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# Chemical, Microbiological and Sensory Characteristics of Wader Fish (*Rasbora argyrotaenia*) Joruk During Fermentation

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**ABSTRACT**

*Joruk is a fermented fish product typical from East Ogan Komering Ulu, South Sumatra. This study aims to determine the chemical, microbiological, and sensory properties of the joruk during fermentation, as well as to get the right fermentation time for joruk production. Research was performed by preparing joruk using wader fish. Observations included pH, total lactic acid, water content, and total volatile base (TVB), total molds and yeasts, total microbes, and total lactic acid bacteria (LAB). Furthermore, the best treatment was observed for sensory properties and protein content. Results showed that during fermentation there was a decrease in the pH value, total mold, total microbes, and water content, while the total amount of lactic acid, LAB, and TVB increased. The best treatment was obtained on joruk stored on the 10$^{th}$ day of fermentation with a pH value of 6.33, total lactic acid 9.48%, water content 67.74%, TVB 93.88%, and total LAB, total mold/yeast, and total microbes was respectively 10.46, 7.21, and 12,13 log CFU/g. Sensory properties for raw joruk was brown with 6.2 scale, fishy (6.4), and intact appearance (5.1). Sensory properties for cooked joruk was brown (4.2), fishy (6.7), salty taste (3.1), sour taste (8.4), and incomplete appearance (2.8).*

## 1. INTRODUCTION

The process of preserving fish by fermentation will involve chemical and microbial enzymatic processes during the fermentation process which will ultimately determine the microbiological and chemical characteristics of fermented fish. Joruk is a fermented fish product originating from East Ogan Komering Ulu Regency, South Sumatra. The raw materials used in making joruk are wader freshwater fish, salt, rice, and palm sugar. During the fermentation process there are several factors that affect the fermented fish products

produced. These factors are raw material variations (Peralta & Serrano, 2014), amount of salt (Andarti & Wardani, 2015; Moede *et al*., 2017; Pratomo *et al*., 2020), and fermentation time (Suyatno *et al*., 2015). Optimization of the process of making joruk can be done by adding palm sugar of 20% w/w of fish weight, salt of 10% w/w of fish weight, and rice of 20% w/w of fish weight (Koesoemawardani *et al*., 2019a). Joruk can be consumed after being incubated for 1-2 weeks, with various products, so it is necessary to know the exact fermentation time to produce quality joruk. Fermentation time is a variable related to the microbial growth phase during the fermentation process so that it will affect the fermentation results. Several previous studies have shown that the duration of fermentation affects the physical, chemical, microbiological and sensory characteristics (Andarti & Wardani, 2015; Dewi *et al*., 2014; Moede *et al*., 2017; Mulyani *et al*., 2021; Pratomo *et al*., 2020; Suyatno *et al*., 2015).

During the fermentation process changes in physical, chemical and microbiological properties occur. Controlled protein hydrolysis in the manufacture of fermented fish products can prevent spoilage, produce pastes and amino acids and peptides that have a "meaty" and "savory" flavor (Steinkraus, 2002). Pratomo *et al*. (2020) stated that during the fermentation process fermented fish products gave different chemical and microbiological properties in each observation period. In addition to chemical and microbiological properties, fermentation also affects the sensory properties of the product (Adawyah *et al*., 2021; Mahulette *et al*., 2017). Koesoemawardani *et al*. (2019b) stated that the joruk observed at 0 days, 1 week and 2 weeks formed 17 amino acids with glutamic acid predominating, the fatty acids formed were saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, and Volatile compounds are formed including hydrocarbons, carbonyls, alcohols, oxides, carboxylic acids, nitrogen and esters. It was further explained that joruk with storage of 0, 1 week and 2 weeks experienced a change in the amount of amino acids, fatty acids and volatile compounds formed.

The formation of these simple compounds comes from the process of hydrolysis of autolytic enzymes and microorganisms. It is important to know the changes in sensory, chemical and microbiological properties that occur during the fermentation of joruk, so that the appropriate fermentation time can be determined to produce good quality and safe to consume joruk. Therefore, this study aims to determine the chemical, microbiological and sensory characteristics of joruk during fermentation with observations up to the 14th day, as well as to obtain the proper fermentation time in the process of making joruk.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The main ingredients used are wader fish (*Rasbora argyrotaenia*), coarse salt, palm sugar obtained from the local market in Bandar Lampung. The chemicals used are distilled water, 0.85% physiological salt, 70% alcohol, MRS-A media, PCA media, Potato Dextrose Agar (PDA) media and other chemicals. The tools used were a pH meter (Lovibond), analytical balance (ES model 08152-CA4ZA10A-A), Kjeldahl flask, Waring blender (Philips) and other analytical tools.

Fermentation was carried out within three replicates. Observations were made periodically based on the fermentation time, namely on day 0, 2, 4, 6, 8, 10, 12 and 14.

The qualitative data obtained was analyzed descriptively and presented in graphical form (Arifin, 2010). Observations made included total lactic acid bacteria, total microbes, total mold, water content, pH value, total lactic acid, total volatile base, sensory test, and protein content.

### 2.2. Joruk preparation

Whole wader fish (without removing the entrails) was cleaned using running water, then drained to remove excess water. Liquid palm sugar was prepared with a ratio between palm sugar and water is 3:1. In this case, 450 g of sliced palm sugar and 150 mL of water was mixed and heated until boiling for ± 5 min. Liquid palm sugar was let to cool off. Then, the wader fish was weighed as much as 100 g and put in a small 150 mL jar, added 20% (w/v) of liquid palm sugar, 10% (w/w) rice, and 10% (w/w) salt. The percentage of liquid palm sugar, rice and salt added was based on the weight of the fish (w/w). All ingredients was mixed by stirring evenly, stirring should be conducted in such a way that the rice did not crumble because it was desirable that the rice was visible until the end of fermentation. The jar that already contains fish, salt, rice, and palm sugar was then closed tightly and then put in a larger jar, put a lit candle and close it tightly. Anaerobic conditions were indicated by an extinguished candle. The sample was fermented for 14 days, because it can be consumed after 1-2 weeks of fermentation. Observations were made every 2 days starting on days 0, 2, 4, 6, 8, 10, 12, 14.

### 2.3. Analysis and Measurement

#### 2.3.1. Total lactic acid bacteria (Fardiaz, 1993)

The total lactic acid bacteria (LAB) was tested using the plate count method (Fardiaz, 1993). Calculation of the total LAB was begun with preparing a sample of the joruk which has been ground so that it is in the form of coarse crushed fish. The joruk sample was then taken as much as 1 gram and diluted with 9 ml of diluent to obtain a $10^{-1}$ dilution. From the $10^{-1}$ dilution, 1 ml was diluted with 9 ml of the second diluent to obtain a $10^{-2}$ dilution, and so on until a $10^{-8}$ dilution was obtained. After that, from the last 3 dilutions ($10^{-6}$ to $10^{-8}$) were taken for plating.

Plating was carried out with MRS agar (MRSA) culture medium. The culture media was prepared by dissolving 67 g MRSA (Baird-Parker Agar Base CM0275B OXOID) in 1000 ml of distilled water. Then the MRSA+1% $CaCO_3$ solution was sterilized by autoclaving at 121 °C for 15 minutes. Sampling was carried out by taking 1 ml of each sample resulting from the dilution and putting it into a petri dish containing ±25 ml of MRSA+1% $CaCO_3$ and then evenly or like the shape of the number 8 on the table so that it was homogeneous. The sampling was carried out in duplicate from the $10^{-6}$ to $10^{-8}$ dilution. After the media in the cup had solidified, the dish was incubated at 37 °C and incubation period of 48 h by placing the dish upside down.

#### 2.3.2. Total microbes (BSN, 2015a)

The total microbial test was carried out based on the modified SNI 2333.3.2015. A total of 1 g of sample was diluted with 9 ml of physiological salt (0.85% NaCl) which had been sterilized. This dilution is calculated as a $10^{-1}$ dilution. Dilution was continued gradually until a $10^{-12}$ dilution was obtained. A total of 1 ml of sample from each dilution ($10^{-10}$, $10^{-11}$, and $10^{-12}$) was pipetted and put into each sterile petri dish, then ±15 ml of sterile PCA was poured (done in duplicate for each dilution) and shaken evenly or like the

shape of the number 8 on the table. After the agar media has solidified, the dish was wrapped in paper and then incubated upside down at 35°C ± 1°C for 48±2 h. The total number of microbes was counted (scale 25–250 colonies) and expressed in CFU/g.

### 2.3.3. Total mold (BSN, 2015b)

The calculation of yeast mold numbers referred to modified SNI 2332.7:2015. A total of 1 g of sample was diluted with 9 ml of physiological salt (0.85% NaCl) which had been sterilized. This dilution is calculated as dilution $10^{-1}$. Subsequent dilutions were carried out gradually until $10^{-6}$ dilution was obtained. A total of 1 ml of sample from each dilution $10^{-4}$, $10^{-5}$, and $10^{-6}$, was made into a pour plate on PDA medium. It was then incubated in an incubator at 25 °C for 3-5 days in an upside down position. Calculations were made with the condition that the number of colonies in each petri dish was between 10-150 colonies, and that no colonies covered more than half the area of the petri dish.

### 2.3.4. Moisture content (AOAC, 2005)

Water content was measured using the gravimetric method. The test procedure begun with drying the crucible in an oven at 105 °C for 1 hour. Then the crucible is cooled in a desiccator for 15 min and weighed to obtain weight (A). Furthermore, the joruk sample in the form of coarse crushed fish was mashed using a blender, then taken as much as 1 to 2 grams and was put into a dry crucible whose weight is known (weight B). The sample in the porcelain cup was dried using an oven at 105 °C for 6 h, after which it was cooled in a desiccator for 30 min and then weighed again and the weight was obtained (C). The water content (KA) was calculated according to:

$$KA = \frac{B - C}{B - A} \times 100\% \quad (1)$$

### 2.3.5. Measurement of pH (AOAC, 2005)

pH measurement was carried out using a pH meter. The sample is weighed as much as 5 g and then put into 10 ml of distilled water, then homogenized. Before use, the pH meter was calibrated for 15-30 minutes until stable. The electrodes were rinsed with distilled water and dried with tissue paper. After that, the electrodes were dipped into the fish extract media. The electrode was left immersed for a while until a stable reading was obtained. Prior to sample measurement, the pH-meter was standardized with phosphate buffer pH 7 and pH 4.

### 2.3.6. Total lactic acid (AOAC, 2005)

Determination of total lactic acid was carried out using the titration method (AOAC, 2005). A sample of 10 g was crushed with a blender, then put into a 250 ml Erlenmeyer. Aquades was added to the Erlenmeyer flask to the right mark, then homogenized and filtered. Into 25 ml of filtrate was added 2-3 drops of phenolphthalein indicator, then titrated with 0.1 N NaOH solution until a pink color was formed. Total acid as a percent of lactic acid (LA) was calculated using the following formula:

$$LA = \frac{V \; x \; N \; x \; FP \; x \; 90}{B} \times 100\% \quad (2)$$

where V is volume of NaOH solution (ml), N is the normality of NaOH solution (in this case 0.1 N), B is sample weight (g), FP is dilution factor (in this case 0.04, namely 10 g in 250 ml or 1g in 25ml), and 90 is molecular weight of lactic acid,

### 2.3.7. Total volatile base (BSN, 2009)

The refined sample was weighed as much as 10 g with a beaker, then 90 ml of 7% TCA (Trichloroacetate) was added. The sample was homogenized using a homogenizer for 2 min. Then the sample was filtered using filter paper to get the filtrate. A total of 50 ml of the filtrate sample was put into the distillation tube, then added 2-3 drops of phenolphthalein indicator and added a few drops of anti-foaming silicon. The distillation tube was installed and 20% NaOH was added until it changed color to red. An Erlenmeyer glass containing 100 ml of 3% $H_3BO_4$ (boric acid) and 3-5 drops of Tashiro indicator was prepared. After that the sample was distilled for ±10 min to obtain 100 ml of distillate so that the final volume reached ±200 ml of green solution. A blank solution was prepared by replacing the sample with 50 ml of 7% TCA. The sample and blank distillate solutions were then titrated using 0.02 N HCl solution until they turned to golden yellow again. The titration results were recorded and the total volatile base was calculated through Equation (3):

$$\text{TVB (mg N/100 g)} = \frac{(Va-Vb) \times N\,HCl \times Ar\,N \times FP \times 100}{W} \text{ x } 100\% \quad (3)$$

where Va is volume of HCl solution in sample titration, Vb is volume of HCl solution in blank titration, Ar N is Atomic weight of nitrogen (14.007), FP is dilution factor, and N HCl is normality of HCl (0.02 N).

### 2.3.8. Sensory test (Setyaningsih *et al.*, 2010)

Sensory properties of joruk was assessed by observing the sensory appearance, aroma, and taste. The test method used is descriptive testing method (Setyaningsih *et al.*, 2010). There were 8 panelists selected from those who have or frequently consume fermented fish (such as “becakam” and “rusip”). The sensory properties testing step is carried out in two stages. In the first stage, a panel discussion was conducted to formulate and equate perceptions regarding the sensory attributes, including color, aroma, taste, and appearance of the joruk to be tested. Then in the second stage the panelists assessed the joruk sensory attributes. Panelists determine the intensity of each parameter tested by using the 1-10 scale lines that have been provided on the questionnaire sheet.

### 2.3.9. Protein levels (Sudarmaji *et al.*, 2010).

Testing for protein levels was carried out using the micro Kjeldahl method (Sudarmaji et al., 2010). The principles of this analysis include destruction, distillation, and titration. The sample of crushed joruk was taken as much as 0.5 - 1 gram and put into a 100 ml Kjeldahl flask. Next, 1 g of $K_2S$ or anhydrous $Na_2SO_4$, 10-15 mL of $H_2SO_4$, 0.1 – 0.3 gram of $CuSO_4$ were added to the sample and then the destruction is carried out on an electric heater in a fume hood. The destruction process was terminated when the liquid becomes clear. Then the mixture was allowed to cool and then added by 100 mL of distilled water and 45% NaOH until the mixture become alkaline. The sample was immediately distilled until all the ammonia has evaporated. The distillation drops were

collected in an Erlenmeyer flask containing 25 mL of 0.1 N HCl which had been given a few drops of 1% PP indicator. The distillation was ended after 150 mL of the distillate were accommodated or after the distillate was no more alkaline. The distillate was then titrated with 0.1 N NaOH solution. The protein content contained in the sample can be calculated using the formula:

$$\%N = \frac{(\text{ml NaOH blank} - \text{ml NaOH sample})}{\text{g sample} \times 10} \times N\ NaOH \times 14.008 \quad (3)$$

where N NaOH is normality of NaOH, 14.008 atomic weight of nitrogen, and 6.25 is conversion factor.

## 3. RESULTS AND DISCUSSION

### 3.1. pH

The results of the analysis of the degree of acidity (pH) in the fermented wader fish joruk showed that during the fermentation process the pH value decreased by 23.14% (Figure 1), starting from day 0, the joruk pH reached 7.91 and decreased to 6.08 on the 14th day of fermentation. Changes in pH of joruk began with a decrease in pH on the 2nd day, and from the 8th to the 14th day it tended to be stable without changing. The decrease in pH value is caused by lactic acid fermentation carried out by lactic acid bacteria (LAB). Lactic acid bacteria are able to convert carbohydrate sources into lactic acid, volatile acids, alcohols, and esters which can reduce pH value of the product (Desniar *et al*., 2012; Yuliana, 2014), as a consequence of increasing total acid. Furthermore, Desniar *et al*. (2012) confirmed that the decrease in pH is because carbohydrates are hydrolyzed into glucose, then by LAB, the glucose is used as an energy material for its activities and produces acid. Alvarado *et al*. (2006) stated that the decrease in pH value is caused by acidification activity that is related to the amount and type of organic acid produced, and varies. LAB produce organic acids such as lactic acid, acetic acid and other secondary metabolites. This was shown in the growth of lactic acid bacteria in this study which increased during the fermentation of joruk.

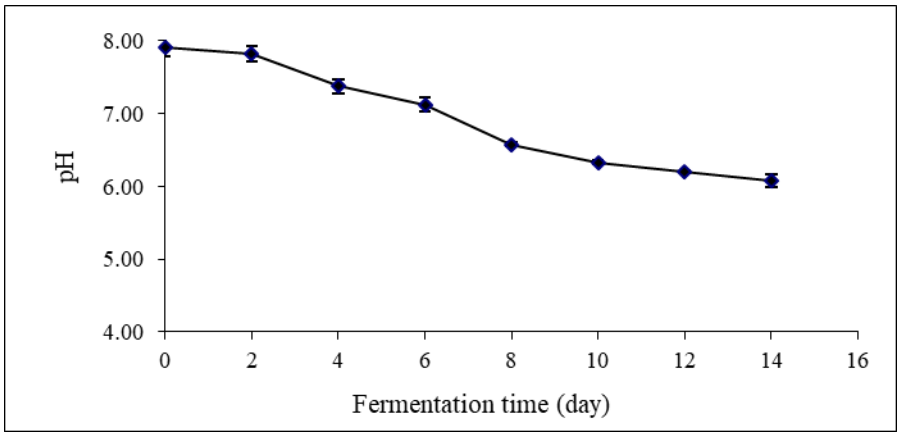


**Figure 1**. Changes in pH of joruk during 14 days of fermentation

The increase in lactic acid will be followed by an increase in H+ concentration which means a decrease in pH. This is due to the fermentation process by lactic acid bacteria to produce lactic acid which is characterized by a decrease in the pH value (Ahillah *et al*., 2017). According to Koesoemawardani *et al*. (2019b), changes in pH during the fermentation of slops occur due to the metabolic processes of carbohydrates

originating from rice and sugar which have an impact on the pH of the environment. This is in line with Wikandari *et al*. (2011), Hadiyanti & Wikandari (2013), and Mani (2018) who state that a decrease in pH value and an increase in total acid begins with the process of converting carbohydrates which are hydrolyzed into glucose and then glucose will be used by lactic acid bacteria as a carbon source to carry out their activities, which produces organic acids such as lactic acid, acetic acid, so as to lower the pH.

### 3.2. Total lactic acid

The results of analysis of total lactic acid during the fermentation of the wader fish joruk showed that during fermentation there was an increase in total lactic acid (Figure 2). On day 0 of the joruk fermentation, the measured total lactic acid was 0.9% and there was an increase to 11.17% on the 14$^{th}$ day of fermentation.

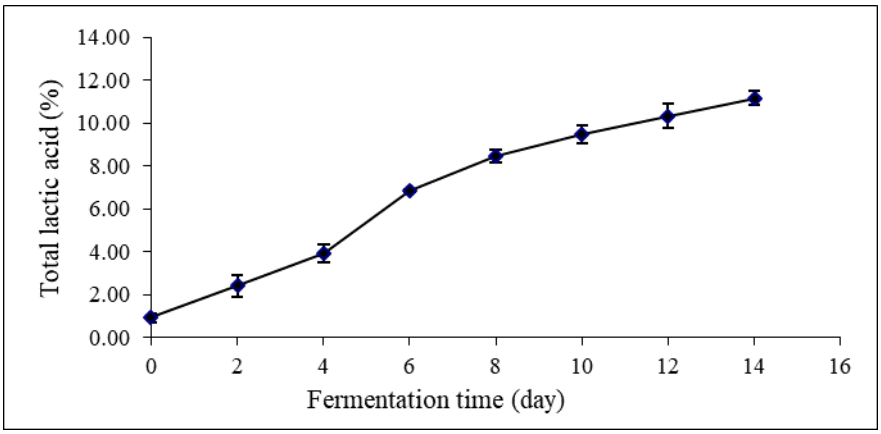


**Figure 2.** Changes in total joruk lactic acid during 14 days of fermentation

The increase in total acid is due to an increase in lactic acid, which is followed by an increase in the breakdown of sugar by lactic acid bacteria. The total lactic acid value is inversely proportional to the pH of the product, the higher the total lactic acid value, the lower the pH value (Desniar et al., 2013). Carbohydrates contained in this process will be hydrolyzed into glucose, then lactic acid bacteria use the glucose as energy for their activities and produce acid (Desniar *et al*., 2012; Nuraini *et al*., 2014). The increase in total acid begins with the process of converting carbohydrates which are hydrolyzed into glucose and then glucose will be used by lactic acid bacteria as a carbon source to carry out their activities, which produce organic acids such as lactic acid and acetic acid (Chadong *et al*., 2015; Kalista *et al*., 2012; Mani, 2018).

The total lactic acid of the joruk until the 14$^{th}$ day of fermentation reached 11.17%, while the total lactic acid in previous studies was only 6.92% (Koesoemawardani *et al*., 2019b) and 2.97% (Koesoemawardani *et al*., 2021). The difference is caused by differences in the number of LAB that play a role during the fermentation process.

### 3.3. Total lactic acid bacteria

The analysis showed that total lactic acid bacteria (LAB) during the wader fish joruk fermentation that on day 0 it was 9.19 log CFU/g and at day 2 it reached 9.34 log CFU/g. This indicates that LAB growth was at slow phase. In this phase, LAB makes adjustments towards its environmental conditions. The increase in total LAB occurred

after day 2 to day 6, indicating the growth of LAB was in the logarithmic growth phase, where LAB grew rapidly until it reached 10.46 log CFU/g. Then during day 8 to day 14 the growth of LAB underwent a stationary phase up to 10.30 log CFU/g. In this phase, LAB did not experience growth, but on the graph it tends to decrease towards the death phase. The death phase occurs due to competition for nutrients, and the accumulation of lactic acid metabolites which contributes to suppressing the number of LAB (Yuliana, 2014).

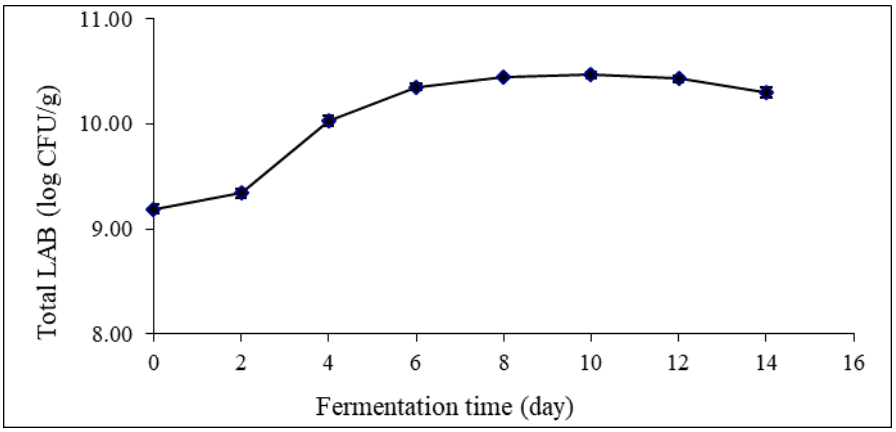


**Figure 3.** Changes in total LAB of joruk during 14 days of fermentation

Carbohydrates contained in this process will be hydrolyzed into glucose, then LAB will use the glucose as energy for their activities and produce acid (Desniar *et al*., 2012). Nuraini *et al*. (2014) added that the addition of carbohydrates serves as a carbon source for LAB which will be broken down into lactic acid. Rhee *et al*. (2011) states that the shelf life of fish can be extended through lactic acid fermentation by adding rice, flour, millet, sugar, or syrup as a source of carbohydrates, whereas according to Fernandes (2009), providing a carbohydrate source in shrimp fermentation allows LAB to ferment. Khairina *et al*. (2016) stated that rice (carbohydrates) was degraded into simple compounds such as acids which resulted in a decrease in the total starch value.

Salt (NaCl) acts as an inhibitor or selects for spoilage and pathogenic bacteria so that LAB dominate more at the beginning of fermentation (Koesoemawardani *et al*, 2021). According to Subagiyo *et al*. (2015) salt levels affect the rate of lactic acid production which is followed by an increase in the growth of LAB. Yuktika *et al*. (2017) stated that NaCl breaks down into $Na^+$ and $Cl^-$ ion molecules. The $Na^+$ ions required by LAB as one of the factors supporting their growth are small, but the amount of $Cl^-$ ions which cause the availability of water in the material to decrease is also small so that free water can be used by microbes for their growth.

The longer the fermentation time, the number of LAB increases. The increase in total LAB during the fermentation process causes an increasingly acidic condition which helps in selecting the number and type of microbes present in the fermented fish. Microbes that are not able to stand with acidic conditions will die while microbes that can withstand acidic conditions will grow well (Putri *et al*., 2014).

### 3.4. Total mold

Mold analysis during the joruk fermentation showed that during the fermentation there was a decrease in the total mold by 0.29 log CFU/g (Figure 4). The total log

number of mold decreased from 7.41 log CFU/g (day 0) to 7.12 log CFU/g (day 14). Other study using *cincalok* (small shrimp fermented product from West Kalimantan) reported similar result with the highest total mold reached log 6.0-7.2 CFU/g (Khairina *et al*. 2016). During the joruk fermentation, mold is another microorganism that grow besides LAB. Mold grows in the joruk fermentation, presumably because there is space in the jar during its preparation. This situation can cause mold to grow in the joruk fermentation. Joruk fermentation is a type of spontaneous fermentation so that the types of microbes that grow are diverse, such as LAB, mold and yeast (khamir). During the fermentation, changes in the growing of microorganism groups include yeast, molds, and LAB (Koesoemawardani *et al*., 2019a). This is in line with research conducted by Dewi *et al*. (2014) which states that mold can grow in acidic conditions.

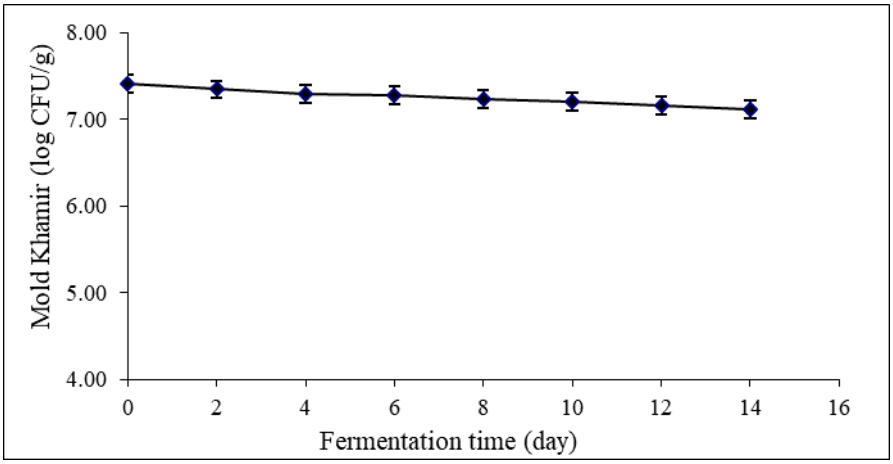


**Figure 4.** Total change of mold during 14 days of joruk fermentation

Temperature can affect mold growth. The optimum temperature for mold growth ranged from 25-30 °C, while in this study the joruk was stored at room temperature (25 -30 °C). The molds grew well at a pH of 2-8.5, while the pH in this study ranged from 6.08-7.83, so the molds still grew well. Meanwhile, oxygen availability of the joruk still supports the growth of the mold because the conditions of the joruk container in this study are facultative anaerobes, so the mold still contains oxygen even in small amounts. Mold nutrients are in the form of carbon sources, nitrogen sources, energy sources and growth factors (minerals and vitamins). All of these nutrients are still present in the joruk, as it is known that the raw materials for making joruk are wader fish, palm sugar, rice and salt.

The addition of palm sugar and rice in the joruk fermentation can also affect the growth of mold. Nurhartadi *et al*. (2018), explained that an excess proportion of carbon sources causes environmental conditions to become hypertonic so that fluids in the microorganism cells flow out resulting in dehydration and cell shrinkage (plasmolysis). This condition supports the growth of mold. Susilowati *et al*. (2014) stated that the reduction in total mold in rusip fermentation occurred in microaerophilic conditions (with little oxygen). This condition causes inhibition of mold growth in rusip due to the reduced amount of oxygen contained in rusip during fermentation. Mold generally grows in an aerobic atmosphere. The number of molds in this study ranged from 7.12 log CFU/g to 7.41 log CFU/g, while the total joruk mold in the study of Koesoemawardani *et al*. (2021) reached 4.27 log CFU/g. This difference is due to the

difference in the amount of material used in the preparation of joruk which also causes differences in the presence of microbes in the joruk. Mahulette *et al*. (2017), Novianti (2016), and Oktarianto & Widawati (2017) mention the type and habitat of fish affect the number of microbes in fermented fish products.

### 3.5. Total microbes

The microbial analysis during the wader fish joruk fermentation showed that during the fermentation there was a decrease in total microbes of 0.41 log CFU/g (Figure 6). The total log number of microbes decreased from 12.46 log CFU/g (day 0) to 12.05 log CFU/g (day 14). The decrease in total microbes is thought to occur due to the growth of LAB which dominates during fermentation which can suppress microbial growth, so that microbes cannot compete and die. The pattern of total microbial growth during the wader fish joruk fermentation can be seen in Figure 5.

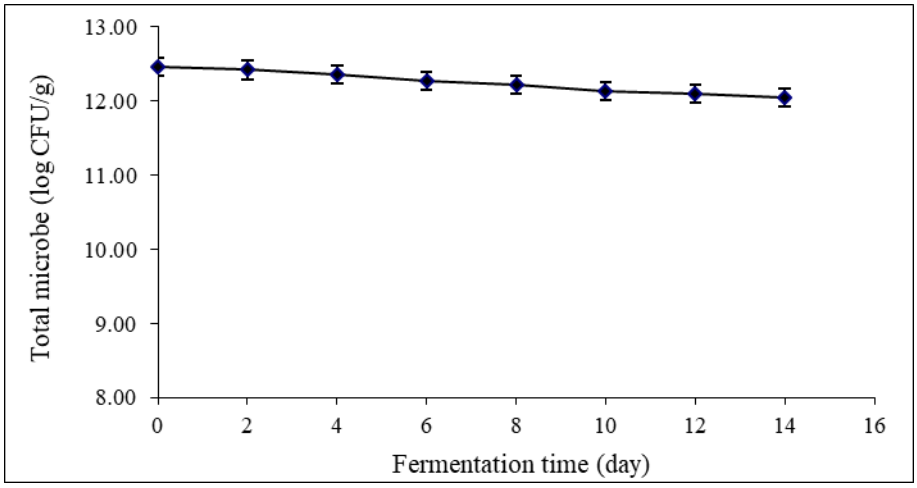


**Figure 5.** Changes in total microbes of joruk during 14 days of fermentation

An increase in the total LAB will cause the total microbes to decrease (Koesoemawardani, 2010). This is because LAB produce secondary metabolites in the form of lactic acid and acetic acid which can lower the environmental pH (Kalista *et al*., 2012). Some microbes will be inhibited by the low pH of the environment. According to Fernandes (2009) providing a carbohydrate source in shrimp fermentation allows the LAB to ferment and produce a low pH which contributes to inhibiting the growth of spoilage and pathogenic bacteria. LAB is able to competitively grow than other bacteria so that it becomes dominant bacteria under mineral stress conditions, such as higher NaCl salts (Singracha *et al*., 2017). LAB is able to produce lactic acid and bacteriocins which inhibit the growth of competing bacteria (Perez *et al*., 2014). The low degree of acidity causes non-acid-resistant competitive bacteria to find it difficult to maintain their energy cycle while at the same time making these bacteria run out of cellular energy quickly (Yang *et al*., 2014). This causes the total microbes and total mold to decrease along with the increasing number of LAB. According to Sulistijowati (2012), the decrease in total microbes during fermentation occurs due to reduced nutrients and the formation of metabolic products that tend to be toxic to microbes. Suprihatin (2010) added that microbial death is because nutrients in the medium have run out and energy reserves in cells have run out.

### 3.6. Water content

During the joruk fermentation the water content decreased by 4.94% from 70.04% on the 0th day to 66.58% on the 14th day (Figure 6). The addition of sugar and salt causes the percentage of solids to increase while the percentage of water decreases. Salt added to the fermentation process will attract water in the fish's body with the principle of osmosis so that the water content in the material will decrease. According to Thariq *et al*. (2014) salt has a higher osmotic pressure than fish, hence it will absorb the water contained in the fish until an equilibrium occurs. In addition, Kalista *et al*. (2012) stated that the addition of salt can inhibit microbial growth and affect water activity in the material. Sugar has the capacity to bind water in foodstuffs due to the presence of hydrogen bonds which result in reduced water activity in foodstuffs (Buckle *et al*., 2009). Restu (2017) stated that carp fermented product (*wadi*) with the addition of sugar has a lower water content compared to that of without sugar.

According to Koesoemawardani *et al*. (2016), similar to salt, the sugar contained in joruk also has the ability to bind water. Muchtadi & Sugiyono (2012) explained that sugar can bind free water in food, so it can be used as a preservative. The breakdown of carbohydrate elements in palm sugar and rice can also cause the binding of water molecules to reach a saturation point at high sugar concentrations (Koesoemawardani *et al*., 2019a).

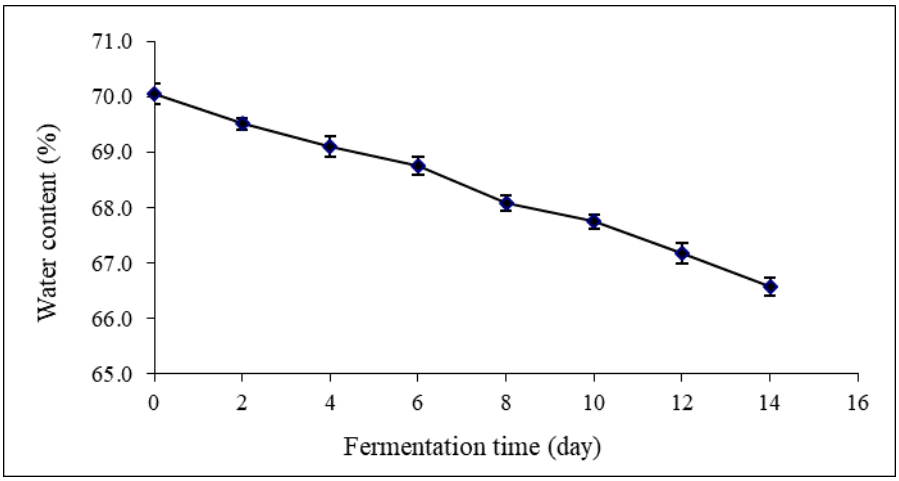


**Figure 6.** Changes in soil water content during 14 days of fermentation

### 3.7. Total volatile base (TVB)

During the fermentation of the wader fish joruk there was an increase in the total volatile base (TVB) as presented in Figure 7. On the 0th day of the joruk fermentation, the TVB value was 23.29 mgN/100 g and it increased to 146.44 mgN/100 g on the 14th day of fermentation. The TVB value for joruk which was stored for 14 days was higher than the TVB for *cincalok* which was stored for 14 days reaching 131.79 mgN/100 g (Novelia *et al*., 2020), but the TVB for joruk was lower than *ronto* (rebon shrimp fermented product from South Kalimantan) stored for 16 days which was 196.77 mg N/100 g (Khairina *et al*., 2016).

Based on the results obtained, the longer the fermentation time, the TVB value will increase. Mahamudin *et al*. (2016) revealed that the TVN (Total Volatile Nitrogen) value of a sample is generally used as an indicator of fish damage, and TVB is part of TVN.

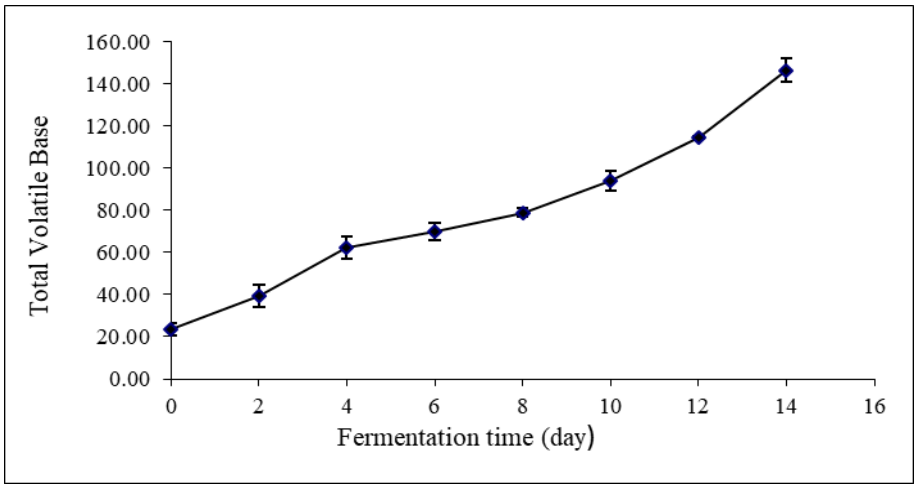


**Figure 7.** Changes in TVB of the joruk during 14 days of fermentation

Fish TVN consists of TVB which is contributed by ammonia, dimethylamine and trimethylamine plus other nitrogen compounds (Jay, 2000). The higher the TVN value, the higher the level of fish damage. This is in line with Karneta *et al*. (2013) stated that the TVN of *pempek lenjer* increased with longer storage. Karungi *et al*. (2004) also stated similarly that an increase in TVB values during storage due to degradation of protein and its derivatives produces a number of volatile bases such as ammonia, $H_2S$, and trimethylamine which smells bad. Farahita *et al*. (2012) stated that the increase in TVB levels occurred due to the work of proteolytic enzymes which break down proteins into short peptide bonds and amino acids which then become amine and ammonia compounds which give a sharp odor and a distinctive taste. On the 10$^{th}$ day TVB achieved 93.88 mgN/100 g, while in the study of Koesoemawardani *et al*. (2019b) and Koesoemawardani *et al*. (2021) TVB of joruk were 84.55 mg/100g and 153.05 mgN/100g, respectively. The maximum TVB value for fresh fish products and suitable for consumption is 300 mg N/100 gram sample, while the acceptable limit for TVB-N values for salted fish products is 200 mg N/100 g sample (Connell, 1995). The TVB value of joruk is 146.44 mgN/100 g indicating that joruk with a fermentation time of 14 days is still suitable for consumption. The Directorate General of Fisheries states that the TVB limit for fermented products that are fit for consumption is 300 mgN/100g (Direktorat Jendral Perikanan, 2000).

### 3.8. Selection of the best treatment

Determination of the best treatment in this study was based on microbiological properties, namely total LAB, total mold/yeast, and total microbes. In addition, other parameters can be observed in chemical properties like pH value, total lactic acid, and water content. The best products can be seen in Table 1.

Koesoemawardani *et al*., (2016; 2021) and Koesoemawardani *et al*. (2019a) optimized the use of joruk raw materials by adding sugar, rice and salt, while the best fermentation time had not been carried out. In those study the best treatment was chosen based on microbiological properties, namely the highest total LAB of 10.46 log CFU/g on the 10$^{th}$ day of fermentation. In addition, sensory tests and protein levels of the wader fish were carried out. The analysis revealed protein content 8.45% in the 10$^{th}$ day of joruk fermentation. The value of protein content is not much different when

compared to snakehead fish *bekasam* with a protein content of 8.65% to 10.6% (Suyatno *et al*., 2015), and seluang fish *bekasam* of 6.20% to 14.68% (Lestari *et al*., 2018).

Table 1. Determination of the best treatment for joruk product

| Parameter | Values on $10^{th}$ day | Literatures | References |
|---|---|---|---|
| pH | 6.33 | 6 – 9 | (Ahillah *et al*., 2017) |
| Total lactic acid (%) | 9.48 | 1.04 – 10.80 | (Nofiani & Ardiningsih, 2018) |
| Total LAB (log CFU/g) | 10.46 | 10.19 | (Koesoemawardani *et al*., 2016) |
| Total mold/yeast (log CFU/g) | 7.21 | 4.16 – 4.44 | (Koesoemawardani *et al*., 2019b) |
| Total mikrobes (log CFU/g) | 12.13 | 11.36 – 12.20 | (Koesoemawardani *et al*., 2016) |
| Water content (%) | 67.74 | 56.23 – 64.14 | (Koesoemawardani *et al*., 2016) |
| TVB (mgN/100g) | 93.88 | 84.55 – 153.05 | (Koesoemawardani *et.al*., 2019b; 2021) |

Sensory properties of joruk was tested at the optimum fermentation time ($10^{th}$ day). Descriptive sensory testing uses a qualitative method of focus group discussion (FGD) techniques (Setyaningsih *et al*., 2010). The testing process includes two stages, namely the formulation of sensory quality attributes for wader fish joruk (Table 2) and an assessment of sensory attributes (Table 3). Cooked joruk is joruk that has been cooked with the addition of spices such as shallots, garlic and chilies.

**Table 2.** The results of the formulation of quality attributes for wader fish joruk

| Parameter | Joruk type | |
|---|---|---|
| | Uncooked joruk | Cooked joruk |
| Color | Brown to dark brown | Brown to blackish brown |
| Aroma | Not fishy to very fishy | Not typical joruk to very typical joruk |
| Taste | Not tested | Not salty to salty |
| | | Sour to very sour |
| Appearance | Not intact to very intact | Not intact to very intact |

**Table 3.** Descriptive sensory properties score of wader fish joruk based on FGD

| Parameter | Perlakuan | |
|---|---|---|
| | Uncooked joruk | Cooked joruk |
| Color | Brwon (6,2) | Brown (4,2) |
| Aroma | Fishy (6,4) | Typical joruk (6,7) |
| Taste | Not tested | Salty (3,1)<br>Highly sour (8,4) |
| Appearance | Intact (5,1) | Not intact (2,8) |

The results of the qualitative descriptive sensory test (Table 3) show that the uncooked joruk of wader fish has quality characteristics, namely brown in color with a color intensity on the scale line of 6.2. Uncooked joruk has a browner color than cooked wader fish joruk which has a brown color with color intensity of 4.2 on the scale line. The results of research conducted by Koesoemawardani *et al*. (2016) showed that uncooked joruk color was brown with an intensity of 6.5 and for the cooked joruk with an intensity of 3.2. The dominant aroma impression of uncooked joruk is fishy with an intensity of 6.4, which is higher than the research conducted by Koesoemawardani *et al*. (2016) with an intensity of 5.4. The fishy smell of joruk comes from fish as the main raw material. According to Murtini *et al*. (2014) the fishy smell in fermented fish together with the volatile components in palm sugar and secondary metabolites will form a distinctive aroma thereby strengthening the aroma of fermented fish. Meanwhile, the joruk of cooked wader fish has a distinctive aroma with an aroma intensity 6.7 on the scale line. The distinctive aroma produced is a mixture of fishy, sardines, sour and aromas that come from added spices such as shallots, garlic and chilies Koesoemawardani *et al*. (2016).

The joruk of fresh wader fish has an intact shape with a wholeness intensity on the scale line of 5.1, while the joruk of cooked wader looks rather intact with a wholeness intensity of 2.8. This can be caused by stirring conducted during cooking the fish that destroyed fish meat. Based on research by Koesoemawardani *et al*. (2016) uncooked joruk has an intensity of 6.8, while cooked joruk looks incomplete (crushed) with a low integrity intensity of 1.4. For taste parameters, cooked joruk has a salty taste with a scale of 3.1 and a very sour taste with a scale of 8.4.

## 4. CONCLUSION

During the 14 days of storage, joruk experienced a decrease in pH value, total mold, total microbes, and water content, while the total amount of lactic acid, LAB, and TVB values increased. Based on the microbiological properties of joruk on the parameters of lactic acid bacteria, the joruk on the 10th day of storage was chosen to be the best joruk with the highest LAB amount, namely 10.46 log CFU/g. Characteristics of the loamy wader fish on the 10th day of storage, namely pH 6.33, total lactic acid 9.48%, total volatile base 93.88%, water content 67.74%, protein content 8.45%, total mold and yeast 7.21 log CFU/g, and total microbes 12.13 log CFU/g. Meanwhile, the sensory characteristics of uncooked joruk on the 10th day had a brown color (6.2 scale), fishy aroma (6.4 scale), and intact appearance (5.1 scale), while cooked joruk had a brown color with lower intensity (4.2 scale), distinctive dirty aroma (6.4 scale), salty taste (3.1 scale), very sour taste (8.4 scale) and incomplete form (2.8 scale).

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