

# Quality Evaluation of Bakasang Processed with Variation of Salt Concentration, Temperature and Fermentation Time

*by Sanusi Gugule*

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## Research Article

# Quality Evaluation of Bakasang Processed with Variation of Salt Concentration, Temperature and Fermentation Time

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## Abstract

**Background and Objective:** Production of bakasang in North Sulawesi has not been standardized. Local people still produce it without considering the optimal conditions and the fermentation is done in a simple way. The fish viscera is traditionally fermented inside a bottle with addition of salt and incubated under the sun for a few days until it turns into a liquid. In this present study, the quality of bakasang was assessed during various fermentation condition. **Materials and Methods:** The viscera of fresh cakalang (*Katsuwonus pelamis*) fish was fermented under various temperatures (30 and 70 °C) for 5, 10 and 15 days with the addition of 10, 20 and 30% of salt. The property of quality included pH, moisture content, free fatty acid (FFA) and thiobarbituric acid (TBA) of the bakasang product. **Results:** The results of this study indicated that moisture content ranged from 16.42-77.98%. Moisture content decreased with increasing the salinity, fermentation temperature and fermentation time. The pH of bakasang ranged from 5.66-6.73. The pH of bakasang decreased with the increasing salinity. The FFA level ranged from 1.42-5.18, it increased with increasing fermentation time but decreased with increasing fermentation temperature and increased salt level. The TBA (expressed in malondialdehyde/MDA) level varied from 0.53-4.81 g/100 g, it decreased with increasing salinity and fermentation temperature but decreased with increasing fermentation time. **Conclusion:** The data revealed that based on the property of quality, the best product was bakasang produced using salt 20-30% at 70 °C which was fermented for 15 days.

**Key words:** Bakasang, fermented fish sauce, malondialdehyde, thiobarbituric acid, salt concentration

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Bakasang is a fermented fish sauce made of viscera<sup>23</sup> of various fishes. It is a typical food of North Sulawesi. Fish sauce is a clear, amber to reddish brown liquid with a predominantly salty taste and characteristic fish flavor. The fermentation<sup>36</sup> process generally takes not less than 6 months. The liquid is obtained from the h<sup>42</sup>olysis of fish natural fermentation. Besides aiming for preservation, fermented foods also have additional benefits, such as enhancing flavor, improve digestibility and increase therapeutic value<sup>1</sup>. This product also contains lactic acid bacteria with antimicrobial property<sup>2</sup>.

There are other fermented fish sauces in other name in several countries, for example Nam-Pla or Plasom (Thailand), Nouc-Mam (Vietnam), Teuktrey (Kamboja), Budu (Malaysia), Patis (the Philippines), Shottsuru or Nukazuke (Japan), Aek-jeot (Korea), Yu lu (China), Lanhouin (Benin and Togo), Momoni (Ghana) and Fessekh (Egypt)<sup>3-5</sup>. The fishes which are<sup>1</sup> used in the process of fermented fish sauces include anchovy (*Stolephorus* sp.), sardines (*Sardinella* sp.), mackerel (*Rastrellinger* sp.), gambusia (*Affinis affinis*), Pacific whiting (*Merluccius* sp.) and other low value fish species<sup>6,7</sup>.

Fish sauce in some countries is made with different formulations and processing techniques, for example proportion of fish-water, salt concentration, storage time length and stirring. The fish sauce processing in Japan involves the use of fish: water 3:1 with 15% of salt and storage time at room temperature<sup>8</sup>. Sanchez<sup>6</sup> used various ratio of salt and fish, which ranged from 1:1 to 1:5. Research of Thariq *et al.*<sup>9</sup> showed that salt concentration gave different effect to parameters such as hedonic scale, glutamic acid and salt concentration, pH as well as its proximate (protein, fat and moisture content). Koochekian and Moini<sup>10</sup> used 20% salt for the fermentation of kilka fish from Caspian sea. Desniar *et al.*<sup>11</sup> showed that during fermentation of peda fish (pickled<sup>19</sup>), the increase of salt content up to 50% resulted in lower total volatile base nitrogen (TVBN) and trimethylamine (TMA) values. However, based on the sensory test, the selected salt concentration was 30%. According to El Sheikha *et al.*<sup>12</sup>, if fermentation process of the fish is done without using salt, it will produce uncontrolled fermentation products.

In addition to salt content, temperature and fermentation time also affect the quality of bakas<sup>43</sup>. Kim *et al.*<sup>13</sup> stated that using controlled fermentation temperature and salt concentration, production of fish sauce from jack mackerel could be performed successfully. The variation of some parameters in the processing of fish fermentation will result in various characteristic of fish sauce and this will depend on raw

material, salt concentration or ratio of salt to fish, processing method, fermentation time and type of fish<sup>7</sup>.

<sup>5</sup> In principle, the processing of fish sauce fermentation is divided into two stages. The first is the diffusion of salt into fishes and elimination of moisture content in fish through osmotic process. The se<sup>5</sup>nd is ripening process which is a slow stage involving a series of complex biochemical processes including proteolysis, lipolysis and lipid oxidation. Ripening stage results in product with<sup>5</sup> long consistency with distinctive flavors. The physical and chemical changes that occur during ripening determine the overall sensory quality of the fermented fish sauce product<sup>14</sup>. El Sheikha *et al.*<sup>12</sup> reported that there were three main techniques of fish fermentation cor<sup>17</sup> only practiced in many African countries. This included fermentation with salting and drying, fermentation and drying without salting and fermentation with salting but without drying.

Anihouvi *et al.*<sup>15</sup> stated that generally fermented fish product is processed spontaneously and the fermentation runs uncontrollably. This resulted in a variety of product quality and the product is vulnerable to da<sup>21</sup>ge. Kakati and Goswani<sup>1</sup> stated that fermented fish product mostly produced in households or small scale industry with limited quality control and product safety. The procedures used for the processing of fer<sup>21</sup>mented fish products are often not accompanied by cooking or pasteurization to destroy pathogens. Products are stored in room temperature and use of low-salt concentration or salt-free so they are very susceptible to<sup>34</sup> high microbiological risks. Based on the above background, the purpose of this study was to determine the quality of the processed bakasang with various processing conditions.

## MATERIALS AND METHODS

The viscera of fresh cakalang (*Katsuwonus pelamis*) fish were bought from a smoked cakalang processing house at North Minahasa Regency. The viscera consisted of liver, intestines, heart and eggs. Approximately  $\pm 250$  g of viscera fish were washed with running tap water, dried, cut into smaller parts ( $\pm 1$  cm) and put inside the fermentor, topped with salt with variation of concentration: 10% (S1), 20% (S2) and 30% (S3). The containers were closed tightly and heated with various temperature variations: 30 °C (T1), 50 °C<sup>40</sup> (T2) and 70 °C (T3) and variation of fermentation time: 5 days (t1), 10 days (t2) and 15 days (t3). The bakasang was shaken once a day inside the fermentor until the appointed time and then taken for analysis. All samples were analyzed by three repetitions but only two closest repeat values were used for statistical tests.

The parameters tested included moisture content, pH, free fatty acid (FFA) and thiobarbituric acid (TBA). The pH value of bakasang was measured using a digital pH meter according to a method such as that employed by Petrus *et al.*<sup>16</sup> Homogenates were prepared by homogeneously mixing 5 g samples with 10 mL of distilled water and the pH recorded with a digital pH meter (La Motte). The moisture content was determined by drying the fresh sample in an oven (Memmert) at 105 °C to a constant, reproducible weight<sup>17</sup>. Analysis of free fatty acid (FFA) was determined base on AOCS<sup>18</sup>. The FFA concentration in bakasang was calculated as a percentage of cis-5,8,11,14,17-Eicosapentaenoic Acid (EPA). Thiobarbituric acid (TBA) value of samples was determined with distillation methods from official methods of analysis of AOAC International<sup>19</sup>. The TBA or TBARS values were expressed in mg of malondialdehyde (MDA) kg<sup>-1</sup> bakasang. Absorbance was measured using spectrophotometer (Shimadzu UV-1800 Serial No. A116351).

**Statistical analysis:** One-way ANOVA analysis was used in this study. The LSD test was conducted if there were significant differences among the groups. All statistical analyses were performed using the statistical software SAS (University Edition). The level of significance was set at  $\alpha$  level of 0.05,  $p < 0.05$ . All values were presented as mean  $\pm$  standard deviation (SD) and statistical significance is indicated with appropriate letters on the data tables.

## RESULTS AND DISCUSSION

In this study, bakasang was processed using 3 variables: Fermentation temperature, fermentation time and salt content. For each variable there are 3 variations of values so that there are 27 overall test samples.

**Overall bakasang quality:** The statistical analysis of the quality of bakasang is presented in Table 1. The data on the

Table 1: The pH, moisture content, FFA and TBA of bakasang processed with variation of salt concentration, temperature and fermentation time

Fermentation time	Salt concentration/temperature	pH	Moisture content (%)	FFA (%)	TBA (mg malondialdehyde kg <sup>-1</sup> Bakasang)
t1	<b>S1</b>				
	T1	6.73 $\pm$ 0.007 <sup>a</sup>	77.983 $\pm$ 0.132 <sup>a</sup>	4.49 $\pm$ 0.004 <sup>b</sup>	3.74 $\pm$ 0.028 <sup>b</sup>
	T2	6.04 $\pm$ 0.048 <sup>b</sup>	70.791 $\pm$ 0.048 <sup>b</sup>	3.83 $\pm$ 0.152 <sup>c</sup>	2.88 $\pm$ 0.031 <sup>cde</sup>
	T3	5.98 $\pm$ 0.000 <sup>c</sup>	70.111 $\pm$ 0.033 <sup>c</sup>	1.80 $\pm$ 0.028 <sup>d</sup>	2.33 $\pm$ 0.001 <sup>fg</sup>
	<b>S2</b>				
	T1	5.95 $\pm$ 0.000 <sup>c</sup>	63.0611 $\pm$ 0.230 <sup>d</sup>	3.18 $\pm$ 0.285 <sup>ef</sup>	3.89 $\pm$ 0.548 <sup>b</sup>
	T2	5.83 $\pm$ 0.028 <sup>d</sup>	48.308 $\pm$ 0.195 <sup>e</sup>	2.86 $\pm$ 0.093 <sup>f</sup>	3.05 $\pm$ 0.057 <sup>cd</sup>
	T3	5.79 $\pm$ 0.014 <sup>d</sup>	41.856 $\pm$ 0.183 <sup>f</sup>	1.60 $\pm$ 0.099 <sup>g</sup>	2.41 $\pm$ 0.042 <sup>efg</sup>
	<b>S3</b>				
T1	5.83 $\pm$ 0.028 <sup>d</sup>	33.811 $\pm$ 0.538 <sup>g</sup>	2.10 $\pm$ 0.019 <sup>h</sup>	4.81 $\pm$ 0.847 <sup>a</sup>	
T2	5.71 $\pm$ 0.000 <sup>e</sup>	31.382 $\pm$ 0.029 <sup>h</sup>	2.00 $\pm$ 0.047 <sup>h</sup>	4.66 $\pm$ 0.383 <sup>a</sup>	
T3	5.66 $\pm$ 0.000 <sup>f</sup>	23.249 $\pm$ 0.377 <sup>i</sup>	1.42 $\pm$ 0.007 <sup>k</sup>	3.10 $\pm$ 0.113 <sup>cd</sup>	
t2	<b>S1</b>				
	T1	6.59 $\pm$ 0.014 <sup>g</sup>	71.409 $\pm$ 0.010	5.16 $\pm$ 1.320 <sup>a</sup>	2.91 $\pm$ 0.243 <sup>cd</sup>
	T2	5.93 $\pm$ 0.014 <sup>g</sup>	69.780 $\pm$ 0.142 <sup>c</sup>	4.33 $\pm$ 0.070 <sup>bc</sup>	2.27 $\pm$ 0.324 <sup>fg</sup>
	T3	5.79 $\pm$ 0.014 <sup>d</sup>	68.008 $\pm$ 0.019 <sup>k</sup>	3.05 $\pm$ 0.141 <sup>f</sup>	1.28 $\pm$ 0.000 <sup>k</sup>
	<b>S2</b>				
	T1	5.79 $\pm$ 0.028 <sup>d</sup>	52.107 $\pm$ 0.035 <sup>l</sup>	3.60 $\pm$ 0.056 <sup>cd</sup>	3.24 $\pm$ 0.056 <sup>c</sup>
	T2	5.77 $\pm$ 0.042 <sup>d</sup>	44.782 $\pm$ 0.059 <sup>m</sup>	3.37 $\pm$ 0.100 <sup>de</sup>	2.90 $\pm$ 0.096 <sup>cd</sup>
	T3	5.72 $\pm$ 0.014 <sup>d</sup>	41.354 $\pm$ 0.109 <sup>n</sup>	1.63 $\pm$ 0.000 <sup>l</sup>	1.53 $\pm$ 0.098 <sup>ij</sup>
	<b>S3</b>				
T1	5.58 $\pm$ 0.028 <sup>h</sup>	29.823 $\pm$ 0.018 <sup>o</sup>	2.28 $\pm$ 0.113 <sup>gh</sup>	3.88 $\pm$ 0.028 <sup>b</sup>	
T2	5.50 $\pm$ 0.014 <sup>i</sup>	24.75 $\pm$ 0.141 <sup>p</sup>	2.04 $\pm$ 0.003 <sup>h</sup>	2.95 $\pm$ 0.042 <sup>cd</sup>	
T3	5.48 $\pm$ 0.014 <sup>i</sup>	21.869 $\pm$ 0.189 <sup>q</sup>	1.43 $\pm$ 0.004 <sup>k</sup>	1.97 $\pm$ 0.000 <sup>gh</sup>	
t3	<b>S1</b>				
	T1	6.60 $\pm$ 0.007	71.153 $\pm$ 0.070 <sup>l</sup>	5.18 $\pm$ 0.152 <sup>a</sup>	2.06 $\pm$ 0.026
	T2	5.98 $\pm$ 0.014 <sup>c</sup>	67.157 $\pm$ 0.082 <sup>r</sup>	4.38 $\pm$ 0.070 <sup>bc</sup>	1.61 $\pm$ 0.070 <sup>gh</sup>
	T3	5.88 $\pm$ 0.000 <sup>j</sup>	54.224 $\pm$ 0.108 <sup>s</sup>	3.25 $\pm$ 0.123 <sup>ef</sup>	0.53 $\pm$ 0.161 <sup>l</sup>
	<b>S2</b>				
	T1	5.85 $\pm$ 0.007 <sup>j</sup>	50.463 $\pm$ 0.252 <sup>t</sup>	3.62 $\pm$ 0.028 <sup>cd</sup>	2.40 $\pm$ 0.127 <sup>efg</sup>
	T2	5.81 $\pm$ 0.014 <sup>d</sup>	44.237 $\pm$ 0.094 <sup>u</sup>	3.39 $\pm$ 0.014 <sup>de</sup>	2.08 $\pm$ 0.345 <sup>gh</sup>
	T3	5.74 $\pm$ 0.028 <sup>e</sup>	39.319 $\pm$ 0.161 <sup>v</sup>	1.87 $\pm$ 0.058 <sup>d</sup>	1.01 $\pm$ 0.014 <sup>k</sup>
	<b>S3</b>				
T1	5.80 $\pm$ 0.000 <sup>d</sup>	21.036 $\pm$ 0.107 <sup>w</sup>	2.41 $\pm$ 0.046 <sup>g</sup>	2.69 $\pm$ 0.028 <sup>def</sup>	
T2	5.62 $\pm$ 0.014 <sup>h</sup>	19.736 $\pm$ 0.095 <sup>x</sup>	2.27 $\pm$ 0.164 <sup>gh</sup>	2.17 $\pm$ 0.014 <sup>g</sup>	
T3	5.61 $\pm$ 0.000 <sup>h</sup>	16.416 $\pm$ 0.062 <sup>y</sup>	1.45 $\pm$ 0.008 <sup>k</sup>	1.24 $\pm$ 0.028 <sup>k</sup>	

<sup>a</sup>Results are means  $\pm$  standard deviation (n = 2), Means within the same column followed by same superscript are not significantly different (p < 0.05)

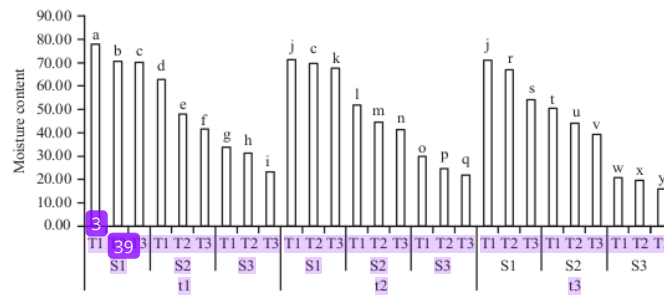


Fig. 1: Moisture content of bakasang processed using variation of salt concentration, fermentation temperature and time\*  
 \*Results with same superscript are not significantly different (p<0.05)

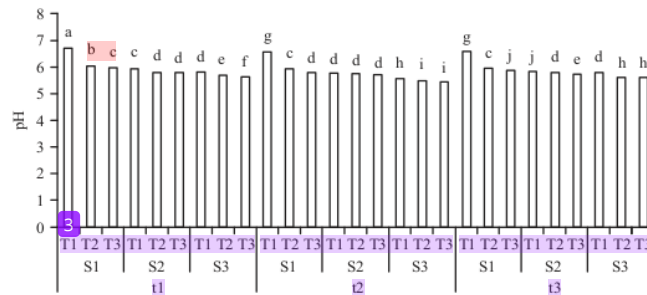


Fig. 2: The pH of bakasang processed using variation of salt concentration, fermentation temperature and time\*  
 \*Results with same superscript are not significantly different (p<0.05)

table showed the result according to variation of salt concentration, temperature and fermentation time. The parameter tested included moisture content, pH, FFA and TBA values. The moisture content of 27 samples of bakasang differed significantly. It varied from  $16.416 \pm 0.062\%$  to  $77.9835 \pm 0.132\%$ . The pH varied from  $5.48 \pm 0.014$  to  $6.73 \pm 0.007$ . The FFA content varied from  $1.42 \pm 0.007$  to  $5.18 \pm 0.152$ . The TBA values varied from  $0.53 \pm 0.161$  to  $4.81 \pm 0.847$  g/100 g (Table 1).

**Moisture content:** Moisture content of bakasang indicated a clear trend (Fig. 1). The moisture content decreased with the increasing of salt concentration, fermentation temperature and time. The highest moisture content (superscript a) was found in bakasang processed with the condition of t1, S1 and T1, i.e., 5 days fermentation time, 10% salt content and fermentation temperature at 30°C, while the lowest moisture content (superscript y) was found in bakasang processed with the condition of t2, S3 and T3, i.e., fermentation time 10 days, 30% salt content and fermentation temperature at 70°C.

**pH:** The pH tended to decrease with increasing salinity, although not all values differed significantly (Fig. 2). The highest pH (superscript a) was found in bakasang processed with t1, S1 and T1, i.e., 5 days of fermentation, 10% salt content and at 30°C, while the lowest pH (superscript i) was found in sample processed with t2, S3 and T3, i.e., 10 days of fermentation, 30% salt content and at 70°C. This condition was not different significantly from pH value from sample processed with t2, S3 and T2 (10 days of fermentation, 30% salt content and at 50°C).

**Free fatty acid:** There is a considerable difference in FFA processed with different conditions. According to Fig. 3, there was a decrease in FFA by increasing salinity, fermentation temperature and fermentation time. The lowest FFA value (superscript k) was found in bakasang under processing conditions of t1, S2 and T3 (5 days fermentation time, 20% salt content and 70°C fermentation temperature) and was not significantly different from the pH value of t2, S2 and T3 (10 days, 20% salt at 70°C) and t3 S3 T3 (15 days, 30% salt at 70°C). Thus, the lowest FFA value was found in

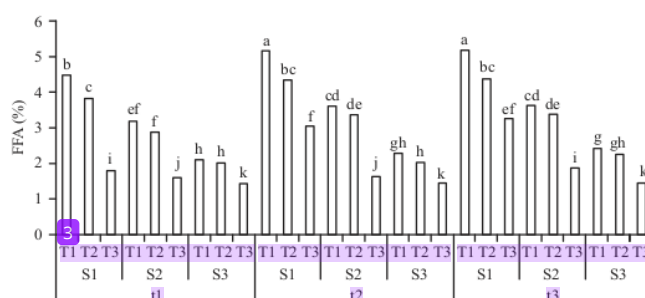


Fig. 3: The FFA values of bakasang processed using variation of salt concentration, fermentation temperature and time\*  
 \*Results with same superscript are not significantly different (p<0.05)

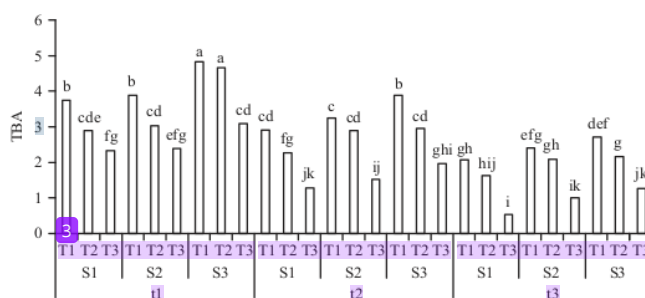


Fig. 4: The TBA values of bakasang processed using variation of salt concentration, fermentation temperature and time\*  
 \*Results with same superscript are not significantly different (p<0.05)

bakasang processed for 5-15 days, 20-30% salt and at 70°C. The highest FFA value (superscript a) was found in bakasang processed with t3, S1 and T1 (15 days, 10% salt and at 30°C). This value was not significantly different from pH value of bakasang processed with t2, S1 and T1 (10 days, salt content 10% and at 30°C).

**Thiobarbituric acid:** The value of TBA (TBARS) was expressed as mg of malondialdehyde in kg of bakasang. As seen in Fig. 4, the lowest TBA (superscript i) was found in bakasang processed with t3, S1 and T3 (15 days, 10% salt and at 70°C). The next lowest TBA was found in the fermentation processed with t3, S2 and T3, i.e., Bakasang fermented for 15 days with 20% salt at 70°C (superscript ik) and t3, S3 and T3 (superscript jk), i.e., bakasang fermented for 15 days with 30% salt at 70°C. The highest TBA value of bakasang (4.81 ± 0.847 g/100 g) was found in fermentation process of t1, S3 and T1 (5 days, 30% salt and at 30°C) (superscript a). This value did not differ significantly from bakasang processed at t1, S3 and T2 (5 days, 30% salt, at 50°C).

**Moisture content:** The lowest moisture content was obtained for bakasang products processed at the highest temperature, i.e. 70°C with 30% salt content and fermentation time of 10 days, while the highest moisture content was obtained for bakasang product processed at 30°C with 10% salt and 5 days fermentation time. Until recently, the best moisture content standards for bakasang or other fermented fish products are not known. However, if it is taken that the best bakasang is the product with medium to lowest moisture content, then the best recommended treatment is the processing at 50-70°C with salt content of 20-30% and fermentation time 5-15 days.

According to Buckle *et al.*<sup>20</sup>, salt is a chemical commonly used as a preservative and flavor enhancer. In its function as a preservative, salt acts as a humectant because it is soluble in water and absorbs water material (hygroscopic), so it can lower moisture content and water activity (A<sub>w</sub>).<sup>14</sup> Anihouvi *et al.*<sup>15</sup> stated that moisture content of cassava fish (*Pseudotolithus* sp.) and king fish (*Scomberomorus tritoi*) (*S. tritoi*) fermentation products were



50.1 and 56.6%, respectively. Furthermore, it was said that variation of moisture content could be used as an exact indicator against susceptibility of a product to undergo microbial spoilage. A major factor which determines the microbial, chemical and enzymatic stability of foods is the water activity. Salt plays a role in lowering the water activity of a system thus making the conditions less favorable for microbial life. To reach an  $A_w$  of 0.90 which inhibits bacterial development, a saline solution of about 15.5% is required. In general, food pathogenic bacteria grow with a water activity condition of 0.92 which is equivalent to 13% NaCl (w/v) concentration.

According to Zaman *et al.*<sup>21</sup>, sodium chloride plays an important role in microbial growth and affects the activity of amino acid decarboxylase. High salt concentrations also affect the growth of lactic acid bacteria. These types of bacteria are generally tolerant to moderate salt concentrations in the range of 10-18%<sup>22</sup>. Majumdar and Basu<sup>14</sup> stated that the fermentation mechanism included salt diffusion in fish and further the elimination of water through the osmosis process. Moisture transfer in fish was due to the osmosis process resulted in a decrease in moisture content, along with the simultaneous increase of ash and salinity in the final product.

Decrease in moisture content with increasing fermentation time is also suspected to be influenced by an increase in salinity content of bakasang products. This is in line with Mueda<sup>7</sup> and Tsai *et al.*<sup>23</sup> that increased fermentation time would increase the salt content of fermented fish products. Tsai *et al.*<sup>23</sup> reported that salt concentration of tuna viscera increased during fermentation, from initial concentration of 10, 17.5 and 25 to 19.20, 29.76 and 31.39%, respectively. For commercial fish paste, salt concentration for samples fermented at 17.5 and 25% fell within the range from 17.5-35.4%. Desniar *et al.*<sup>24</sup> also showed that the value of moisture content of fish sauce decreased (70, 88, 67, 86 and 64, 12) along with the increase of salt concentration (20, 30 and 40%) used during the process of fish sauce making.

**pH:** The pH of bakasang in this study ranged from 5.66-6.73 and it was in the range which is required by Codex Alimentarius. Codex Alimentarius<sup>25</sup> stated that a standard pH ranged from 5.0-6.5 for fish sauce products. While according to El-Sheikha *et al.*<sup>12</sup>, there were no literatures available on the recommended range of African fermented fish products. The pH above 6.5 only occurred in bakasang products processed with 10% salt content with a temperature of 30°C. The pH for fermented fish products above 6.5 was also reported by Anihouvi *et al.*<sup>15</sup>, for example the pH of fermented cassava fish (*Pseudotolithus* sp.) and king fish

(*Scomberomorus tritor*) varied between 6.7-7.9. Based on this, then the poor processing conditions for bakasang was 10% salt content with a temperature of 30°C with a fermentation time of 5-15 days because it resulted in a pH above 6.5. The recommended processing condition is 20-30% salt concentration at 50-70°C for 5-15 days.

The decrease in pH with increasing salinity is also in line with the results of Besas and Dizon<sup>22</sup> which stated that the pH of the sample decreases as salt concentration increased during the fermentation period of tuna viscera. However, the fermented tuna viscera using 17.5 and 25% salts had comparable pH values (5.33 and 5.32) and was significantly more acid (2.84 and 3.08% lactic acid) compared with tuna viscera fermented using 10% salt (pH 5.83 and 2.34% lactic acid). The increase in salt concentration caused the pH of the fermentation products to decrease or became more acidic. A decrease of pH was significantly affected by microorganisms, sugar content, salt concentration and temperature<sup>26</sup>.

The pH of bakasang was also affected by fermentation time. Decrease in pH of bakasang was seen in the first 5 days to the second 5 days and increased again in the third 5 days. Mueda<sup>7</sup> also reported a decrease in pH in the first 2 weeks of the fermentation process then increased in the following week. This is also in line with the results of El Hag *et al.*<sup>27</sup> and Kilinc *et al.*<sup>28</sup>, where the pH values in the *Labeo* sp. fermentation sample decreased in the early stages of fermentation with the addition of 25% salt and in sardines with the addition of 10% salt. Similarly, Besas and Dizon<sup>22</sup> stated that the pH value of viscera tuna fermented fish products with different salt concentrations decreased throughout the fermentation period due to increased levels of citric acid or lactic acid. At the end of the fermentation period, the viscera tuna pH decreased from 6.13-5.61. According to Sanchez<sup>5</sup>, pH reduction in fermentation products is due to the dissociation of amino acids and peptides in the presence of salt.

According to Mueda<sup>7</sup>, the pH in fermented fish sauce is a very important characteristic in identifying product quality. Good quality fish sauce can only be produced if using good quality fish raw materials where fresh fish pH is almost neutral. After the fish is dead, decomposition occurs through the enzymatic digestion of the fish muscle and gradually decreases the pH. The use of appropriate salt concentrations in the fermentation process inhibits the decomposition of fish.

The pH values of most commercially available Southeast Asian fermented fish sauces were in the range of 4.66-5.91<sup>29</sup>. The pH of traditional fermented fish product Lona ilish of Northeast India was 5.66±0.06<sup>14</sup>. The pH of fermented anchovy fish sauce significantly decreased over

the 270 days of fermentation period, from initial pH of  $6.99 \pm 0.08$  to final value of  $6.05 \pm 0.03$ <sup>7</sup>. The pH difference may be due to protein hydrolysis which resulted from free hydrogen ions, free amino acids and amino acid of oligopeptides<sup>30</sup>. According to Majumdar and Basu<sup>14</sup>, the reason for low pH value of the product might be attributed to the fact that samples were undergoing fermentation and not spoilage.

**Free fatty acid:** The FFA value decreased with the increasing of fermentation temperature and salt content (Fig. 3). Majumdar and Basu<sup>14</sup> reported the similar results on mackerel and pink perch products<sup>5</sup> at room temperature. In the fermentation process, a series of complex biochemical processes occur, including proteolysis, lipolysis and lipid oxidation (also known as ripening stage). Ripening stage produces products with strong consistency and has a distinctive aroma and flavor. The physical and chemical changes that occur during the ripening stage determine the overall sensory quality of fermented fish products<sup>14</sup>.

The FFA value of fermented fish has been reported by others. Zhou<sup>15</sup> for example reported that FFA of fermented cassava fish (*Pseudotolithus* sp.) and king fish (*Scomberomorus tritor*) were 11% and 14%, respectively. It is further said that the high FFA is an indication of microbial spoilage activity. According to Majumdar and Basu<sup>14</sup>, high FFA values were in consequence of the production of different organic acids. This implied that the fish had undergone sufficient fermentation with endogenous and/or exogenous microbes.

Chemically, the fermentation process begins with a post mortem process in fish, where lactic acid formation occurs due to the anaerobic situation in muscle tissue, followed by protein and lipid autolysis and microbial flora formation<sup>31</sup>. Many autolytic enzymes are found in muscle and intestinal tissue. Together with bacterial decomposition, organic acids also form, such as propionic acid, butyric acid and acetic acid. Decreasing of value of FFA caused by increased salt concentration in this study was thought to be associated with decreased of moisture content. Majumdar and Basu<sup>14</sup> suggested that salt did not inhibit the action of lipase enzymes in freeing free fatty acids but decreased moisture content would result in decreased lipase enzyme activity. Lipase enzyme activity in fish meat occurs by hydrolyzing lipid but the action of lipase enzyme depends on salinity and fermentation time<sup>12,32</sup>. The best bakasang products then were those with the lowest FFA content. The lowest FFA was found in the processing at 70°C (Fig. 3) with 20-30% salt content and for 5-15 days of fermentation.

**Thiobarbituric acid:** The TBA content of bakasang in this study was quite low compared to other fish fermentation products. It was reported that TBA from Gambusia fish sauce was 1.55 mg MA/100 mL<sup>33</sup>. The TBA from two fermented fish products, hentak and ngari, were 0.20 and 0.47 mg/100 g, respectively. The TBA of fermented fish product of cassava fish (*Pseudotolithus* sp.) and king fish (*S. tritor*) varied from 5.3-6.1 and 7.2-9.4<sup>15</sup>. Furthermore, it was said that TBA was a reflection of chemical decay activity in the presence of acidity<sup>15</sup>.

The TBA content of bakasang decreased with the increasing of fermentation temperature and time (Fig. 4). The highest TBA content was found in products processed at 30°C with 30% salt for 5 days of fermentation. While the lowest TBA level was found in bakasang products with 10% salt at 70°C for 15 days of fermentation. Fish oil is very unsaturated and therefore is very susceptible to oxidation. Some prooxidant, such as haem in proteins, can catalyze oxidation reactions. Similarly, the iron content contained in the salt also accelerates auto-oxidation<sup>12</sup>.

Fish fermentation products such as lona ilish which contain high unsaturated fatty acids produced high oxidation product, it was characterized by high value of PV and FFA that was 41.3 meq O<sub>2</sub> kg<sup>-1</sup> fat and 31.84%, respectively<sup>14</sup>. In this study, TBA levels increased with increasing levels of salt. This is in line with Majumdar and Basu<sup>14</sup> that sodium chloride acted as a prooxidant. Lee *et al.*<sup>34</sup> also stated that the strength of Cl<sup>-</sup> ions could decrease the muscle-extracted enzyme activity of catalase, superoxide dismutase and glutathione peroxidase. It was further said that the ability of NaCl to reduce the activity of the antioxidant enzymes could be partially responsible for the lower oxidative stability of salted muscle foods. The results of Nurmahdi *et al.*<sup>35</sup> study stated that extract peptide with dose of 200 mg kg<sup>-1</sup> body weight decreased level of malondialdehyde (MDA) level in sera and inducible nitric oxide synthase (iNOS) expression in cardiac tissue.

Although the presence of salt may act as a prooxidant but in this study, TBA content of bakasang was quite low (0.53-4.84). This is thought to be related to the content of natural antioxidant component produced by fermentation products. According to Giri *et al.*<sup>36</sup>, several traditional fermented foods possess many advantageous properties, such as free radical-scavenging activity and reducing power and ion-chelating abilities. Storage of fermented fish products at low temperatures might inhibit the development of potent antioxidant active compounds<sup>36</sup>. The presence of these components in fermented fish products is also suspected to be present in bakasang products. Bakasang processed at high temperatures had lower TBA levels and on the contrary,

bakasang processed at low temperatures had higher TBA levels. TBA is a parameter of oxidation product. Based on the results of research and discussion above, the best bakasang products are bakasang that have the lowest TBA levels. Thus, the best processing conditions of bakasang are 15 days of fermentation and the selected temperature is 70 °C.

### CONCLUSION

The results of this study indicated that moisture content of bakasang ranged from 16.42-77.98%. Moisture content decreased with increasing of the salinity, fermentation temperature and time. The pH of bakasang ranged from 5.66-6.73. The pH of bakasang decreased with the increasing salinity. The FFA valued ranged from 1.42-5.18% and the values increased with increasing fermentation time but decreased with increasing of fermentation temperature and salt content. The MDA levels varied from 0.53-4.81 g/100 g. The TBA content of bakasang product decreased with increasing salinity and fermentation temperature but decreased with increasing fermentation time. Based on the parameters of moisture content, pH, FFA and TBA, the selected processing conditions of bakasang were 20-30% salt at 70 °C with a fermentation time of 15 days.

### SIGNIFICANCE STATEMENT

This study discovers that in order to achieve optimal fermentation of bakasang, the salt content, temperature and time of fermentation needed to be adjusted properly. This study finds that the best condition for cakalang fermentation to produce bakasang is 30% salt, 70 °C and 15 days. This finding will contribute to the knowledge in making fermented fish sauces.

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