

Microbiological Quality and Proximate Composition of *Bakasang Laor*, a Traditional Fermented Fishery Product in Maluku

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Abstract. *Bakasang laor* is one of the traditional fermented fishery products in Maluku, Indonesia. There are two types of *bakasang laor*, i.e. with and without vinegar. The microbiological research of *bakasang* processed use *laor* as raw material has never been done before. The research aimed to analyze the microbiological quality and proximate composition of two types of *bakasang laor*. The proximate composition can determine the nutritional value of this product for consumption by the community. The sample of *bakasang laor* was taken from traditional producer in Latuhalat village, Ambon. The total number of halotolerant and coliform bacteria in *bakasang laor* without vinegar were 1.7×10^6 CFU/g and 4.1×10^6 CFU/g, respectively, while the total of lactic acid bacteria in *bakasang laor* with vinegar was 6.0×10^6 CFU/g. The carbohydrate, protein, fat and ash contents of *bakasang laor* with vinegar was higher than *bakasang laor* without vinegar. The carbohydrate, protein and fat contents of *bakasang laor* with vinegar were 19.64 %, 16.23 %, and 2.42 %, respectively. Generally, microbiological quality and proximate composition of *bakasang laor* with vinegar was better than *bakasang laor* without vinegar. The bacteria that play a role in fermentation can be used as a starter in the fermentation of various food products. The results of this research to improve the quality of this fermentation product in the future.

Key words: *bakasang laor*, coliform, halotolerant bacteria, lactic acid bacteria, proximate composition

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INTRODUCTION

Fishery product is the main source of protein consumed by people in Maluku. One of the fishery product in this area is *laor*, a colony of sea worms that swarming periodically in March and April in Maluku Seas (Figure 1a). The worms are usually found in Ambon, Uliaser (Lease) Islands, Ternate, Banda Islands, and Tanimbar with different names. *Laor* is also found outside the seas of Maluku, such as in Lombok and Sumba (Mahulette, 2020). The worms are often caught abundantly, making their preservation becomes necessary. One of the *laor* preservation techniques with fermentation is called *bakasang laor* (Figure 1b), a local wisdom of Maluku communities. This product is used as a food reserve during lean times.

Bakasang consists of *bakasang* and *bakasang laor*. *Bakasang* is processed using viscera of cakalang fish (*Katsuwonus pelamis*, Lin) as raw material and it is a traditional food consumed by the people in Maluku and North Sulawesi (Purwaningsih et al., 2013), while *bakasang laor* is processed using *laor* worm and only consumed by people in Maluku. This fermented product is very popular in the community. In processing *bakasang laor*, salt and vinegar are usually added, However, in certain conditions, vinegar is not

always added in producing *bakasang*. *Bakasang* with vinegar (*bakasang V*) uses vinegar and salt as preservatives and *bakasang* without vinegar (*bakasang NV*) uses only salt. *Bakasang laor* processing in Ambon Island usually only uses salt, whereas in Uliaser Islands usually uses salt, vinegar, and certain spices. Both have distinct sensory characteristics and different shelf life. The shelf life of *bakasang laor V* is usually more than a year (Latumahina, 2011)

The sensory characteristics and shelf life of a fermented product are strongly influenced by microbial diversity. *Bakasang* fermentation occurs spontaneously and involves various types of microbes. The main microbes involved in fishery product fermentation is lactic acid bacteria (LAB) (Noonpakdee et al., 2009). The bacteria belong to the Generally Recognized as Safe (GRAS) category, making it safe to be in a food product (Pringsulaka et al., 2012). Other bacteria are also found in various fermentation product, such as halotolerant and coliform bacteria. Both of these bacteria are pathogenic, so their number is limited in food. The halotolerant bacteria, such as *Staphylococcus* are able to produce cytotoxin that kill innate immune cells (DuMont et al., 2011). Coliform is often used as an indicator of water and food contaminant (Ananchaipattana et al., 2012)

A microbiological quality and proximate analysis of *bakasang* processed using *laor* as raw material has never been done, even though this fermented product has been consumed for a long time by the community. The objective of this research was to study the microbiological quality and proximate compositions of *bakasang laor* NV and *bakasang laor* V. LAB might potentially produce antimicrobial compounds that function as biopreservative in food to reduce the use of chemical preservatives that are potential health risks (Pringsulaka et al., 2012). Some of these bacteria have been used as probiotics. LAB found in *bakasang laor* can be used as a starter to improve the quality of this product and other fermented products. Proximate compositions are used to determine the nutritional value of this product for consumption. Fermented products with high protein content can be used as food substitutes that are sufficient for people nutrition.

METHODS

Processing of *Bakasang Laor*

The *bakasang laor* used in this study was taken from traditional producer in Latuhalat village, Ambon Island consisting of *bakasang laor* NV and *bakasang laor* V. The processing of *bakasang laor* was done by the producer. A total of 2 kg of *laor* worms as raw material was put into basin and added with 400 g of table salt then dried in the sun for 10 hours. The basin was covered with mosquito net, so that it was not entered by flies and other insects. The *laor* worm was then put in a bottle after drying. The bottle was closed and *laor* was allowed to fermentation at room temperature for 12 weeks to produce *bakasang laor* (without vinegar). In the processing of *bakasang laor* with vinegar, 5% of vinegar was added (Latumahina, 2011). The research was conducted in Laboratory of Biology Education, Pattimura University.

Isolation and Characterization of Bacteria in *Bakasang Laor*

A total of 25 g of sample was mixed with 225 ml of sterile peptone solution and homogenized using a stomacher bags. One ml of the homogenized and diluted samples were poured into Petri dishes, then de Man, Rogosa and Sharp Agar (MRSA) (KGaA, Germany) containing 1% CaCO₃ and 3% NaCl were poured on it and incubated at room temperature for 48 hours (Fan & Song, 2013). For isolation of halotolerant and coliform bacteria, 100 µl from the homogenate samples were inoculated on Mannitol Salt Agar (MSA) (KGaA, Germany) (Kateete et al., 2010) and Eosin Methylene Blue Agar (EMBA) (KGaA, Germany) (Colclasure et al., 2015), respectively, using the spread plate technique with a sterile L-shape glass

rod then incubated at 37°C for 24 hours. All the isolates obtained were stained with Gram and spore staining and catalase test (Fan & Song, 2013).

Physicochemical Analysis

Physicochemical analysis was carried out at the beginning and after 12 week of fermentation. The pH measurement was conducted using pH meter (CT 6022 Kedida, China). The proximate analysis was performed based on Lee et al. (2013). The protein and fat content were determined using Kjeldahl and Soxhlet methods. The total of carbohydrate was determined using carbohydrate by difference method. The moisture and ash contents were measured using the gravimetric methods. The proximate analysis was conducted in the Integrated Laboratory of IPB University.

RESULT AND DISCUSSION

The Number and Characteristics of Bacteria in *Bakasang Laor*

Bacteria play a role in *bakasang laor* fermentation. The bacteria were also contaminants in the food product so the number was limited. The main bacteria that play a role in *bakasang* fermentation were lactic acid bacteria (Ingratubun et al., 2013), while contaminant bacteria include halotolerant bacteria, such as Staphylococcus, and coliform bacteria, such as Escherichia and Enterobacter. The main factor influencing the presence of bacteria in fishery fermentation was salt as a preservative. In *bakasang laor* V, vinegar also acts as a preservative that limits microbial growth.



Figure 1. *Laor* (a), *bakasang laor* (b)

A total of 7 isolates of bacteria were isolated from *bakasang laor* NV, and 6 isolates from *bakasang laor* V. Isolates found in *bakasang laor* NV were also found in *bakasang laor* V, except on EMBA media. The highest total number of bacterial colonies in EMBA media was 3.0x10⁵ CFU/g. (Table 1). Two

bacterial colonies was found on MRSA media. One of these colonies were white color and negative catalase so that were considered as lactic acid bacteria. Total of lactic acid bacteria in *bakasang laor* V was higher than *bakasang laor* NV after fermentation for 12

weeks. Vinegar is a weak acid which can reduce the pH of medium and support the growth of lactic acid bacteria. The characteristics of colonies in EMBA media that had different color can be used to identify the types of coliform (Table 1).

Table 1. Macroscopic characteristics of bacteria isolated from *bakasang laor*

Sample	Media	Code	Characteristics of Colony			Catalase test	Total of Colony (CFU/g)
			Forms	Pigments	Margins		
Without vinegar	MRSA	BK-01	Circular	Yellow	Entire	+	1.0x10 ⁵
		BK-02	Circular	White	Entire	-	2.0x10 ⁴
	MSA	BK-03	Circular	Yellow	Entire	+	1.1x10 ⁵
		BK-04	Circular	Pink	Entire	+	3.0x10 ⁴
		BK-05	Circular	White	Entire	+	3.0x10 ⁴
	EMBA	BK-06	Circular	Red	Entire	+	2.3x10 ⁵
		BK-07	Circular	Green	Entire	+	1.8x10 ⁵
With vinegar	MRSA	BK-01	Circular	Yellow	Entire	+	4.0x10 ⁴
		BK-02	Circular	White	Entire	-	6.0x10 ⁴
	MSA	BK-03	Circular	Yellow	Entire	+	1.0x10 ⁵
		BK-04	Circular	Pink	Entire	+	3.0x10 ⁴
		BK-05	Circular	White	Entire	+	3.0x10 ⁴
	EMBA	BK-08	Circular	Pink	Entire	+	3.0x10 ⁵

The result of microscopic characterization showed that the bacteria found in *bakasang laor* were rods and cocci shaped. These bacteria were grouped into Gram positive and negative bacteria. Two bacterial isolates was found on MRSA media. One of these isolates formed spore, so that it was considered as Bacillus, while the other isolate formed cocci pairs and no spore formation. This last isolate was considered as Leuconostoc. Three bacterial isolates were

found on MSA media. The characteristics of these isolates were cocci shape, Gram positive and no spore formation, so they were considered as Staphylococcus. The characteristics of isolates in EMBA media were cocci shape, Gram negative and no spore formation, so they were considered as Enterobacter and Escherichia because they had different colony colors (Table 2). The selected isolates had different shape and sizes (Figure 2).

Table 2. Microscopic characteristics of bacteria isolated from *bakasang laor*

Sample	Media	Code	Gram	Spore	Shape of cell	Genus of Bacteria (Estimation)
Without vinegar	MRSA	BK-01	+	+	Rod	Bacillus
		BK-02	+	-	Cocci	Leuconostoc
	MSA	BK-03	+	-	Cocci	Staphylococcus
		BK-04	+	-	Cocci	Staphylococcus
		BK-05	+	-	Cocci	Staphylococcus
	EMBA	BK-06	-	-	Rod	Enterobacter
		BK-07	-	-	Rod	Escherichia
With vinegar	MRSA	BK-01	+	-	Rod	Bacillus
		BK-02	+	-	Cocci	Leuconostoc
	MSA	BK-03	+	-	Cocci	Staphylococcus
		BK-04	+	-	Cocci	Staphylococcus
		BK-05	+	-	Cocci	Staphylococcus
	EMBA	BK-08	-	-	Rod	Enterobacter

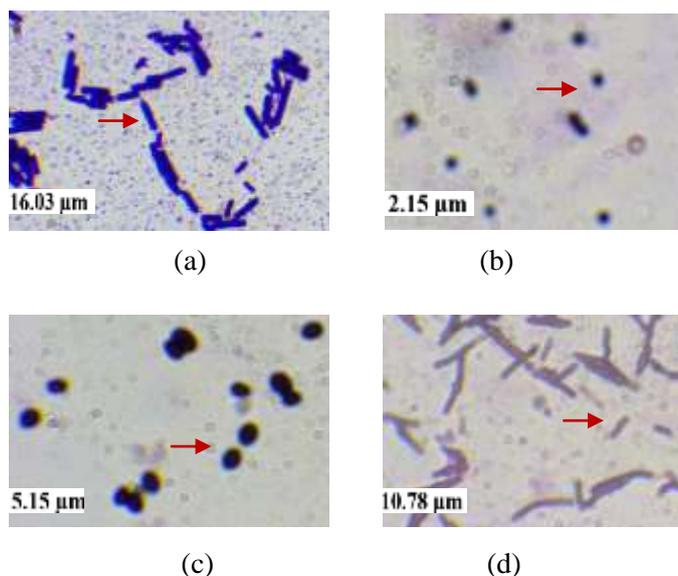


Figure 2. Microscopic characteristics of bacteria isolated from *bakasang laor* on different media (Magnification 1000x). Isolates of BK01 and BK02 on MRSA (a,b), BK05 on MSA (c), and BK06 on EMBA (d).

The Changes of Phycochemical Characteristics in *Bakasang Laor* Fermentation

The pH of both types of *bakasang laor* were showed noticeable changes as the increase of fermentation time. At the beginning of fermentation, the pH value of *bakasang laor* NV and *bakasang laor* V were 6.70 and 5.80 respectively. The pH value of *bakasang laor* NV and V sharply decreased at the fourth weeks of fermentation and continued to reach 5.1 and 4.5, respectively at the end of fermentation. The decrease was not sharp after 4 weeks of fermentation in *bakasang laor* NV (Figure 3). The high microbial numbers in this fermented product cause the degradation of protein into peptides and volatile base substances more quickly (Purwaningsih et al., 2013). An increase of volatile base substances causes the pH of *bakasang laor* to be relatively stable at the end of fermentation.

The pH value of *bakasang laor* V was lower than *bakasang laor* NV. The accumulation of acid compounds from vinegar causes a more acidic medium in *bakasang laor* V and support the growth of lactic acid bacteria. The pH greatly affects the presence of lactic acid bacteria in a fermentation product. Coccus-shaped lactic acid bacteria, such as *Leuconostoc* usually grow at an optimum pH above 4.5, different from *Lactobacillus* which grow optimally at the pH below it. The bacteria normally grow in neutral and acid condition (pH 5.0-7.0) (Li et al., 2012). The pH of *bakasang laor* was relatively high compared to the fermentation of other fishery products because there was no addition of carbohydrate source to accelerate the acid compounds formation.

Proximate analysis of both types of *bakasang laor* at the beginning and after 12 weeks fermentation showed that the moisture of *bakasang laor* NV in-

creased until the end of fermentation, whereas in *bakasang laor* V it decreased. The moisture of *bakasang laor* NV at the beginning of fermentation was 38.82 % then increased to 60.35 % at the end of fermentation, whereas in *bakasang laor* V, it had decreased from 54.28 % to 51.15 %. The moisture content was essential for microbial growth and enzyme activity that play a role in fermentation (Anggo et al., 2015). *Laor* that used as a raw material in *bakasang* processing was a sea worm with fragile body segments (Mahulette, 2020). Preservation of *bakasang laor* using salt only causes very soft and slimy texture which indicates high moisture.

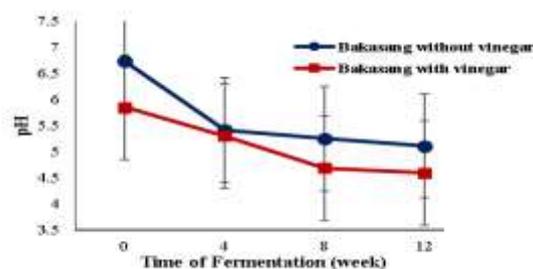


Figure 3. The changes of pH during *bakasang laor* fermentation

The protein content of *bakasang laor* NV was 25.72 % at the beginning of fermentation and then decreased to 15.29 % at the end of fermentation, whereas in *bakasang laor* V it increased from 13.45 % to 16.23 %. The fat content also decreased in *bakasang laor* NV. The fat of *bakasang laor* NV was 3.62 % at the beginning and then decreased to 0.17 %, while the fat content of *bakasang laor* V increased from 0.07 % to 2.42 % at the end of fermentation. *Bacillus* and *Staphylococcus* were more commonly found in *bakasang laor* NV. Both of these bacteria

have high proteolytic and lipolytic activities in fish fermentation (Marti et al., 2010; Marsiello et al., 2016). Tolerance to high salt content causes degradation of protein to amino acids more quickly by *Staphylococcus* in fermented product (Mahulette et al., 2018b).

Although the carbohydrate content of both types of *bakasang laor* decreased, it was no significant in *bakasang laor V*. The carbohydrate content of *bakasang laor NV* at the beginning of fermentation was 26.69

% then sharply decreased to 16.65 % at the end of fermentation, whereas in *bakasang laor V* it had decreased from 21.35 % to 19.64 %. Double preservation of fermented product with salt and vinegar causes only a few microbes to grow and utilize carbohydrate as an energy source in *bakasang laor V* fermentation. Thus, the carbohydrate, protein and fat contents of *bakasang laor V* were higher than *bakasang laor NV* at the end of fermentation (Table 3).

Table 3. The result of proximate analysis of *bakasang laor* at beginning and after 12 week fermentation (wet basis)

Parameter	Time of Fermentation (week)			
	<i>Bakasang laor NV</i>		<i>Bakasang laor V</i>	
	0	12	0	12
Moisture (%)	38.82±0.04	60.35±2.01	54.28±0.02	51.15±0.02
Carbohydrate content (%)	26.69±0.01	16.65±0.01	21.35±0.17	19.64±0.12
Protein content (%)	25.72±0.23	15.29±1.01	13.45±0.25	16.23±0.12
Fat content (%)	3.62±0.07	0.18±0.23	0.07±0.02	2.42±0.01
Ash content (%)	5.15±0.11	7.53±0.02	10.85±0.02	10.64±0.03

Lactic acid bacteria found in *bakasang laor* can increase the collection of microbes that play a role in fermentation of fishery product. These bacteria can be used as a starter to improve the quality of *bakasang laor* and other fermented product. The large amount of contaminant bacteria in *bakasang laor* indicates that this fermented product was less safe for consumption. Aseptic processing was needed to reduce the amount of contaminant bacteria in this product. The nutrition value of *bakasang laor V* was higher than *bakasang laor NV* at the end of fermentation. *Bakasang laor V* can be used as food substitutes to provide nutrition for people. The result of this study can be used as a reference to improve quality of *bakasang laor* as one of the popular local food in Maluku.

CONCLUSION

Bakasang laor without vinegar and with vinegar have different microbiological characteristics and proximate composition. The total number of halotolerant and coliform bacteria was higher in *bakasang laor* without vinegar, while the total number of lactic acid bacteria was higher in *bakasang laor* with vinegar. The carbohydrate, protein, fat and ash contents of *bakasang laor* with vinegar was higher than *bakasang laor* without vinegar. Generally, microbiological quality and proximate composition of *bakasang laor* with vinegar was better than *bakasang laor* without vinegar.

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