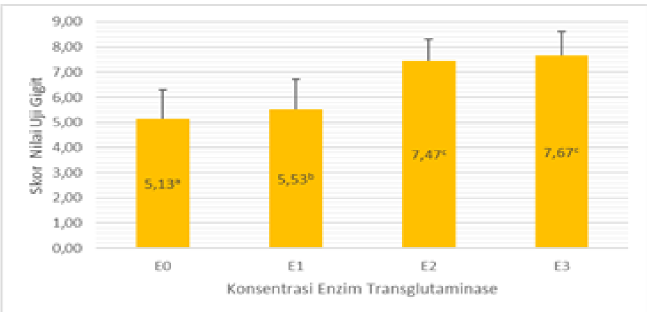

Figure 6 Mackerel Kamaboko bite test graph with addition of transglutaminase enzyme

The results of sensory testing of the bite test showed that the treatment of the concentration of transglutaminase enzyme had an influence on the bite test. This is stated in the graph of the bite test scoring results on kamaboko E0 of 5.13, it shows that mackerel kamaboko without the addition of transglutaminase enzyme has a slightly mushy texture. Kamaboko E1 showed a slight increase in scoring results with a score of 5.53, this showed that the addition of transglutaminase enzyme as much as 0.4% gave a significant increase in the bite test. This is directly proportional to the results shown in kamaboko E2 and E3 which show significant results respectively, 7.47 and 7.67, this shows that kamaboko E2 and E3 have very strong suppleness product specifications.

The results of sensory testing of mackerel kamaboko on the bite test specifications carried out further tests with Mann Whitney ($p < 0.05$) found that E0 treatment had a significant effect on E1. The results were also found in E1 and E2 that the treatment of E1 and E2 had a noticeable effect. The results of further tests on kamaboko found that E2 and E3 had an intangible effect.

Mackerel Kamaboko Gel Strength Test Results

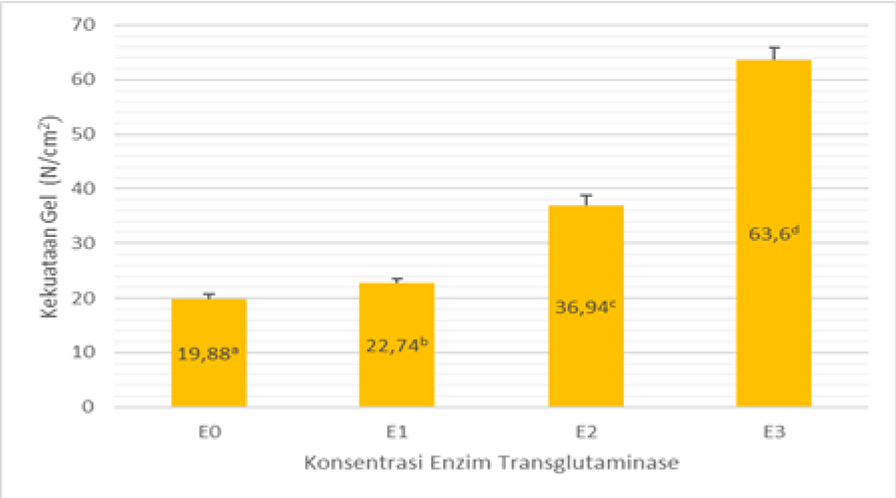

Figure 7 Mackerel Kamaboko gel strength test graph with addition of transglutaminase enzyme

Based on the results of further DMRT (Duncan Multiple Range Test) tests that the strength of the gel in mackerel kamaboko E0, E1, E2, and E3 had a significant effect ($p < 0.05$) on mackerel kamaboko in each treatment with the addition of transglutaminase enzyme.

Based on statistical tests on sensory testing, it can be seen that the treatment of transglutaminase enzyme concentration has a significant influence on the appearance of mackerel kamaboko. The increase in appearance scores in mackerel kamaboko was in line with the increased use of transglutaminase enzyme concentrations. The increased appearance of kamaboko can be caused by the heating process. The heating process is carried out after the surimi doughing process mixed with sugar, salt, and transglutaminase enzymes using a food processor to form surimi sol then a heating process is carried out to become kamaboko.

According to Fadhilatunnur et al., (2022) the heating process allows surimi sol to become denser so that kamaboko products are not fibrous. due to damaged or denatured pigments. In addition, it is also affected by incomplete oxidation of myoglobin in meat.

Myoglobin is composed of protein (globin) and nonprotein (heme molecule parts) molecules that play an important role in structural changes in meat..

The results of testing the water content of mackerel meat indicate that mackerel has a fairly high water content. Mackerel meat is then processed into surimi experiencing a decrease in water content. This is due to the decreased water content in mackerel due to the repeated washing process. Washing is a critical stage in the process of making surimi, a large amount of water is used to remove sarcoplasmic proteins, blood, fat, and binding water content that can affect the quality of surimi (Wawasto et al., 2018).

Based on the water content graph in figure 6, it can be seen that the higher the concentration of transglutaminase enzyme, the lower the water content in mackerel kamaboko. Transglutaminase enzyme treatment in each treatment of E0, E1, E2, and E3 had a significant effect on changes in the fat content of mackerel kamaboko products ($p > 0.05$). The decrease in water content in kamaboko is in accordance with the research of Kaewudom et al., (2012) that the addition of transglutaminase enzyme induces bonds between matrix gels, so that the gel will release water.

A certain amount of water in the muscle of fish meat contained in myofibrils is in the space between thick filaments of myosin and thin filaments of actin (tropomyosin) (Lovell et al., 2010).The interaction between these water-binding myofibril proteins plays an important role in gel formation. This is in accordance with the research of Kaewudom et al., (2012) which states that the addition of 0.3% transglutaminase enzyme in fish meat gel significantly affects the water content in beef homogenate.

Based on the results of fat content testing shows that there is a decrease in the fat content of fish meat processed into surimi. The decrease in the fat content of mackerel meat occurs due to the washing process repeatedly so that the fat dissolves with water. These results are in line with those stated by Tanuja et al. (2014), Washing is a critical stage in the process of making surimi, a large amount of water is used to remove sarcoplasmic proteins, blood, fats and other nitrogen components that can affect the quality of surimi.

Based on the results of the significance test ($p > 0.05$), it can be seen that the treatment of adding transglutaminase enzyme has a significant effect on changes in mamaboko fat levels. The results of testing the fat content of mackerel kamaboko along with the increase in the concentration of transglutaminase enzyme, the increasing fat content in mackerel kamaboko. Changes in fat content in kamaboko can be caused by heating or cooking factors so that it can evaporate large amounts of product fat. Based on Figure 7, it can be seen that the kamaboko content in the E0 treatment results in lower product fat content compared to the E1, E2, and E3 treatments.

According to (Wibowo et al., 2020) suggested that crosslinking between proteins formed by transglutaminase enzymes is a covalent crosslink (NH). This crosslinking can inhibit the release of fatty acids resulting from fat liquefaction and adipose tissue fragments. Such crosslinking can be broken by physical treatment (heating). The N-H covalent bond is polar, meaning it is opposite to a nonpolar substance (fat/oil).

The decrease in protein levels in mackerel meat that is processed into surimi is caused by the surimi washing process which dissolves soluble protein in water, resulting in a decrease in protein levels in surimi. Surimi reprocessed into kamaboko and added transglutaminase enzyme did not provide a noticeable increase in protein levels. Based on the results of the mackerel and surimi meat protein content test showed a significant decrease in protein levels caused by the mackerel meat washing process in the surimi making process. According to Ismail et al., (2010), the washing process in making surimi also aims to remove water-soluble proteins, namely sarcoplasmic proteins. Sarcoplasmic protein is a protein that can inhibit gel formation and can reduce product quality. Sarcoplasmic proteins are present in the liquid in meat fibers and are associated with many enzyme metabolites. These proteins can reduce the quality of enzymes during the surimi storage process.

Based on the results of the protein content test, transglutaminase enzyme treatment had no real effect ($p > 0.05$) on changes in mackerel kamaboko protein levels. Compared to the E0 treatment, it was seen that the addition of transglutaminase enzyme concentration in each treatment could significantly increase the protein content in mackerel kamaboko, but the treatment did not show a significant difference when compared to the protein content of mackerel raw materials ($p > 0.05$).

The increase in protein levels in the E2 to E3 treatment occurs because the transglutaminase enzyme is able to catalyze the transfer reaction of acyl acyl as-carboxamide groups from glutamine residues to proteins or peptides and other primary amines (lysine) to produce polymers and intramolecular cross-reactions of isopeptide forms, such as ε-(γ-glutamil) lysine. The more concentration of transglutaminase enzymes, the more crosslinked covalent isopeptides are formed, so that the amino acids in the proteins that form gelation in kamaboko bind and form gelation.

Based on statistical tests, it can be seen that the treatment of transglutaminase enzyme concentration has a significant effect ($p < 0.05$) on the folding test. This is in accordance with the results of research by Ali et al. (2012), namely the addition of enzymes with concentrations of 0.5% and 1% when compared to enzyme concentrations of 0% (without the addition of enzymes) has a significantly different effect on the strength of kamaboko gel.

An increase in the score of folding test results in each treatment showed that the quality and chewiness of the gel from kamaboko increased with the addition of the enzyme transglutaminase. The transglutaminase enzyme can increase the chewiness of kamaboko gel by increasing and modifying the functional properties of milkfish protein, namely gelation (gelling) properties. The textural properties of kamaboko in this case, the chewiness of the gel can be enhanced by the addition of the enzyme transglutaminase (r-glutaminylpeptide: amine γ-glutamyltransferase; EC 2.3.2.13) which catalyzes muscle cross-linking reactions of fish proteins, especially myosin (Yongsawatdigul et al., 2002). The γ-carboxyamide group of the glutamine residue peptide bond acts as an acyl donor, while the primary amino acids, including the amino acid lysine, act as acceptors. This is in accordance with research data from Kaewudom et al., (2012) which states that by adding transglutaminase enzyme at the level of 1.2 units / g transglutaminase enzyme in the process of making surimi gel, all texture parameters of surimi increase.

Based on the results of the bite test and continued further tests, DMRT showed that the addition of transglutaminase enzyme gave significant results ($p < 0.05$) against the bite test. Sitompul et al., (2018), stated that the washing process affects the high and low bite test value produced by kamaboko. The washing process can improve gel characteristics because trpomiosin, troponin and light chain myosin in washes one and two will disappear, mixed with protein interactions that promote gel formation (Ismail et al., 2010). The addition of transglutaminase enzyme also plays a role in giving mackerel kamaboko products suppleness. This is in line with the theory put forward by Uresti et al., (2004) increased suppleness can occur because the use of transglutaminase enzymes induces covalent crosslinking between protein polymers and acts as a catalyst for acyl transfer between γ-carboxyaminda groups of glutamine residues in proteins. The formation of ε-(γ-glutamyl) crosslinking causes the polymer to be denser resulting in a thicker and chewier texture.

Mackerel kamaboko gel strength testing uses punch method to measure gel strength from kamaboko. Measurement using the Tensile Strength tool produces N/cm2 units. The quality requirements for frozen surimi based on SNI 10-2694-1992 standards are the strength of surimi gel at least 300 g / cm2, so it is converted to 300 g / cm2 = 2.941995 N / cm2 (1 g / cm2 = 0.0098 N / cm2). The strength of mackerel kamaboko gel is 19.83-63.6 N/cm2 or

2022.09 – 6485.2 g/cm2 (1 N/cm2 = 101.97162 g/cm2) higher than the minimum gel strength requirement in frozen surimi.

Based on the results of the significance test ($p<0.05$), treatment with the addition of transglutaminase enzyme in mackerel kamaboko has a significant effect on changes in the strength of mackerel kamaboko gel each treatment. The results of the tensile strength test show that the tested treatment shows a noticeable difference between one another. The addition of transglutaminase enzyme has a significant effect on increasing the strength value of the product gel, because the E3 treatment shows the largest value of 63.6 (N/cm2). Transglutaminase enzymes form crosslinks between protein molecules, including myofibril proteins and protect damage to gelling proteins such as myosin from denaturation due to heating shortly after the setting process (Margareta, 2020). According to Nahariah & Hikmah, (2021), thermal energy will only result in breaking noncovalent bonds that exist in the natural structure of myofibril proteins and not breaking covalent bonds in peptide bond structures.

The presence of myofibril protein in surimi which is processed into kamaboko, the addition of transglutaminase enzymes will increase the stability of the gel by forming crosslinks of protein molecules and increasing the strength of the surimi gel. The transglutaminase enzyme catalyzes the formation of ε-(γ-glutamil) lysine crosslinks between actomyosin molecules, which results in improved gel texture. Yongsawatdigul et al., (2002), suggested that an increase in the strength of kamaboko gel is seen at the time of formation of suwari gel, namely after pre-incubation of surimi (sol formation) at a certain temperature range between 5-40 °C.

The textural properties of kamaboko in this case the strength of the gel can be enhanced by adding the enzyme transglutaminase which catalyzes the crosslinking reaction of muscle proteins, especially myosin (Yongsawatdigul et al., 2002). The γ-carboxyamide group of the glutamine residue peptide bond acts as an acyl donor, while the primary amino acids, including the amino acid lysine, act as acceptors. The lyyl isopeptide bond ε-(y-glutamil) is stronger than the hydrogen and hydrophobic bonds. Crosslinks in myosin proteins are formed resulting in high gel elasticity. The more transglutaminase enzymes are added, the more crosslinks between proteins are formed, and the myosin protein structure in myofibril proteins is more stable, so the gel strength is higher

CONCLUSION

The conclusions from the study of the effect of adding transglutaminase enzyme on the physical and chemical properties of mackerel kamaboko (Rastralliger kanagurta), Testing the chemical properties of mackerel kamaboko which includes water content, protein, and fat content shows that the higher the addition of transglutaminase enzymes in kamaboko produces better kamaboko quality.

Testing the physical properties of mackerel kamaboko which included sensory tests and gel strength on mackerel kamaboko found that mackerel kamaboko in accordance with SNI was obtained in E2 and E3 kamaboko.

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