



ARTICLE Review, Quality of Fish Sauce from the Use of Different Enzymes

Junianto¹, Andini Elpania Diyobel Purba², Devi Fitriani², Muh. Ragam Padlu Rochman² and Tarisa Rusmana²

- 1) Lecturer Staff of the Department of Fisheries, Padjadjaran University, Bandung-Indonesia**
- 2) Students of the Undergraduate Fisheries Study Program, Padjadjaran University, Bandung-Indonesia**

ABSTRACT

The use of enzymes has been widely carried out in various aspects of life, one of which is in the field of fisheries, be it cultivation, or processing of fishery products. The addition of enzymes to the process of processing fishery products, more specifically what will be discussed in this article is fish sauce, can give its own quality to the products produced. This article aims to find out how it works as well as what are the effects of giving different enzymes to one type of product, namely fish sauce. The enzymes that will be discussed this time include the enzymes bromelin, papain, pepsin, and trypsin. Has a result of studies from several sources shows that there are significant differences in product quality in the use of different enzymes. So it can be concluded that the use of different enzymes in the processing of fish sauce provides a different quality of fish sauce.

Keywords: Fish sauce; fermentation; enzymes; bromelin; papain; pepsin; Trypsin

INTRODUCTION

Fish sauce is a fermented product with fish ingredients. Hydrolyzed clear brown liquid is the color of fish sauce and is commonly used as a flavor enhancer or salt substitute in various foods. The production process involves mixing salt with fish in a ratio of 1:2 or 1:3 (Lopetcharat et al., 2001). The shortcomings of making fish sauce often take a lot of a long time and the taste of the product is very salty. Food fermentation can be shortened by adding the enzyme papain and reducing salt intake.

The manufacture of fish sauce with enzymes has several advantages, including: in a short time, (Subroto et al., 1985), conducting research on the manufacture of fish sauce turns out to be with enzymes, the production process can be shortened to 3 days. (Muliati, 1985), analyzing the protein content of fish sauce by enzyme hydrolysis has a higher protein content, which is 10.52 grams / 100 ml, while the fermented soy sauce is only 2.14g / 100ml. This article aims to find out the differences in the influence of various enzymes on the quality of fish sauce.

Fermentation

Fermentation comes from the Latin "fervere" which means to boil (to boil). The meaning of the word from latin can be attributed to the condition of the liquid bubbling or boiling. As technology develops, the definition of fermentation also expands into all processes involving microorganisms to produce a product called primary metabolite and secondary in a controlled environment. In addition, fermentation can also be defined as a way of processing through the process of utilizing the decomposition of compounds from complex protein materials. These complex proteins are found in the body of fish which are converted into simpler compounds with the help of enzymes derived from the body of fish or microorganisms and take place in controlled or regulated circumstances. (Hidayat *et al.* 2006)

Broadly speaking, fermentation can be divided into two, namely:

- Fermentation process that allows decomposition or transformation that will later be able to produce a product with a completely different shape and properties or change from the state originally. For example, the processing of fish sauce and shrimp paste.
- The fermentation process that produces compounds, in fact, will have the ability or durability in the product, for example the manufacture of peda fish.

The fermentation process that occurs in fish is a process of biological or semi-biological decomposition of complex compounds, especially proteins, into simpler compound compounds under the circumstances of controlled. During the fermentation process, fish protein will be hydrolyzed into amino acids and peptides, then amino acids will decompose further into other components that play a role in the formation of amino acids taste of the product. As for the factors that can affect the fermentation process, namely temperature, oxygen, substrate and water.

Fish Sauce

Fish sauce is one of the fermented products with fish raw materials. Fish sauce has a different taste from soy sauce made from soybeans. Fish sauce is widely used as an additive in cooking and food by various countries such as China, Korea, and Thailand. Fish sauce has a distinctive taste and aroma. The color is clear yellowish to light brown with a relatively salty taste and contains a lot of nitrogen compounds. In addition to the nitrogen component, fish sauce also contains minerals that are important for the body, for example NaCl salt or calcium salt. Fish sauce has a high nutritional content because it contains nitrogen. In the process of processing fish protein soy sauce will be hydrolyzed. Based on the results of research during the process, amino nitrogen will increase but there will be a decrease in total nitrogen. Amino nitrogen is a good nutritional element for the body because it is easily digested. The manufacturing process is to mix salt with fish in a ratio of 1:2 or 1:3 (Lopetcharat *et al.* 2001).

Enzyme

Enzymes are biocatalysts that are able to increase the speed of specific reactions without reacting and do not produce by-products, are much more efficient than other catalysts, because enzyme molecules have a high specificity to their substrate. The molecular size of the enzyme is much larger than the size of its substrate because the enzyme consists of hundreds or even more than a thousand amino acids. Enzyme bonds with substrates usually occur around the active site, besides that enzymes have a regulatory side that functions as a regulator to increase or decrease the work activity of enzymes. This side of the regulator will bind small molecules or substrates directly or will re-form free enzymes and products (Lehninger 1997).

According to Palmer (1985), the reaction between enzymes and substrates can occur according to the following two hypotheses:

a. *Lock and Key Hypothesis*

Enzyme specificity including the presence of a complementary structure between the enzyme and the substrate occurs if the substrate has a spatial shape compatibility with enzymes in the active side structure of the enzyme.

b. *Induce Fit Hypothesis*

The substrate does not have the suitability of the space with the active side of the enzyme in the enzyme-substrate complex, but in the process of binding the substrate, the enzyme

undergoes conformation changes so that it corresponds to the substrate . This process is called the induction process.

Papain Enzyme

Papain is a substance (enzyme) obtained from the sap of young papaya and papaya plants. Almost all of them contain sap on part of the papaya plant, excluding the roots and seeds. The most papain content is in young papaya fruits. Papaya sap (papain) contains quite a lot of proteolytic enzymes (protein decomposers) (Warisno, 2003). Papaya sap contains up to 10% papain, 45% chymopapain, and lysozyme increases by 20% (Winarno, 1993).

Papain enzyme is a protease enzyme that can reconstruct the primary structure of proteins, the bonds between amino acids in the amino acid polymer chain. This enzyme is classified as sulfhydryl protease and contains a fairly large element, namely (1.2%) of the amino acids that make up papain are: lysine, arginine, aspartic acid, Asparagine, acid glutamate, glutamine, theanine, serine, proline, alanine, valine, Isoleucine, leucine, tyrosine, phenylalanine, tryptophan, cysteine, cystine (Wirahadikusumah, 1989).

Currently papain is a product that is very beneficial for human life, both in the household and in industry (Purnomo, 2005). Several uses of the enzyme papain are being studied, including as a meat vaporizer, making protein concentrates, protein hydrolysis processes , and its role as an antifreeze agent in the beer industry (Yuniwati et al. 2008).

The combination of enzymatic hydrolysis and fermentation can be used to produce fish sauce in a relatively short time so as to produce quality fish sauce (Suparman, 1993).

Aniqoh (2017) in his research concluded that the addition of papain enzymes and the duration of fermentation have an influence on protein levels, amendments and pH of fish sauce. However, it does not have an effect on salt levels. The best results on the addition of the enzyme papain are as follows:

Table 1. Quality of Fish Sauce with the Addition of Papain Enzyme

No	Test Type	Unit	Result
.			

1.	Ph	-	5,63
2.	Protein content	%	4,84
3.	Salt content	%	23,28
4.	Amendments	%	90,54

Bromelin Enzyme

Efforts to speed up the process of hydrolysis of fish meat proteins are mostly carried out by adding proteolytic enzymes from the outside, both enzymes derived from animals and plants. The use of pure enzymes requires a high cost given the very expensive price of pure enzymes. Therefore, in this study, a source of proteolytic enzymes was sought that is cheap and easy to hydrolyze fish meat protein with a fairly high dissolved nitrogen yield, then tried using pineapple fruit extract which is known to contain a lot of bromelin enzyme (Iskandar and Widyasrini 2009).

The enzyme bromelin is a proteolytic enzyme derived from pineapple fruit. Pineapple fruits contain vitamins (A and C), calcium, phosphorus, magnesium, iron, sodium, potassium, dextrose, sucrose (cane sugar), and the enzyme bromelin. Bromelin is anti-inflammatory, helps soften food in the stomach, interferes with the growth of cancer cells, inhibits platelet aggregation, and has fibrinolytic activity. The enzyme bromelin belongs to the group of sulfhydryl protease enzymes which means that it has a sulfhydryl residue at its active location. As a proteolytic enzyme, bromelin is able to break down proteins into amino acids (Hamidi, 2008). The optimum temperature for the enzyme bromelin is 50° C, above and below that temperature the activeness of the enzyme becomes lower. The enzyme bromelin has an average molecular weight of 31,000. The enzyme bromelin can be activated by cysteine and KCN. Inhibition by HgCl₂ can be reactivated with the addition of cysteine, since it will again become a reducing compound that has a sulfhydryl group at its active location (Reed 1986). The results of the study (Hamidi 2008) showed that the more mature the fruit, the bromelin enzyme in the fruit is less active.

Based on research conducted by Pramita *et al.* (2020), it was concluded that the addition of the enzyme bromelin to fish sauce produces the following qualities:

Table 2. Quality of Fish Sauce with the Addition of Bromelin Enzyme

No	Test Type	Unit	Result
1.	Color	-	2,738
2.	Smell	-	2,425
3.	Viscosity	%w/b	41,364
4.	Dissolved nitrogen	%	0,8600
5.	Total dissolved solids	%	27,5
6.	Fluid volume	%	17

Pepsin and Trypsin Enzymes

Pepsin and trypsin enzymes can convert proteins into smaller peptides. One of the sources of pepsin and trypsin enzymes that can be utilized is fish offal. Pepsin enzyme can be obtained from the stomach of fish, while the intestines of fish can be used as a source of trypsin enzyme. (Nurjanah *et al.* 2021)

The results showed that the use of different types of enzymes (trypsin and pepsin) in the processing of fish sauce from the contents of the abdominal cavity of Manyung fish caused a noticeable influence on the activity of enzymes, colors, percentage of amendments as well as hedonic values of their products. The use of trypsin and pepsin enzymes increases the activity value of enzymes, the percentage of amendments and the hedonic value of their products noticeably, but lowers the pH value.

Table 3. Quality of Fish Sauce with the Addition of Pepsin and Trypsin Enzymes

No	Test Type	Unit	Result
1.	Color	-	Brownish-yellow
2.	Ph	-	5.48
3.	Enzyme activity	$\mu\text{mol ml}^{-1}$	0.84
4.	Hedonic value	-	6.97
5.	Percentage of amendments	-	48.54

Fish Sauce Processing Process

Fish sauce is one of the fermented products with fish raw materials. Fish sauce is a clear brown liquid resulting from the hydrolysis of salted fish and is usually used as a flavor enhancer or salt substitute for various types of food. The manufacturing process is by mixing salt and fish and using a ratio of 1:2 or 1:3 (Lopetcharat et al., 2001).

Making fish sauce which is carried out by saline fermentation takes a long time, namely 4-12 months (Desniar, 2004) then for 6-12 months or more which is fermented at temperature space (30-40°C) (Lopetcharat et al., 2001). Things that can be done to overcome the manufacture of fish sauce that requires a long fermentation process are by adding enzymes, using papain, bromelin and ficin (Basmal, 1974), viscera and koji (Dissaraphong et al., 2005) and lactic acid bacteria (Tilarsih, 2008). Several studies have shown that the addition of enzymes from the outside (e.g. papain and bromelin) can shorten the fermentation time (Gildberg, 1993 and Haard et al., 1994 in BBRPKP, 2010).

One of them is using papaya on a large scale is one of its uses for the production of papain enzymes. Papain is a proteolytic enzyme resulting from isolation from papaya sap. The enzyme can be produced in the form of flour as well as solution. The advantage of the papain enzyme is its ability to degrade collagen or elastin found in meat so that softer tissue is obtained due to the hydrolyzing process (Hidayat, 2005).

According to Rachmi et al., (2008) and Purwaningsih and Nurjanah (1995) the best fermentation results of fish sauce with a papain concentration of 8% and 3% with an incubation duration of 4 days. Meanwhile, according to Hasan (1991) the best results were obtained in fish sauce with a concentration of papain 8% and an incubation duration of 10 days.

From the literature study, no data on the quality of trash fish sauce has been obtained. Based on these problems, research has been carried out on the effect of fermentation duration (10, 20, and 30 days) on the total amount of N, TVBN, yield and sensory with the use of 20% salt content and the addition of papain enzymes 3%, 6%, and 9%. The enzymatic manufacture of fish sauce has several advantages, including:

1. - Taking a shorter time, Subroto et al., (1985), have conducted research on the enzymatic manufacture of fish sauce and the result is that the manufacturing process can be shortened to 3 days;
2. - The protein is higher, Muliati (1985), has analyzed the protein content of fish soy sauce by enzymatic hydrolysis has a higher protein content, which is 10.52 grams / 100 ml, while fermented soy sauce only 2.14 grams/100.

The quality of fish sauce according to SNI is presented in the following table :

Table 4. SNI Fish Sauce Quality Requirements In Accordance with SNI 01-0222-1995

No.	Test Type	Unit	Requirement
1	Circumstances :	-	
1.1	● Sightings	-	Clear
1.2	● Smell	-	Distinctive
1.3	● Taste	-	Distinctive
1.4	● Color	-	Usual
5	Ph	-	5-6
6	Amino Nitrogen	% w/b	Min. 5
7	NaCl	% w/b	19-25
8	Food Additives		
8.1	● Food Preservatives	%	0,1%
8.2	● Food Coloring	%	0,1%
9	Metal Contamination :		
9.1	● Lead	Mg/kg	Max. 2.0
9.2	● Copper	Mg/kg	Max. 20.0
9.3	● Zinc	Mg/kg	Max. 100.0
9.4	● Mercury	Mg/kg	Max. 0.5
10	Arsenic contamination	Mg/kg	Max 1.0

11	Microbial Contamination		
11.1	● Total plate numbers	Colony/g	Max.10 ⁴
11.2	● Coliform	APM/g	< 3
11.3	● <i>Salmonella</i> / 25 ml	-	Negative
11.4	● <i>Staphylococcus aureus</i> / ml	-	Negative
11.5	● mold	-	Negative

Conclusion

The use of different enzymes in the processing of fish sauce provides a different quality of fish sauce .

BIBLIOGRAPHY

- Aniqoh, M. 2017. *Effect of Crude Papain Enzyme Administration (Crude Papain) and Fermentation Duration on the Quality of Lemuru Fish Soy Sauce (Sardinella longiceps)*. Thesis. Department of Biology, Faculty of Science and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang.
- AMGT. Briani, S. , Darmanto Y., Irianingsih, L. 2014. *Effect of Papain Enzyme Concentration and Fermentation Duration on the Quality of Trash Fish Sauce*. Journal of Processing and Biotechnology of Fishery Products Volume 3, Number 3, Year 2014, Pages 121-128
- Hasnan, M. 1991. *Effect of Papain Enzyme Use During The Fish Soy Sauce Hydrolysis Process*. Thesis. Faculty of Agricultural technology, Bogor Agricultural University, Bogor.
- Hidayat, N., Miniarti, I., Setyahadi, S., Pato, U., Susanti, E., Padaga, M.C., Purwandari, U. 2018. *Microbiology of the Agricultural Industry*. Universitas Brawijaya Press.
- Iskandar, T., Widyasrini, D.A. 2009. Effect of Bromelin Enzyme and Incubation Time on the Hydrolysis Process of Lemuru Fish into Soy Sauce. Buana Science, 9 (2): 183-189.
- Krisniawati, F., Ibrahim, R., Irianingsih, L. 2014. Addition of Different Enzymes to Fish Soy Sauce Processing From the Contents of the Stomach Cavity of Manyung Fish (*Arius thalassinus*) To Product Quality. *Journal of Fisheries Science*, 9(2): 24-32.

- Lehninger, A. L. 1997. *Fundamentals of Biochemistry*. Volume I (Revised Edition). Jakarta: Erlangga.
- Lopetcharat, K., Choi, Y. J., Park, J.W., Daeschel, M. A. 2001. Fish Sauce Product and Manufacturing: a review. *Food Reviews International*, 17, 65-68.
- Nurjanah., Baharuddin, T.I., Nurhayati, T. 2021. Collagen Extraction of Yellowfin Tuna Fish Skin (*Thunnus albacares*) Using Pepsin and Papain Enzymes. *Journal of Indonesian Fishery Products Processing*. 24(2): 174-187.
- Muliati, T. 1985. *Studying the Process of Making Mackerel Soy Sauce (Rastrelliger sp.) by Hydrolysis and Fermentation*. Scientific Papers. Faculty of Agricultural Technology Bogor. Bogor.
- Palmer, T. 1995. *Understanding Enzyme 3rd*. New York: Ellishorwood Publisher.
- Pramita, E.A., Finarti., Renol., Ula, R. 2020. Chemical and Organoleptic Quality of KiteFish Soy Sauce (*Decapterus Spp.*) With the Addition of Bromelin Enzyme. *Journal of Fisheries*, 2(2): 139-146.
- Purnomo, H. 1997. *Studies on the Protein Stability of Dried Meat and Jerky During Storage*. Malang: Faculty of Animal Husbandry, Universitas Brawijaya.
- Purnomo, E. 2005. *Utilization of Residual Materials in an Effort to Minimize Solid Waste*. Thesis. Master Program in Environmental Science, Postgraduate Program, Diponegoro University Semarang, Semarang.
- Reed, G. (1975). *Enzymes in Food Processing*. New York: Academic Press.
- Simanjorang, E. , N. Kurniawati, and Z. Hasan. 2012. *Effect of Papain Enzyme Use with Different Concentrations on the Chemical Characteristics of Soy Sauce Tutut*. *Journal of Fisheries and Marine Affairs*, 3 (4) pp 209-220
- Subroto, W.L, Hutu Cyl, N.N, Haerudin, and A. Purnomo. 1985. *Preliminary Research on Fish Sauce by Enzymatic Hydrolysis*. Fisheries Technology Research Report, BPTP. Jakarta.
- Suparman, A. 1993. *Making Fish Sauce by Means of a combination of Enzymatic Hydrolysis and Fermentation*. Thesis. Faculty of Agricultural Technology. Bogor Agricultural Institute, Bogor.

Warisno. 2003. *Papaya Cultivation*. Yogyakarta: Kanisius.

Winarno, F.G. 1993. *Introduction to Food Technology*. Jakarta: PT. Gramedia.

Wirahadikusumah. 1989. *Biochemistry : Proteins, Enzymes and Nucleic Acids Edition II*. ITB. Bandung. Pp : 43-69.

Yuniwati, M, et al. 2008. *Utilization of Papain Enzyme as a Coagulator in Cheese Making*. Journal of Science and Technology.

© GSJ