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FISH SAUCE FERMENTATION OF SKIPJACK FISH *(Katsuwonus pelamis* L.) AT VARIOUS INCUBATION TIMES AND CONCENTRATIONS OF CRUDE PAPAIN ENZYME

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ABSTRACT

This study aims to determine the differences in variations of crude papain enzyme concentration and optimal fermentation time on the microbiological quality of skipjack tuna (*Katsuwonus pelamis* L.) fish sauce. This research is an experimental study with two variables: 2 different enzyme concentrations (5% and 10%) and 3 different periods (3, 7 and 11 days). The microbiological tests include the *Escherichia coli* contamination test, total microbial test, and total lactic acid bacteria test. Physical tests include water content tests, pH tests, and organoleptic tests. The results showed that the enzyme concentration and fermentation time affected the microbiological quality of skipjack tuna sauce. The sample with an enzyme concentration of 10% and a fermentation time of 11 days was the best sample of skipjack tuna fish sauce because no *E. coli* bacteria were found (negative), the total BAL test result was 8.2 x 103 cfu/mL, and the TPC result was 7.1 x 103 cfu/mL which meets SNI standards for Fish Sauce No. 01-4271-1996. This sample also had the best organoleptic test results: a non-fishy aroma, savory taste, and dark brown color. Based on the pH test, samples with enzyme concentrations of 5% and 10% and a fermentation time of 3 days have a pH value that meets the requirements, namely 5.88 (pH requirement 5-6). The results of the water content test showed that all samples did not meet the standards.

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1. INTRODUCTION

Skipjack tuna is easy to find at Sadeng Beach, Gunungkidul and has a high protein content. Skipjack tuna causes an allergen in the form of an itchy tongue for some people who consume it. Consumption of skipjack tuna can be more optimal if it is produced in the form of fish sauce. Fish sauce is a fermented fishery product in liquid form, derived from the process of salting fish, and is brown to clear in color. Traditional fish sauce production has the disadvantage of requiring a long production process, namely several months.

The fish sauce production process can be accelerated by enzymatic fermentation. The addition of enzymes in making fish sauce, such as papain, bromelain, and ficin, or the addition of protease bacteria, can digest fish muscle tissue in a short time (Prasetyo, 2012). The crude papain enzyme has advantages in terms of cost, and the crude papain enzyme contains papain and chymopapain, which can break down the bonds in protein molecules (Aniqoh, 2017). The enzyme concentration and incubation time in making skipjack tuna sauce have not been found.

Research regarding various concentrations of the crude papain enzyme and the optimal incubation time in making skipjack tuna sauce needs to be carried out. Fish sauce that is good or fit for distribution from a microbiological perspective has a total microbial value below 104 and is

negative in the *Escherichia coli* test. Physical tests in pH, water content, and organoleptic tests are also carried out to support microbiological tests.

This research aims to determine the optimal incubation time and concentration of the crude papain enzyme in making skipjack tuna sauce. The results are useful as a reference in similar research regarding differences in papain enzyme concentration and effective fermentation time in making fish sauce. The public can also use the results of this research as a reference in the production of skipjack tuna sauce.

2. RESEARCH METHODS

2.1 Types of research

This experimental research used a randomized block design method.

2.2 Time and Place of Research

This research was carried out from September 2021 to March 2022. The research was carried out at the Microbiology Laboratory, FMIPA UNY.

2.3 Samples

The research sample was skipjack tuna, cleaned of organs, heads, and bones. The research sample was skipjack tuna meat produced into fish sauce by treating crude papain enzyme concentrations of 5 and 10% and incubation times of 3, 7, and 11 days.

2.4 Procedure

Skipjack tuna sauce was prepared by mixing 75 grams of clean skipjack tuna meat with 75 mL water and 15 grams (20%) of table salt. The fish sauce samples were treated with different concentrations of the crude papain enzyme, namely 3.75 grams (5%) and 7.5 grams (10%). Samples were incubated in airtight glass bottles at 50^{o}C with incubation periods of 3, 7, and 11 days. The sample was filtered and obtained with skipjack fish sauce.

The microbiological tests carried out on the skipjack fish sauce sample test were the *Escherichia coli* test, total microbial test, and total lactic acid bacteria test. The *E. coli* test was carried out by isolating fish sauce samples with a 10-2 dilution of 1 mL on EMBA (Eosyn Methylene Blue Agar) media using the pour plate method for 24 hours. The results are positive if metallic green colonies are on the EMBA media. The total microbial test was done by isolating fish sauce samples with a 10-2 dilution of 0.1 mL on NA (Nutrient Agar) media using the pour plate method for 24 hours. Then, the existing colonies are counted using a colony counter. The total lactic acid bacteria test was carried out by isolating fish sauce samples with a 10-2 dilution of 1 mL on MRSA (de Mann Rogosa Sharpe Agar) media using the pour plate method for 48 hours. Then, the existing colonies are counted using a colony counter.

The physical tests are pH, water content, and organoleptic tests. The pH test was carried out using a pH meter on a sample of skipjack tuna sauce. The water content test was carried out by pouring 10 mL of fish sauce sample into porcelain, drying it in an oven, and cooling it in a desiccator. The final weight minus the initial weight of the porcelain when empty is the percentage of the existing water content. Organoleptic tests were carried out by comparing existing fish sauce samples with commercially available fish sauce (Finna brand). Fish sauce that resembled control fish sauce in color, aroma, and taste was said to have the best value.

2.5 Data, Instruments, and Data Collection Techniques

Primary data was collected from the results of observations of microbiological tests, including the total microbial test, total lactic acid bacteria test, and *E. coli* test. Secondary data was collected from literature studies from relevant sources.

2.6 Data analysis technique

Data obtained from laboratory analysis is presented in a qualitative descriptive manner. The results of the microbial contamination test were compared with SNI Soy Sauce Ikan No. 01-4271-1996.

3. RESEARCH RESULTS AND DISCUSSION

3.1 The presence of Escherichia coli bacteria

Bacterial growth. shown by a shiny metallic green color on Eosyn Methylene Blue Agar (EMBA) media. The results of the skipjack tuna sauce test on 6 types of samples can be seen in Figure 1.

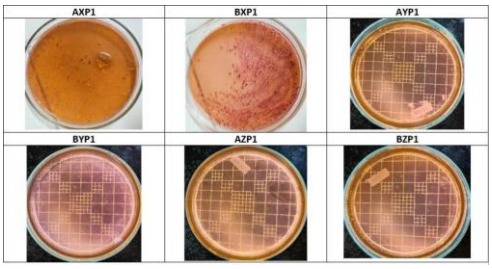

Figure 1. E. coli test for skipjack tuna sauce

Samples with code A are samples with a crude papain enzyme concentration of 5%, and samples with code B have a crude papain enzyme concentration of 10%. Samples with code X are samples with an incubation period of 3 days, samples with code Y are samples with an incubation period of 7 days, and samples with code Z are samples with an incubation period of 11 days. All samples were not found to be metallic green with black spots on EMBA (Eosyn Methylene Blue Agar) media. So, it can be concluded that the skipjack fish sauce in all samples did not contain the pathogenic *Escherichia coli* bacteria or meet the requirements for being fit for distribution.

3.2 Total Microbes

Based on SNI Fish Sauce No. 01-4271-1996, the maximum total microbes in the fish sauce was 104. The total microbes in the skipjack tuna sauce samples can be seen in Table 1.

Table 1. Total Microbial Results for Skipjack Tuna Fish Sauce (cfu/mL)

	3 days (X)	**7 days (Y)**	**11 days (Z)**
Papain 5% (A)	33 x 10^2	10 x 10^2	112 x 10^2
Papain 10% (B)	63 x 10^2	18 x 10^2	71 x 10^2

The observation showed that all samples showed a total microbial test result of no more than 104. All samples met the standards based on the total microbial test or total plate count. So it can be concluded that all fish sauce samples meet the requirements for being fit for distribution based on the total microbial test. Adding 15-20% salt can inhibit the growth of bacteria such as *Staphylococcus aureus* and other putrefactive bacteria. By adding 20% salt, spoilage bacteria in fish sauce can be prevented. Based on research by Rianingsih (2016) regarding differences in salt concentration in making fish sauce, the best results were obtained from adding 20% salt. Adding 15-20% salt can inhibit the growth of bacteria such as *Staphylococcus aureus* and other putrefactive bacteria.

3.3 Total Lactic Acid Bacteria

An increase in the total number of lactic acid bacteria indicated the success of the fish sauce fermentation process. Table 2 shows the results of total lactic acid bacteria measurement in 6 samples of skipjack tuna sauce grown on MRSA (de Mann Rogosa Sharpe Agar) media.

Table 2. Results of Total Lactic Acid Bacteria for Skipjack Tuna Fish Sauce (cfu/mL)

	3 days (X)	**7 days (Y)**	**11 days (Z)**
Papain5% (A)	5 x 10^2	16 x 10^2	85 x 10^2
Papain10% (B)	57 x 10^2	43 x 10^2	82 x 10^2

Based on the results of the total BAL test, the sample treated with an enzyme concentration of 5% for 7 days of fermentation had a decreased total amount of LAB compared to the fermentation time of 3 days. The total LAB increased in the sample with a fermentation time of 11 days. The total microbial yield tends to increase with the length of the fermentation process. There was a change in the structure of a community over some time interval. The high salt content in fish sauce will inhibit spoilage and pathogenic bacteria as fermentation progresses (Nur, 2010).

Samples treated with an enzyme concentration of 10% experienced an increase in total lactic acid bacteria along with the length of fermentation time used. All samples with 10% enzyme treatment yielded total acid bacteria higher lactate than fish sauce with 5% enzyme treatment. This is due to the theory that the higher the enzyme concentration, the better the fermentation process (Sari, 2018).

3.4 Water content

The water content measurement in fish sauce aims to determine the number of free water molecules in the sample. According to Sudarmadji et al. (1997), good soy sauce contains 6% protein, 1% fat, 9% carbohydrates, and 63% water content. The test results for the water content of skipjack tuna soy sauce can be seen in Table 3.

Table 3. Water Content Test for Skipjack Tuna Fish Soy Sauce

	3 days (X)	**7 days (Y)**	**11 days (Z)**
Papain5% (A)	80.8%	75.3%	76.6%
Papain10% (B)	79.2%	75.3%	75.3%

Based on the results of the essay water, all samples cannot be considered as good fish sauce. All samples of skipjack tuna sauce had water content far above the standard, namely 63%. The samples with the lowest water content were samples AY, BY, and BZ, namely 75.3%. The water content of the control sample was 62.6%. When the water content is higher, the growth of putrefactive microorganisms will be higher, resulting in accelerated decay (Azhari, 2018).

3.5 Acidity levels

Lactic acid bacteria produce lactic acid, which causes the fermentation conditions for skipjack tuna sauce to become sour. Table 4 shows the test results for samples of skipjack tuna sauce.

Table 4. pH Test for Skipjack Tuna Fish Sauce

	3 days (X)	**7 days (Y)**	**11 days (Z)**
Papain 5% (A)	5.93	6.24	6.06
Papain 10% (B)	5.88	6.14	6.01

The research results showed that the higher the concentration of crude papain enzyme given, the pH of the fish sauce would decrease. Meanwhile, the length of fermentation time shows that the pH at the beginning of fermentation was low, rose at a fermentation time of 7 days, and fell again at 11 days. According to Anjarsari (2010), when fish die, it will accumulate lactic acid and decrease the fish's physiological pH. This is indicated by the low pH at the start of fermentation.

According to Anggraini (2015), the longer the fermentation time, the longer the enzyme will work to carry out the hydrolysis process so that it will release H^+ ions, which lowers the pH. This is indicated by the decrease in acidity levels in fermentation with 7 and 11 days of incubation.

3.6 Organoleptic Test

Organoleptic tests include sensory or aroma tests, fish sauce color, and taste. The organoleptic test results are in Table 5.

Organoleptic tests on fish sauce samples include aroma, taste, and color. Based on the test results, the sample with an enzyme concentration of 10% and a fermentation time of 11 days had the best organoleptic results, which resembled the control sample. Based on existing data, the longer the fermentation time and the higher the enzyme concentration, the better the fish sauce results. The fish sauce's color difference can be seen in Figure 2.

The results of the organoleptic test show that the longer the fermentation and the higher the concentration of the crude papain enzyme, the darker the color of skipjack fish sauce will be. The sample with an enzyme concentration of 10% and a fermentation time of 11 days looks most similar

to the control sample, namely dark brown. The fish sauce aroma closest to the control sample is the sample with a fermentation period of 11 days with a more savory aroma and no foul smell. This sample also had the tastiest taste and was closest to the control sample.

Table 5. Organoleptic Test of Skipjack Tuna Fish Sauce

		3 days (X)	7 days (Y)	11 days (Z)
Papain 5% (A)	Aroma	Fishy	A bit fishy	Nofishy or not stings
	Flavor	Salty	Salty	Salty and tasty
	Color	Less clear	Deep orange	Chocolate
Papain 10% (B)	Aroma	Fishy	A bit fishy	Doesn't sting
	Flavor	Salty	Salty	Salty and tasty
	Color	Bright orange	Deep orange	Dark chocolate

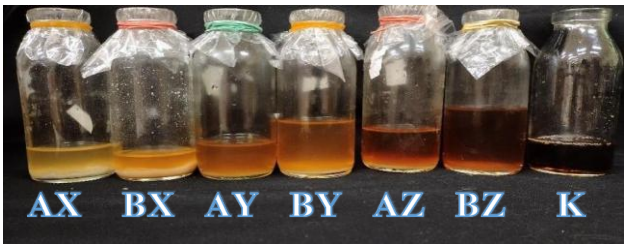

Figure 2. Organoleptic Test of Skipjack Fish Sauce

According to Ardiansyah (2015), the longer the fish sauce is fermented, the more reducing sugar and amino acid reactions are produced, which cause changes in the color and aroma of the fish sauce. According to Irianto (2012), increasing the amount of papain also encourages the formation of nitrogen compounds. Myofibril proteolysis produces protein fragments with shorter peptide chains. Proteins that break down into free amino acids will be converted into aroma-forming compounds.

4. CONCLUSIONS

Variations in the crude papain enzyme concentration influence the microbiological quality of skipjack tuna sauce. Fish sauce with a crude papain enzyme concentration of 10% has more optimal microbiological quality than fish sauce with a crude papain enzyme of 5%. Variations in the length of fermentation time for skipjack tuna sauce influence the microbiological quality of fish sauce. Fermenting skipjack tuna sauce for 11 days produced the best results in terms of microbiological tests. Further tests with higher enzyme concentrations and longer fermentation times are needed to determine the maximum environmental conditions in making skipjack tuna sauce. It is necessary to test skipjack fish sauce samples' protein and fat content with variations in enzyme concentration and fermentation time. Further testing is needed to see if the fish sauce from skipjack tuna can cause allergic reactions in people.

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