Restructuring steak from flakes of yellowfin tuna meat using low salt microbial transglutaminase (MTGase)

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Abstract. Restructuring of fish meat can be done by using fragments of by-product processing such as fillet, loin, and steak of yellowfin tuna. The binder agent that may be the most efficiently used in restructuring the product is microbial transglutaminase (MTGase). Salt is needed to optimize the binding process of the restructuring product. The objective of the study was to determine the effect of low salt MTGase concentration as a binding agent on the quality of restructured yellowfin tuna steak compared to commercial yellowfin tuna steak. Research was designed with completely randomized design using 5 treatments in the 1.5% salt concentration, namely 0%, 0.3%, 0.5%, 0.7%, and 0.9%. The analyzed parameters were proximate (water content, protein, fat, carbohydrate, and ash), texture, colour and water holding capacity. The sensory test was conducted to compare restructured products with commercial tuna steak. The results indicated that the best treatment to the addition of MTGase was 0.5% based on the physical properties of the product, the nutritional content and sensory test. In sensory, MTGase was able to maintain the colour (brightness) of the products as they were darker than the commercial tuna steak and improve the product’s texture (hardness) in the addition of 0.9% MTGase.

Keywords: concentration, low-salt, MTGase, restructuring, yellowfin tuna

1. Introduction

Yellowfin tuna (Thunnus albacares) is a type of tuna that is widely caught in Indonesian seawater. These catches are generally processed in the form of frozen loin, fillets and steak and being marketed locally or globally. The high volume of production and exports, of course, followed by the increasing number of residues (sludge) meat produced. According to Widijantoro (2012), each tuna steak processing produced less than 12% residues of the total weight. So far, the use of wastes from the processing of tuna steaks and other types of pelagic fish has been limited as raw materials for processing shredded fish and pastel seasonings. Technological advancement in the processing of fishery products has a significant impact on the utilization of by-products and wastes from fisheries industry into a variety of processed products with competitive tastes and prices in the market, as well as being able to create alternative business fields (especially culinary), which are quite large and favoured by the community (Sahubawa 2010). In the early 1970s, restructuring technology appeared as a new concept to enhance meat utilization (Mandigo 1988). The definition of restructuring refers to
the use of manufacturing steps to create a consumer-ready product which resembles an intact steak, chop or roast. When the meat is restructured, the form of the meat is changed because consumers are concerned about intaking too much fat and salt, this creates an increasing market for lean restructured meat products. Restructured meat products include any meat products that are completely disassembled and then being reformed into the same or a different form. Therefore, this definition consists of all sectioned and formed meat products, as well as all sausages and a variety of other products. In restructured meat products, several serious problems, i.e., fat oxidation and colour instability, are encountered and considered as the main limitation of consumers’ acceptance (Mandigo 2008).

The processing of restructuring fish steaks with raw materials of meat flakes (residues) from the by-products of processing loin, filet and steak is one alternative technology to increase economic value through diversification of economically important and valuable products using binders. The restructuring process makes it possible to obtain high commercial value products from different fish, such as noncommercial fish, and small size fish obtained from by-catch, by-products, and by-products of industrial fish fillet processing (Ramírez et al 2006). According to Bello et al (2011), the most effective binding agent in the manufacture of restructured products is MTGase. MTGase is already widely used in the food processing industry to catalyze the covalent cross-linking of meat proteins. MTGase catalyzes the reaction of the transfer of acyl groups in the ok-carboxyamidina group of protein bonds to the amino acid residues of glutamine that functions as acyl donors and lysine primary amino acids as acceptors (Whitehurst and Oort 2010).

An alternative process involves obtaining fish paste by mechanical separation of the flesh, using a deboning machine, and then preparing restructured fish products. This process produces fish products with a high commercial value from different sources, such as non-commercial fish, fish which is smaller than commercial species (such as by-catch of shrimp fishing) and by-products of fillet processing of commercial fish (Ramírez et al 2007). Although several methods of restructuring have been developed, the most commonly used are cutting, tumbling and massaging (with or without vacuum). All these techniques use salt (20-30 g/kg) to solubilize and extract myofibrillar proteins which form a sticky exudate to bind the product (Tellez-Luis et al 2020). It was demonstrated, in other fish species, that it is not feasible to obtain restructured products in the absence of salt (Ramírez et al 2007). On the other hand, consumers are demanding healthier foods, and there is a potential interest in lightly-salted products to prevent and control adverse blood pressure levels in humans. Proteins have been used as binding agents or as additive to improve mechanical and functional properties of fish products, e.g. egg white (Ramírez et al 2000), casein and beef plasma-thrombin (Ramírez et al 2002, Rami rez et al 2003), casein and whey protein concentrate (WPC) and MTGase (Uresti et al 2004).

MTGase does not require NaCl to induce protein crosslinking, but myofibril protein requires salt (less than 20 mg/kg of raw material) to optimize the dissolution and exposition of residual groups (Ramírez et al 2006). The use of too much salt can cause the risk of hypertension for consumers. According to the USDA and HHS (2010), the salt consumption limit (NaCl) is 2.3 g/day or equivalent to 6 g of salt per day, so the use of salt should be reduced to optimize the restructuring process. Whitehurst and Oort (2010) state that the minimum amount of salt added to restructuring process is 1.5%. The objective of the study was to determine the effect of low salt MTGase concentration as a binding agent on the quality of restructured yellowfin tuna steak compared to commercial yellowfin tuna steak.
2. Materials and methods

2.1. Materials.
The main raw materials were meat flakes and yellowfin tuna steaks purchased from the loin processing industry and large pelagic fish steaks (tuna, marlin and skipjack) in KUB Fresh Fish Bantul Yogyakarta. Meat binder (Microbial Transglutaminase-Activa TG-TI, Ajinomoto USA, Inc., Teaneck, NJ, composition 1% pure enzyme + 99% maltodextrin with activity ±100 U/g) (Modernist Pantry, York Maine USA), table salt (Refina, PT. Uni Chemcandi Indonesia), warm water and commercial cooking oil (tropical Indonesia).

Types of equipment used in the restructuring process include: Windy oven (EYELA WFO-601 SD. Japan), 250 ml measuring cup, printing equipment (aluminum), polystyrene plastic, sealer (Impulse sealer, model pfs-200. China), thermometers, freezers, analytical scales (Denver Instrument Company AA-200, USA), digital scales (Shimadzu bx 6000. Japan), thermometers, thermocouples (Hanna Instrument hi 98701k-jt. USA), Colourimeter, LLOYD Instrument Texture Analyzer (Zwick instrument type: DO-FBO-STS. Germany), water bath (Sibata WS-240. Japan), gas stove (Covina Superflame CX-2000 EXT, Indonesia), desiccator and meat grinder (Multiuse Chopper, Kind Future kf-808p. China ).

2.2. Methods
The research was conducted in the Laboratory of Fish Technology, Laboratory of Nutrition and Natural Feed and Hydrobiology Laboratory, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University (UGM) and Laboratory of Food and Nutrition Chemistry, Faculty of Agricultural Technology Gadjah Mada University (UGM).

2.3. Preparation
Yellowfin tuna flakes were purchased from KUB Fresh Fish, Bantul Yogyakarta in a frozen. Before processed, the meat was thawed at room temperature for approximately 2 hours, then its chemical quality including colour, content of water, fat, protein, and ash was tested.

2.4. Product quality processing and testing
The flakes of frozen yellowfin tuna that has been thawed at room temperature, then mashed using a grinding machine. After the initial testing (chemical composition and colour), the meat was then weighed at 55 g for the observation of the quality of raw materials. Subsequently, 1.5% of salt binder and MTGase powder were mixed with the treatment of p1 (0% of MTGase concentration), p2 (0.3% of MTGase concentration), p3 (0.5% of MTGase concentration), p4 (0.7% of MTGase concentration) and p5 (0.9% of MTGase concentration) in 3 replications. The material mixture was then put into a plate (size 7 cmx6 cmx1.7 cm) which has been given commercial cooking oil and has been heated at 40ºC for 1 hour and 90ºC for 15 minutes using a water bath. After that, the product was cooled in the cold water at ±4ºC for 30 minutes before being wrapped in polystyrene plastic and stored at 4ºC for one night. The chemical test of the product incorporated water content (AOAC 1990), protein (AOAC 2005), ash (AOAC 1990), fat (AOAC 2005), WHC (Cardoso et al 2010 modified), texture (Instrument Llyod Texture Analyzer, type TA-TX1 plus), breaking force, gel strength, colour (Hunter Colourimeter/ Photoelectric Colourimeter with values L*, a*, b*) and the sensor.

2.5. Data collection
Testing of colour and chemical quality (water, fat, protein and ash content) of yellowfin tuna flakes was followed by product processing (restructuring the flakes of tuna steak) following Ram´ırez et al (2006). The next step was product quality testing including texture tests (hardness, breaking strength and gel strength), colour testing, chemical testing (water, fat, protein and ash content) as well as water-holding capacity test. Before being tested, the restructured product was wrapped using aluminium foil
and heated in an oven at 62.7°C or 63°C as suggested by USDA and HHS (2010), hence the product can be sliced easily. The next step was sensory testing which was carried out using a scoring method (Kartika et al 1988) based on the commercial yellowfin tuna steaks as the benchmark. This process involved 15 trained panellists, which were selected by the triangle method. The test parameters incorporated odour, colour, texture and taste.

2.6. Data analysis
The research data were statistically tested using a single factor as a treatment sources using randomized complete design (RCD) with three (3) replications and analyzed using analysis of variance (ANOVA), followed by a multiple comparison test (Duncan Multiple Range Test, DMRT) (Gomez and Gomez 1994) at a 95% of confidence levels. The sensory quality test of the product used the Tukey's Test (Kartika et al 1988) and SPSS version 18.

3. Results and discussion

3.1. Proximate analysis
3.1.1. Moisture content (%). Based on observations, the optimal cooking time for restructuring was ±110 minutes. According to the statistical test (ANOVA), it was seen that the MTGase treatment did not have a significant effect on the changes of moisture content of the restructured product (p>0.05). The results of the water content analysis showed that in the final product (restructured yellowfin tuna flakes), the water content was not significantly different within all treatments, however compared to raw materials (fresh tuna flakes), the water content showed differences with a significant level of 95% (table 1).

3.1.2. Protein content (%). Based on the results of statistical tests (ANOVA), the MTGase treatment did not have a significant effect on the changes of the protein content of the product (the restructured steak) (p>0.05). Compared to p1 treatment (without MTGase concentration), it can be seen that the addition of MTGase + salt concentration in each treatment could significantly increase the protein content in the restructured product, however the treatment did not show a significant difference when compared to the protein content of raw materials (fresh flakes of yellowfin tuna) at a 95% significance level (p<0.05) (table 1). Increasing protein levels in p2 to p5 treatments because MTGase is able to catalyze the as-carboxyamide acyl group transfer reaction from glutamine residues to proteins or peptides and other primary amines (generally lysine) to produce polymers and intramolecular cross-reactions of isopeptide forms, such as ε-(γ-glutamyl) lysine. The more concentrations of MTGase, the more cross-linking covalent isopeptides are formed, so that the protein becomes denser. In addition, the heating takes longer which results in protein denaturation and makes protein is more stable.

3.1.3. Fat content. Based on the results of the statistical analysis (table 1), it is seen that the treatments have a significant effect on changes in the fat content of the restructured products (p<0.05). Changes in fat content in the product may be caused by non-treatment factors, namely heating in the water bath so that it can evaporate the product fat in significant quantities. Based on table 1, it can be seen that the fat content of the restructured product in p1 treatment (0% MTGase + 1.5% salt) results in lower product fat content compared to p2, p3, p4 and p5 treatments. Jiang et al (2000) states that cross-links between proteins formed or induced by MTGase are covalent (NH) cross-links. These cross-links could inhibit the release of fatty acids resulted from fat melting and adipose tissue fragments. The cross-links can be broken down by physical treatment (heating). N-H covalent bonds are bond with polar properties, which means that they are opposite to nonpolar substances (fats/oils).
Table 1. Average value of chemical, physical and organoleptic quality parameters of the product (restructuring steak of sliced yellowfin tuna) of each treatment.

| No | Quality Parameters | p0 | p1 | p2 | p3 | p4 | p5 | Value Ranges |
|----|--------------------|----|----|----|----|----|----|--------------|
| A  | Chemical quality   |    |    |    |    |    |    |              |
|    | 1. Water content (%) | 73.55\(^a\) | 70.04\(^b\) | 70.31\(^b\) | 71.06\(^b\) | 71.16\(^b\) | 71.12\(^b\) | 70.04-73.55 |
|    | 2. Protein content (%) | 85.46\(^a\) | 80.89\(^b\) | 85.10\(^a\) | 85.01\(^a\) | 86.73\(^a\) | 85.03\(^a\) | 80.89-86.73 |
|    | 3. Fat content (%) | 7.96\(^a\) | 1.83\(^b\) | 7.78\(^a\) | 4.91\(^d\) | 4.04\(^d\) | 5.49\(^f\) | 1.84-7.96 |
|    | 4. Carbohydrate content (%) | 0.79\(^a\) | 11.42\(^a\) | 1.16\(^a\) | 3.90\(^a\) | 1.79\(^a\) | 1.81\(^a\) | 0.79-3.90 |
|    | 5. Ash content (%) | 5.78\(^a\) | 5.86\(^b\) | 5.95\(^a\) | 6.17\(^a\) | 7.44\(^b\) | 7.67\(^b\) | 5.78-7.67 |
| B  | Physical quality   |    |    |    |    |    |    |              |
|    | 1. Breaking strength (kg) | - | 0.27\(^a\) | 0.30\(^a\) | 0.32\(^a\) | 0.47\(^a\) | 0.61\(^a\) | 0.27-0.61 |
|    | 2. Gel strength (g cm\(^{-2}\)) | - | 123.05\(^a\) | 118.91\(^a\) | 178.19\(^b\) | 227.68\(^ab\) | 326.68\(^b\) | 118.91-326.68 |
|    | 3. Hardness | 5.00\(^a\) | 8.40\(^b\) | 7.87\(^a\) | 7.33\(^a\) | 6.00\(^a\) | 6.20\(^b\) | 5.00-8.40 |
|    | 4. Colour: | 5. | 7.90\(^a\) | 3.45\(^d\) | 1.68\(^b\) | 1.53\(^a\) | 1.99\(^c\) | 1.48\(^d\) |
|    | 5. a* | 6.84\(^b\) | 10.41\(^b\) | 10.52\(^c\) | 10.35\(^b\) | 10.37\(^b\) | 11.28\(^d\) | 6.84-11.28 |
|    | 6. Taste | 5.00\(^b\) | 5.13\(^b\) | 5.20\(^b\) | 5.00\(^b\) | 5.13\(^b\) | 5.13\(^b\) | 5.00-5.20 |
|    | 7. Smell | 5.00\(^b\) | 5.27\(^b\) | 5.07\(^b\) | 5.13\(^b\) | 5.20\(^b\) | 5.33\(^b\) | 5.00-5.33 |

Note: p0 = fresh tuna meat steak (without MTGase), p1 = (0.0% MTGase + 1.5% salt / NaCl), p2 = (0.3% MTGase + 1.5% salt), p3 = (0.5% MTGase + 1.5% salt), p4 = (0.7% MTGase + 1.5% salt) and p5 = (0.9% MTGase + 1.5% salt)

3.1.4. Carbohydrates. Based on the results of statistical analysis (table 1), the treatment had a significant effect in carbohydrate content (p<0.05). Treatments p0 and p2 to p5 did not differ from one to another at a significance level of 95%, except for the treatment p1 which has a higher value than other treatments. This is due to the fact that the restructuring process does not change the chemical composition drastically so it does not affect the changes in carbohydrate levels that are strongly bound (chemically) in fish meat.

3.1.5. Ash content. Based on the results of the statistical analysis (table 1), the treatments did not have a significant effect on changes in ash content (p>0.05). Treatments p0 to p3 were not significantly different from one to another, except for p4 and p5 treatments. Significant increase of ash content occurred in treatment p4 with a value of 7.44% and p5 with a value of 7.67%, which was allegedly influenced by the use of MTGase (p<0.05).

3.2. Hardness
Based on the results of statistical tests (ANOVA) it appears that the MTGase treatment in the restructuring process does not have a significant effect on changes in product hardness (p>0.05). According to the DMRT test, the hardness values in p1, p2, p3 and p4 treatments show insignificant different (table 1) at a significance level of 95% (p>0.05). Significant increase occurred in the treatment of p5 with a value of 0.99 kg. According to Uresti et al (2004), increasing hardness can occur because the use of MTGase induces covalent cross-bonds between protein polymers and acts as a catalyst for the transfer of acyl between the γ-carboxyamida groups of glutamine residues on proteins. The formation of e-(γ-glutamyl) crosslinking causes more dense polymer which results in thicker and harder texture.

3.3. Breaking strength
Based on the results of statistical tests (ANOVA), the MTGase treatment did not have a significant effect on changes in the breaking strength of the restructured product (p>0.05). Results showed that a substantial increase in the nature of the breaking strength of the product occurred in treatment p5 with
a value of 0.61 kg (the highest value compared to other treatments) (table 1). The addition of MTGase can catalyze the formation of covalent crosslinks (Motoki et al and Takinami et al cit. Sun 2009). This crosslink causes polymerization of protein molecules and increases molecular mass. According to Ramírez et al (2007), polymerization resulted in an increase in the breaking strength properties of the product and an impact on the mechanical properties of the product (including breaking force). The stronger protein bonds in the product, the more difficult product to break so higher strength and pressure are required.

3.4. Gel strength
Based on the results of statistical tests (ANOVA), the MTGase treatment had a significant influence on changes in the gel strength of the restructured product (p>0.05). The results of the DMRT test showed that the tested treatments did not show significant different from one to another. The addition of MTGase produced a significant effect on the increase of the gel strength value of the product, as p5 treatment shows the greatest value (326.68 g cm⁻²) at a significance level of 95% (table 1). MTGase forms cross-links bonds between protein molecules, including myofibril protein and protects the damage of gel-forming proteins such as myosin from denaturation due to heating shortly after the setting process. According to Ophart (2003), thermal energy will only result in the breaking noncovalent bonds that exist in the natural structure of proteins and does not break the covalent bonds in the peptide bond structure. The more MTGases added the more cross-bonds between proteins are formed, and the structure of the myosin protein in the myofibril protein is more stable, so the gel strength is higher.

3.5. Brightness
Based on the results of statistical tests (ANOVA), the MTGase treatment has a significant influence on changes in the level of brightness (whiteness) of restructured products (p<0.05). The DMRT test reveals that in general, the tested treatments did not show a significant difference, except for the treatment p0. The increase in the brightness of the restructured products is not in line with the rise in the use of MTGase concentrations, which can be seen from significant fluctuation in the brightness value (p<0.05). The brightness fluctuation can be caused by incomplete denaturation during the heating process in each sample. The heating process enables the colour of the product to be brighter due to damaged or denatured pigments. In addition, it is also influenced by incomplete oxidation of myoglobin in the meat. Myoglobin is composed of protein molecules (globin) and nonprotein (heme) molecule parts, which has an essential role in the change of colour in the meat which is determined by the chemical state and the Fe group in the heme ring. When the meat is cooked, the heat will coagulate protein (globulin) so that the heme in the protein molecule allows the oxidation of red meat to turn into hemin (darker colour) (Rospiati 2006).

Compared to samples without MTGase, it can be seen that the addition of MTGase was able to increase product brightness significantly (p<0.05). According to Uresti et al (2003), MTGase can induce covalent cross-linking between adjacent proteins and encourage the formation of strong gels, as well as modify product brightness. This can be caused by the cross-linking of proteins formed by MTGase which is able to inhibit the oxidation of oxymyoglobin to darker-coloured metmyoglobin. When compared to a sample from commercial tuna steak, all samples of restructured products had a darker colour (p<0.05). This is due to the oxidation of oxymyoglobin to darker-coloured metmyoglobin.

3.6. Colour
Based on the results of statistical tests (ANOVA), the MTGase had a significant influence on the colour change of the restructured products (p<0.05). The DMRT analysis showed that in general, the colour from all treatments is different at the 95% significance level. The results of the colour testing showed that the brightness of yellowfin tuna steak from fresh conditions increased significantly after
the restructuring process (p<0.05) (see table 1). The increase occurs because of the denaturation of globin pigments during the heating process during the restructuring process (Hultin 1993 cit. Rospiati 2006). Myoglobin and haemoglobin become the main pigments in red meat.

The colour parameter a* in the restructured products had decreased significantly compared to fresh product (p<0.05). According to Hultin (1993) cit. Rospiati (2006), heating can result in the formation of a number of denatured globin pigments. The colour parameter b* which showed chromatic blue-yellow produces a significant increase in yellow colour in the restructured product compared to fresh steak (p<0.05) (table 1). The rise of MTGase concentrations in the restructuring process had no significant effect on p3 and p4 treatments, yet it greatly affected p1 and p5 treatments (p<0.05). This is due to covalent bonds between protein molecules formed from MTGase activity in protecting carotenoid pigments in fish meat from damage processes.

3.7. Water holding capacity
Based on the results of statistical tests (ANOVA test), the MTGase treatment did not have a significant effect on the water holding capacity of the restructured products (p>0.05). This is also shown from the results of the DMRT test, where in general, the treatments do not make any difference from one to another. Significant increase in the value of water holding capacity occurs in treatment p5 with a value of 64.85% (p<0.05) (table 1). According to Bello et al (2011), MTGase is able to increase interactions between protein molecules and subsequently form a three-dimensional structure (triple helix) that will trap or bind water molecules so that the water holding capacity of restructured products increases.

3.8. Taste
Based on the results of statistical tests (table 1), the MTGase treatment does not have a significant effect on changes in the taste of restructured products (p>0.05). Similarly, the DMRT and sensory tests show that all tested treatments were not significantly different. Furthermore, when compared to samples from commercial tuna steak, all samples have the same criteria as the standard.

3.9. Smell
Based on the results of statistical tests (ANOVA), it was seen that the MTGase treatment did not have a significant effect on the change in the smell of restructured products (p>0.05). Likewise, the results of DMRT and the sensory test showed that the addition of MTGase concentration did not significantly influence the formation of the scent of restructured products. Compared to the commercial yellowfin tuna steak, all samples had an aroma that was classified as "the same as the standard" (typical of tuna).

4. Conclusions
It was revealed that only 5 out of 13 quality parameters of the restructured product were affected by experimental treatment (low salt MTGase concentration), namely fat content, carbohydrate content, gel strength, brightness and colour. The addition of MTGase with a low salt concentration in the product can increase protein content, ash content, hardness, breaking strength, gel strength and water holding capacity in p5 treatment (0.9% MTGase+1.5% salt) (p <0.05). The addition of low salt MTGase to restructuring process sensorily improved the texture (hardness) of the product (p<0.05) and yet it did not affect the taste and smell of the product (p>0.05) when compared to commercial yellowfin tuna steaks. The addition of MTGase in the restructuring process was able to maintain the colour (brightness) of the product and the product was still darker than the commercial yellowfin tuna steak. Based on the physical properties of the product, important and influential nutrient content in the product, economic value of the product and sensory test results, the best treatment is p3 (0.5% MTGase+1.5% salt).
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