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UTILIZATION OF TRANSGLUTAMINASE ENZYMES IN PROCESSED FISH PRODUCTS

Junianto[1], Arifiyana Saefurahmat[2], Hafsoh Nur Muthmainnah[2], Faried Affandy[2], Muhammad Farhansyah[2], Abdul Aziz[2] and Ratu Cholilah Liutamima Andika Putri[2]

[1] *Lecturer at Fisheries Science Department Padjadjaran University, Bandung-Indonesia*
[2] *Bachelor Student at Fisheries Science Department Padjadjaran University, Bandung-Indonesia*

ABSTRACT
Enzymes have been widely utilized in various food product industries including fish processing. This article aims to review the use of the Transglutaminase enzyme in processed fish products in improving the quality of the products it produces. Based on the results of the literature review, information was obtained that the addition of transglutaminase enzymes to the manufacture of surimi, kamaboko and fish balls can increase the strength of the dough gel so that the texture of the product becomes more preferred.

Keywords *: quality, fish balls, surimi, kamaboko, gel strength.*

INTRODUCTION

Enzymes have an important role in various areas of human life, including in the processing of processed fish products. Enzymes are products of cell metabolites from both microbial, plant, animal and human cells. Enzymes are composed of proteins and minerals and vitamins. Enzymes that play a role in the manufacture of processed fish products, one of which is the transglutaminase enzyme. This enzyme belongs to the transferase group, meaning that it has a function in the elasticity and formation of product gels, especially those whose raw materials contain a lot of protein. Transglutaminase enzyme or commonly known as TGase.

According to Fawzya et al (2011), the enzyme transglutaminase is used in modifying the structure of proteins. Martins et al. (2014) also stated that the enzyme transglutaminase or Tgase has a function to increase gel strength, stability, viscosity, reduce syneresis (water loss) in yogurt, and improve rheological properties in meat. The transglutaminase enzyme works intra- and intercellularly which then produces cross-links between proteins. Transglutaminase catalyzes the reaction between a peptide bound to glutamine residues and a primary amine. This article aims to review the use of the Transglutaminase enzyme in processed fish products in improving the quality of the products it produces.

Enzyme Transglutaminase

Transglutaminase (TGA-ase, EC 2.3.2.12) is an enzyme that catalyzes the formation of cross-links between protein molecules (the formation of polymers between protein molecules). The transglutaminase enzyme has a systematic name, namely aminγ-glutyltransferase which belongs to the class of transferase enzymes (E.C.2), acyltransferase (E.C.2.3), aminoacyltransferase (E.C.2.3.2), protein glutamine-γ-glutamyl transferase and has an alternative name, namely fibrinoligase. The transglutaminase enzyme in the commercial market is known under the trademark ActivaTG. ActivaTG products have been marketed abroad for example Europe, USA, Korea, Japan, Thailand, and several other countries, including Indonesia.

The work of the Transglutaminase enzyme is influenced by pH, temperature, enzyme concentration, and substrate concentration. Transglutaminase enzymes work optimmune in the pH range between 5-8, but at pH 4 or 9, transglutaminases still show enzymatic activity. Its optimum temperature in the range of 50-55^{o}C. Transglutaminase loses enzymatic activity at heating 70^{0}C. Transglutaminase still gives off enzymatic activity at a temperature of 10^{0}C, and still shows some activity at temperatures slightly above freezing (Motoki et al., 1986)

According to Nielsen, GS et al. (1995) the mechanism of action of the transglutaminase enzyme is to catalyze the reaction between the amino acid residue of lysine and the amino acid residue of glutamine and the formation of a -(-glutyl) bond of lysine isopeptide that results in the merging of inter- or intramolecular covalent bonds that cross-bind to food proteins. This can improve the physical properties and shape of food. TG-ase can be used to catalyze the binding reaction of amino acids-amino acids added to protein molecules. This is indicated by the binding of methionine to various food proteins. The mechanism of action of the transglutaminase enzyme is found in Figure 1.

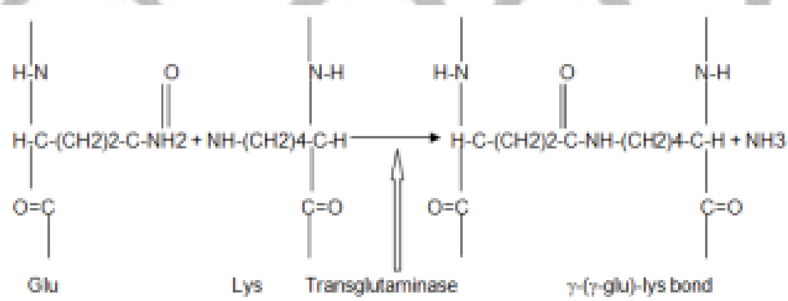

Figure 1. Crosslinking reaction using microbial transglutaminase
(Source : Nielsen, GS et al. 1995)

The mechanism of action of MTGase in general is that it is involved in polymerization reactions, which produce changes in the hydrophobicity of molecules (Gaspar and de GóesFavoni, 2015). The formation of non-disulfide covalent bonds in proteins, in particular cross-links ε-(γ-glutyl) lysine, is catalyzed by MTGase through the transfer of acyl between the γ-amide group of glutamine residues and the ε-amino groups of lysine residues, thereby contributing to the improvement of gel quality in surimi (Chanarat and Benjakul, 2013). MTGase modifies proteins by introducing amines, and affects intra- and intermolecular crosslinking or deamidation, causing major changes in their molecular structure (Celis, 2009). The acyl transfer reaction occurs through the transfer of the c-carboxamide group from peptides bound to glutamine residues (acyl donors) to various primary amines (acyl acceptors), including the e-amino group of lysine residues (Bagagli, 2009). When the e-amino group of the lysine residue on the protein molecule acts as an acyl acceptor, intra- and intermolecular e-(c-glutyl)-lysine (G-L) crosslinks may form. These isopeptide bonds form important stable protein tissues, as in the formation of gels (Parkin, 2010), which generate changes in the hydrophobicity of the surface of proteins, affecting the solubility and other functional properties determined in their characteristics, such as: glassing,

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emulsification, foam formation and viscosity. When lysine is generated, free lysine or primary amines are not in the reaction system, in which water becomes an acyl acceptor, while hydrolytic dehydration of glutamine residues occurs, thus becoming glutamic acid. This reaction changes the protein charge, which also causes a change in the solubility of the protein (Damodaran, 2010). Whether by deamidation or polymerization, the use of MTGase affects the structure of the protein system. This causes changes that occur in its functional properties, that is, to make proteins an efficient agent of transformation, so as to be able to produce products with better texture characteristics (Gaspar and de Góes-Favoni, 2015).

Application of Transgulataminase on Surimi

The transglutaminase enzyme has been applied in the manufacture of surimi to increase gel strength, water holding capacity (WHC), and white degree. These three parameters are factors for assessing the quality of surimi. This enzyme serves to improve solubility and improve gelling, emulsification, foaming, viscosity, and water holding capacity (Gaspar and de Góes-Favoni, 2015).

Table 1, is the surimi characteristic data obtained related to the level of addition of MTgase concentrations as reported by Seighalani *et al.*, (2017). The results of his research show that the addition of MTGase can strengthen the surimi gel by increasing the strength value of the gel, so that the texture of the surimi is not easily broken. The addition of MTGase also results in a stronger surimi texture. In addition, the white degree of surimi added with MTGase is higher than without MTGase.

Table 1. Comparison between characteristics of surimi gel at different concentrations of MTGase (Source : Seighalani e*t al.* 2017)

MTGase concentration (unit/g surimi)	Gel strength (g)	Gel deformation (mm)	White degree	Expressible water (%)
0,00	434,36	10,50	76,53	11,70
0,10	435,17	10,60	78,38	11,25
0,20	438,00	13,13	78,02	11,13
0,30	763,70	14,79	79,73	10,50

In the process of making surimi gel, the addition of MTGase can improve the characteristics of surimi gel. With the presence of MTGase, the ability of gels in surimi to occur gel bond breaking is getting lower (Kaewudom *et al.* 2013). Compact and dense gel tissue is produced by myofibrillar proteins that form cross-bonds with the addition of MTGase so that the quality of surimi is increasing (Kaewudom *et al.* 2013).

Application of Transglutaminase on Kamaboko

Application of Transglutaminase on Kamaboko to obtain a product with a more chewy texture. Kamaboko which has a chewy texture is what consumers like. The texture of kamaboko is obtained from the fusion of gel strength and elasticity.

The results of Anggraeni's research (2015) inform that the rate of addition of transglutaminase enzymes affects the strength of the kamaboko gel produced, as shown in Figure 2.

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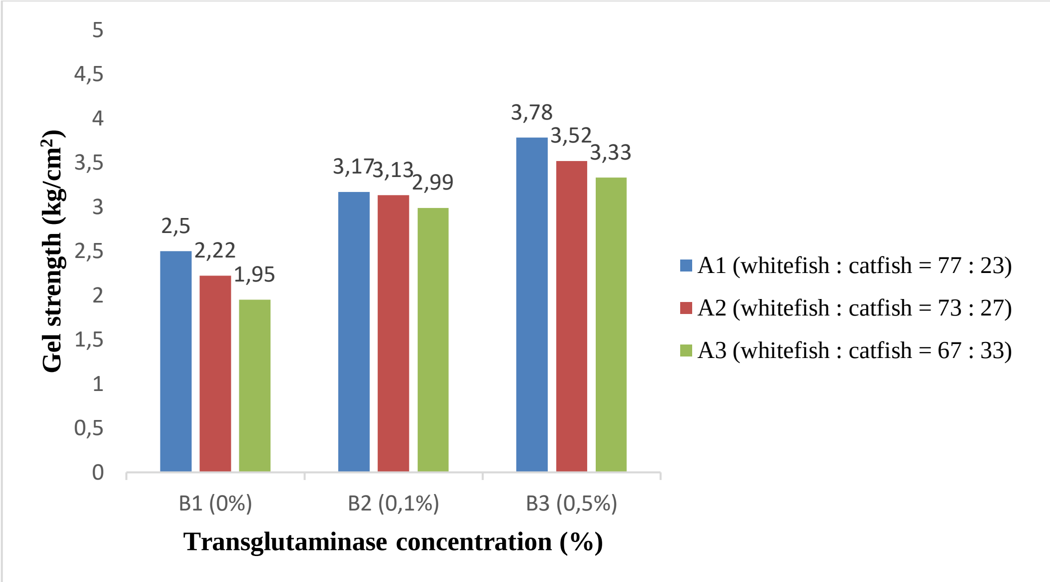

Figure 2. Graphic of average gel strength due to proportion of whitefish : catfish and addition of Transglutaminase enzyme (Source: Anggraeni 2015)

Based on Figure 2, the higher the addition of the transglutaminase enzyme, the higher the strength of the kamaboko gel. The addition of MTGase can induce crosslinks between long chains of myosin so as to increase the strength of the gel (Hsieh *et al.* 2006).

Overall, the results of Anggraeni's research (2015) inform that the addition of transglutaminase enzyme concentrations in the manufacture of kamaboko has a significant effect on chewiness, gel strength, white degree value, and water holding capacity, water content, fat content, salt soluble protein content (PLG), and pH value. The best treatment of kamaboko product based on physical and chemical properties is found in the treatment of the proportion of whitefish: catfish = 77: 23 with the addition of the transglutaminase enzyme 0.5%. The results of the physical and chemical properties of the best treatment are suppleness 5 (AA); gel strength 3.78 kg/cm^2; white degree 68.89; water-binding power 70.88%; moisture content 70.99%; fat content 3.29%; PLG content 18.04%; and a pH value of 6.77.

Application of Transglutaminase on Fish Balls

Fish balls include fish jelly products. According to Muttaqin *et al.* (2016), fish balls are processed products made from meat lumatan which are added with flour, spices, and food additives and then carried out the process of crushing meat, making dough, molding, and boiling.

In general, meatballs are composed of binders and fillers. One of the fillers that are often used in making meatballs is tapioca flour. However, tapioca flour is considered not yet qualified to increase the strength of the gel (chewiness) contained in meatballs so that other binding materials can improve the chewiness quality of meatballs. Kusnadi *et al*. (2012) state that the parameters of good meatball chewiness are meatballs that have the ability to break down due to pressure forces, and the nutritional content contained in good quality meatballs.

Research conducted by Nugroho *et. al* (2019) tried to identify the characteristics of trash fish balls added with the enzyme TGase with different concentrations. TGase concentration treatment in fish balls, namely treatment A (0.3%), B (0.6%), C (0.9%), and K (0%). The result is on the degree of organoleptic favorability of meatballs as found in Table 2.

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Table 2. Panelist acceptance rate result for fish balls (Source : Nugroho et al. 2019)

Treatment	Parameter			
	Appearance	Aroma	Flavor	Texture
K	6,10 ± 0,76	6,76 ± 0,73	6,06 ± 0,69	5,67 ± 0,68
A	6,96 ± 0,61	7,03 ± 0,76	6,23 ± 0,77	6,37 ± 0,67
B	7,50 ± 0,57	7,13 ± 0,63	6,30 ± 0,60	7,37 ± 0,56
C	7,60 ± 0,67	7,23 ± 0,64	6,34 ± 0,67	7,83 ± 0,70

Based on Table 2 above, the higher the concentration of transglutaminase enzymes, the higher the degree of preference for the organoleptic properties of fish balls, especially in texture. Overall, the results of research by Nugroho *et al.* (2019) informed that the concentration of TGase on meatball dough affected the level of liking of panelists. Meatballs with a concentration of C were ranked first with the most favored by the panelists, followed by meatballs with concentrations of B, A, and K. TGase levels affected the appearance, aroma, taste, and texture of fish balls. The greater the concentration of TGase added, the more taste, aroma, and chewiness of fish balls will increase.

CONCLUSION

Based on the results of the literature review, information was obtained that the addition of transglutaminase enzymes to the manufacture of surimi, kamaboko, and fish balls can increase the strength of the dough gel so that the texture of the product becomes more preferred.

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