

Characteristics and effectiveness identification of restructured product of snakehead fish (*Channa Striata*) with transglutaminase enzyme addition

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Abstract. The existence of fishery waste problem shows that the innovation of fishery processing not yet maximized in Indonesia. Both waste and fresh cuts of fish can be utilized by restructuring with transglutaminase enzyme addition as a crosslinking agent. This microbial transglutaminase (MTG) enzyme can alter protein functionalities by forming stable covalent bonds between residues of the amino acid of protein. Accordingly, this study focused on Snakehead Fish (*Channa striata*) is used as the protein source and variations of treatment conditions include incubation duration (2 days and 7 days) and the composition of enzyme and processed fish meat (0.0%, 0.5%, 1.0%) and. The results of this study showed the strong performance of crosslinking by MTG on processed fish meat. This is confirmed by an increase in texture profile parameters (hardness, cohesiveness, and elasticity), the detection of myosin changes, also wave number increment of C-N and an increase of intensity of C=O bonds. The highest effectivity value in the sample was achieved by variation of incubation duration of 7 days and the addition of 1.0% MTG enzyme with a value of 129,78% in hardness parameter in sample.

1. Introduction

Along with the increasing number of world population and the need for better nutrition source, the demand for fish which has a significant nutritional value continue to increase every year. Indonesia with a large population is fortunate enough to have abundant fishery resources with the position as the fifth largest fish producer according to FAO statistics. Indonesia's fishery products in public waters covering an area of 54 million hectares with a production potential of 0.9 million tons a year. The potential value of Indonesian marine and fishery products reaches 3000 trillion per year, however there was only about 225 trillion or about 7.5% that has been utilized. The government-driven potential must be supported by all stakeholders, including in terms of innovative processing of all fishery products [1].

One of the innovation of fishery product processing is chemical binding method which is done by bonding agent with various kinds of treatment. One of the most widely used is transglutaminase enzyme [4]. The use of transglutaminase enzymes that is specifically produced by microbes or known as microbial transglutaminase (MTG) species is expected to increase the value of meat scraps from previous product processing as well as to innovate the fresh fish processing. MTG can produce fish meat with reconstruction of a new and chewier structure because of its working principle as an agent forming of cross-linked covalent peptides between protein surfaces in enzyme-added fish tissues.



Previous studies [2-6] have shown that each species of protein source will have a different response to MTG and can produce various products (polymers) that vary in morphological, rheological, and physiochemical properties. In general, by adding enzymes in the re-structuring of fish meat, meat processing can be done more easily and the yield of marketable fishery products can be maximized.

2. Material and Methods

2.1. Materials

The materials used in the study are snakehead fish (*Channa striata*), Microbial Transglutaminase enzyme (MTG), water and alcohol.

2.2. Preparation Tools and Materials

Preparing the necessary tools in the experiment involves the provision of materials and tools, as well as the sterilization of tools used such as scales and gloves with alcohol. The transglutaminase enzyme was provided by a manufacturer in Spain that has been available since November 2016. Before being used, the enzyme was stored in a sterile and vacuum condition of a refrigerator at about -16°C.

2.3. Preparation of Fish Meat and Enzymes

After preparing the required tools and ensuring the availability of the ingredients, fish meat was cleaned by cutting the meat carefully off the bones and unused body parts such as head and skin before washing. There are two types of treatment for fish meat based on their use for different analysis test.

- Treatment I: Cut fish meat into dice with dimensions of about 3 cm x 5 cm for texture profile samples
- Treatment II: Puree the fish meat and separate into 10 grams each as the sample unit.

2.4. Fish Meat Processing with Enzymes

On sample of texture profile analyzing, the enzyme was applied evenly on one surface of the meat, then stack one piece of other meat on top. As for other tests, the enzyme with adjusted dosage was mixed evenly through mixing. After that, the samples were wrapped up with plastic wrap until it was airtight and covered all the parts perfectly. All samples then are labeled before being stored and incubated in a chiller with an adjusted duration. Specifically, for the texture profile test, samples were made for three duplicates.

2.5. Product Analysis

The tests taken were Acidity Test Level, Texture Test with Texture Profile Analyzer, Detection of Functional Groups/Secondary Structure with Fourier Transform Infra-Red, Electrophoresis Profile Test with Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis.

3. Result and Discussion

In the initial processing of fish, fish meat that has been cut off from other parts or called fillet washed with water as washing can affect the degree of acidity of fish. The washing stage was done for removing the natural components of fish, such as water-soluble proteins, blood and other components, which can affect the decay processes (lipid oxidation and microorganisms) during the storage process at low temperatures [4]. Table 1 shows 15 samples used in this study with various MTG addition and given incubation duration.

Table 1. Prepared Samples

Analysis Types	% Enzyme	Duration (days)	Samples
Texture Profile	0.0; 0.5; 1.0	2 and 7	6
FTIR*	0.0	7	1
	1.0	2 and 7	2
SDS-PAGE*	0.0; 0.5; 1.0	2 and 7	6

* all samples have passed the acidity test

**Figure 1.** One of incubated sample

After being incubated during given time to react, a description of restrutured samples are shown on Figure 1. The chopped and processed meat that has been incubated with MTG addition turned into a larger and solid meat.

3.1. Analysis of Acidity Level (pH)

To analyze the acidity level of the sample, the pH meter measurements with digital pH meters were performed on prepared samples beforehand. All incubated samples were thawed before the measurement. The results of pH values are shown in Table 2.

Table 2. The Result of pH measurements

Incubation Duration (days)	Enzyme Concentration (w/w)	pH
7	1.0%	6.1
7	1.0%	5.8
2	1.0%	6.3
2	0.0%	6.4

The optimum pH of MTG was ranged from 5 to 8, but the enzymatic activity is still processed at pH 4 or 9 [7]. Thus, all data show that the performance of this enzyme reaches optimum under this study conditions. The increased pH value as the MTG addition shows the results of crosslinking reactions in sample proteins, which chemically produce ammonia molecules [8]. Consequently, the content of more alkaline can affect the pH value of meat.

3.2. Texture Profile Analysis

Texture profile analysis was performed to find out the characteristics of fish meat texture that have been modified with MTG. The measurement conditions have been also adjusted according to a certain research reference [9]. Texture profile analysis was done on fish products, both with MTG enzyme or without MTG enzyme which used as control addition. The test was carried out at room temperature about 20-22°C [10,11].

Table 3. Sample Variations in Texture Profile Analysis

Incubation Duration (days)	% Enzyme	Parameter		
		Hardness (N)	Cohesiveness (-)	Elasticity (%)
2	1	39.02	0.178	66.06
	0.5	26.25	0.275	66.12
	0	16.98	0.205	61.87
	1	75.59	0.354	62.39
7	0.5	59.82	0.266	57.59
	0	55.14	0.195	55.59

The results in Table 3 briefly showed the changes on texture profile of samples after MTG was added and incubated in 2 or 7 days. These condition was developed from several previous researches on fish [3-5,7,10,12].

MTG allows the myofibril protein to dissolve and enable to induce the formulation of protein crosslinking. This has a major effect on changes in texture profile of various parameters in which each addition of MTG improves binding, hardness, cohesiveness, chewing and meat elasticity [11-12]. All values are compared and expressed on Figure 2.

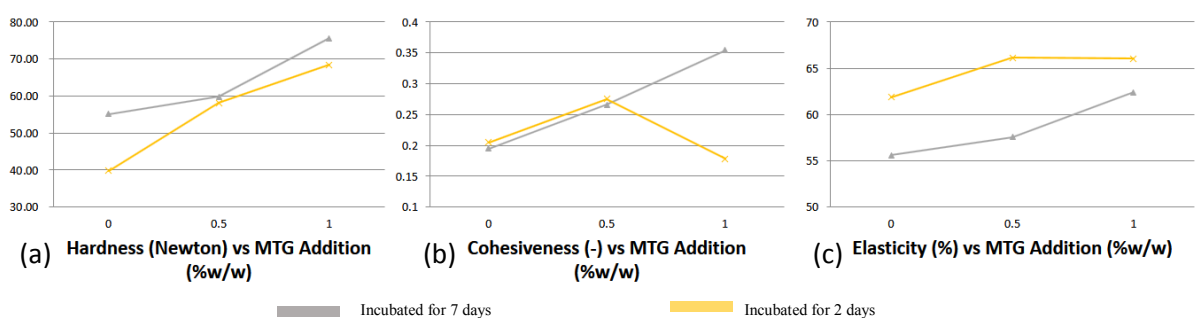


Figure 2. Results of Profile Texture Analysis

3.3. Level of Hardness

The level of hardness is the maximum force required to suppress the sample [11] and has units of force such as Newton (N) or gram-force (gf). The change of hardness value in snakehead fish only ranged from 16.98 to 75.59 N as expressed in Figure 2(a). The effect of MTG addition on samples that was incubated for 7 days shows higher hardness values. This supports various studies on other fish species such as silver goldfish [2], White Gulati Fish [4], Siamese catfish [5] and European hake and rainbow trout [11].

3.4. Level of Cohesiveness

Cohesiveness can be defined as how far a sample can undergo deformation before dehiscent [11]. To express the cohesiveness, no dimension number used. As for this study, the cohesiveness value is very low in all samples, which varied from 0.178 to 0.354 and somehow shows no significant changes of cohesiveness in each MTG addition (Figure 2(b)). In general, this shows a significant difference based on the incubation duration. However, based on different concentrations of MTG used in each sample, the values tend not to be as significant as those obtained by other studies [8,10,12].

3.5. Level of Elasticity

Measurement of springiness or elasticity values has the goal of determining a sample's ability to return to its original state after first pressure [11]. This elasticity level is expressed in a percentage that ranged from ~55% to ~66%. In Figure 2(c), it was shown that elasticity level in snakehead fish presented a significant change in both variations of incubation duration generally. For incubated samples of 2 days, the range of elasticity values ranged from 61.87% to 66.12%. While on the duration of 7 days, the value ranged from 55.59% to 62.39%. Both results showed that more MTG addition gave a higher value.

3.6. Analysis of Enzyme Effectiveness on Sample

As a whole, an increase in the value of hardness, elasticity and cohesiveness observed in this study can explain that MTG enzymes may result in protein crosslinking of fish. This affects the formation of larger protein polymer molecules between fish meat particles [2]. The formulae used in the calculation of the percentage of effectiveness and the results (Table 4) are as follows:

Table 4. Result of Samples Effectiveness

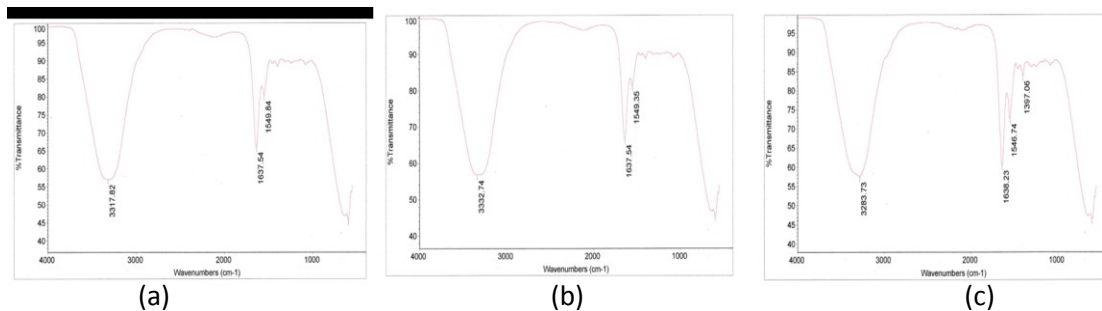
Incubation Duration (days)	% Enzyme	Parameter		
		Hardness (N)	Cohesiveness (-)	Elasticity (%)
2	1	2.10	(13.17)	6.77
	0.5	46.10	36.41	6.87
7	1	37.09	81.54	12.24
	0.5	8.49	34.15	3.60

$$\% \text{ Effectivity} = \frac{(\text{Sample parameter values with MTG addition})}{(\text{Sample parameter values without MTG addition})} \times 100\% \quad (1)$$

The effectiveness analysis was done based on the variation of the concentration of MTG (0.5% and 1,0%) and the incubation duration (2 and 7 days). In the term of incubation duration almost all samples with longer time showed more effective results. The highest effectiveness value was achieved by 7 days-incubated sample with MTG addition of 1.0% with 129,78% of hardness value.

3.7. Functional Group Analysis

In general, based on the results of this study, it was found that each group of atoms in the sample showed changes in the vibrational band with almost the same wave value. To verify the formation of crosslinking by MTG, there are two concerned waves. The preferred wave was assigned to Amide I vibrational mode, which ranges from 1600 cm^{-1} to 1700 cm^{-1} that involves mainly C=O stretching [11]. This mode will show an increase in the band intensity as the addition of MTG applied on samples. Figure 3 shows the result of functional group analysis results, which (a) shows sample with 1,0% MTG addition with 7 days-incubation duration; (b) shows sample with 1,0% MTG addition with 2 days-incubation duration; and (c) shows sample with 0,0% MTG addition with 2 days-incubation duration.

**Figure 3.** Result of Functional Group Analysis

As in Figure 3 show the corresponding result of wave intensity $\sim 1637 \text{ cm}^{-1}$ in (c) has a higher band intensity than (b). In addition, it was analyzed that the formation of new C-N bonds caused a decrease in the number of detected waves at $\sim 1500 \text{ cm}^{-1}$. However, different results were obtained when comparing Figure 3(b) and (c), where samples with enzymes addition had slightly higher wave values than those without enzyme. Figure 3(a) and (b) was to compare the difference of incubation duration of samples, however did not show any significant result.

Therefore, each dominant peak that previously discussed is found in all sample variations. However, some of the results of the analysis show similar conclusions with previous studies [11] and concluded if FTIR test is useful to detect changes on protein binding as the result of MTG addition.

3.8. Analysis of Electrophoretic Profile with Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis

The protein bands found in the performance analysis of MTG enzymes were in the 45 to more than 200 kDa band range. This result shown in Figure 4 is corresponding to the molecular weights of myosin respectively (200 kDa). There 3 lanes provided for samples with each different incubation duration, in which each lane was filled with different MTG addition while Lane 6 was used as protein marker,



Figure 4. The Result of SDS-PAGE

Note: Lane 1-3 (2 days), Lane 6 (Marker), Lane 4,5,7 (7days)

In each samples shown in figure 4, the intensity of the myosin band was not clearly different. MHC (myosin heavy chain) molecule is crosslinked by MTG which ultimately complicates the separation of the molecule during gel analysis. The covalent bonds that occur cannot be disrupted by the electrophoresis reagent during analysis, so the detection becomes lessened [2]. This respect was an explanation of different result of SDS-PAGE based on its incubation duration, where samples with longer period have the decreasing protein band density.

4. Conclusion

The addition of microbial transglutaminase (MTG) enzyme in snakehead fish causes cross-linking of myosin proteins as seen from the results of functional group analysis with increasing C-N wave value and wave intensity C=O. Physically, the changes were also observed on the changes of final restructured samples. Also, the addition of MTG concentration and longer incubation duration were directly proportional to the increase in texture profile parameters (hardness, cohesiveness, and elasticity) of the sample and inversely proportional to the intensity of the protein band on the electrophoretic profile. The highest effectiveness value was achieved with variation of incubation duration for 7 days and addition of 1.0% MTG with effectiveness value of 129,78% in hardness parameter.

5. Reference

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