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Microbiological and physicochemical characteristics of *bakasang laor*, a traditional fermented fishery product from Maluku, Indonesia

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Abstract. *Mahulette F, Kurnia TS. 2020. Microbiological and physicochemical characteristics of* bakasang laor, *a traditional fermented fishery product from Maluku, Indonesia. Biodiversitas 21: 2216-2223. Bakasang laor* consisted of two types, i.e. with and without vinegar. The microbiological research of *bakasang* processed use *laor* as raw material is very limited therefore these investigations are necessary to be conducted. The research aimed to analyze the microbiological and physicochemical characteristics of two types of *bakasang laor*. The microbiological characteristics are used to determine product safety whereas the physicochemical characteristics, amino acids, and fatty acids contents can determine the nutritional value for consumption. The sample of *bakasang laor* was taken from traditional producers in Latuhalat, Ambon. Microbiological analysis using plate count method. From the measurement, the total number of halotolerant and coliform bacterial in *bakasang laor* without vinegar were 6.2 log CFU/g and 6.5 log CFU/g, respectively, while the total of lactic acid bacteria in *bakasang laor* with vinegar was 6.6 log CFU/g at the end of fermentation. The total amino acids and fatty acids contents of *bakasang laor* without vinegar at the end of fermentation were 11.25% and 32.23%, while *bakasang laor* vinegar was 9.38% and 32.72%, respectively. The bacteria found in *bakasang laor* were *Leuconostoc mesenteroides, Bacillus subtilis, Staphylococcus arlettae, Staphylococcus petrasii, Escherichia coli*, and *Enterobacter cloacae*. Generally, microbiological and physicochemical characteristics of *bakasang laor* with vinegar were better than *bakasang laor* without vinegar. This research can improve the quality of this fermentation product in the future.

Keywords*:* Amino acid, *bakasang laor*, fatty acid, lactic acid bacteria, plate count method

INTRODUCTION

Seafood has become part of human diet in many countries. This is an important source of nutrients, especially protein that is easily digested. However, seafood can be a source of toxins that are transmitted through food, so it needs to be controlled for its microbiological characteristics (Costa 2013). One of seafood consumed by the people of Eastern Indonesia is *laor. Laor* is various types of Polychaeta worms that appear in several places in Nusa Tenggara to the Pacific Islands, including in Maluku. These worms are dominated by *Eunice fucata* and *Palola viridis* and appear only in March and April in Maluku seas (Liline et al. 2016). The swarming of *laor* is abundant only at certain times, a few days after the full moon, so that people often process these worms by fermentation into *bakasang laor* (Latumahina 2011). Fermentation is oldest fish preservation method to provide the basic component of food with diverse characteristics of nutrition, flavor, and texture. Fermentation can also increase digestibility of food (Kakati and Goswami 2013).

Bakasang laor is often used as a side dish at famine time when fisherman could not go to sea. This product is usually consumed with raw vegetables, like eggplants. *Bakasang laor* is also used as a natural seasoning when cooking in Lombok (Sukenti et al. 2016). The processing of *bakasang laor* is generally added salt (*bakasang laor* Non Vinegar, NV), but in certain areas, this fermented product is processed using salt and vinegar (*bakasang laor* Vinegar, V). *Bakasang laor* NV is generally processed in Ambon island, while *bakasang laor* V is processed in the Uliaser islands (Haruku, Saparua, and Nusalaut Islands). Processing of *bakasang laor* in Uliaser Islands has often added seasonings for specific taste.

The main microbes involved in *bakasang* fermentation is lactic acid bacteria. The bacteria is safe to be in a food product (Ingratubun et al. 2013). *Bakasang laor* is still produced on a household scale, so there is no control over the quality and nutrition of this product. *Bakasang* processing does not go through cooking to kill pathogens and the product is only stored at room temperature or placed near the fireplace in the kitchen until consumed. Uncontrolled processing with high salt content causes *bakasang laor* is very high microbiological risk. Halotolerant and coliform bacterial can be found during fermentation of this product. Both groups of bacteria are generally as spoilage or pathogen, so that it's limited in food. Several types of halotolerant and coliform bacterial are pathogen opportunistic that associated with different types of infection.

Besides the microbiological quality, the sensory characteristics of the two types of *bakasang laor* are different. *Bakasang laor* NV usually has a fragile texture and less flavorful, while *Bakasang laor* V has a more solid and compact texture with a specific flavor. The sensory quality is determined by amino acids and fatty acids content in a fermentation product (Yu et al. 2014). Amino acids and fatty acids contribute to the taste of the food

products (Gao et al. 2011). Therefore, this study aimed to analyze the microbiological and physicochemical characteristics, especially amino acids and fatty acids contents during the fermentation of both types *of bakasang laor*.

MATERIALS AND METHODS

Fermentation process of *Bakasang Laor* sample

The *bakasang laor* used in this study consist of *bakasang laor* NV and *bakasang laor* V. The processing of this product was done by traditional producer in Latuhalat village, Ambon Island. A total of 2 kg of *laor* worms as raw material was put into basin and added 400 g of table salt then dried in the sun for 10 hours. The basin was covered with mosquito net, so that it doesn't enter by flies and other insects. After drying, the *laor* put in a bottle and closed. The *laor* allowed to fermentation at room temperature for 12 weeks to produce *bakasang laor* NV. In the processing of *bakasang laor* V, added 5% of vinegar (Latumahina 2011). The research was conducted in Laboratory of Biology Education, Pattimura University.

Microbiological measurements

Microbiological analysis uses the plate count method to count the number of total bacteria, lactic acid bacteria, halotolerant bacteria, and coliform bacteria during *bakasang laor* fermentation. The fermentation time observed was 0, 4, 8, and 12 weeks. 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) (Merck KGaA, Germany) containing 1% $CaCO_3$ and 3% NaCl were poured on it and incubated at room temperature for 48 hours (Fan and Song 2013). Colonies that have clear zones around them were considered lactic acid bacteria. For isolation of halotolerant and coliform bacterial, 100 µl from the homogenate samples were inoculated on Mannitol Salt Agar (MSA) (Merck KGaA, Germany), and Eosin Methylene Blue Agar (EMBA) (Merck KGaA, Germany), respectively, using the spread plate technique with a sterile L-shape glass rod then incubated at 37°C for 24 hours (Rattanasuk et al. 2015). For calculate of the total plate count using the spread plate technique on Nutrient Agar (NA) (Himedia, India). Lactic acid bacteria play a role in the fermentation process while halotolerant and coliform bacteria are microbial contaminants, so these bacteria need to be characterized. All the isolates obtained were stained with Gram and spore staining and catalase test. Catalase test to confirm the characteristics of lactic acid bacteria. Gram-positive bacteria with a negative catalase test were most likely lactic acid bacteria (Nurhikmayani et al. 2019).

Molecular identification of bacteria in *bakasang laor* fermentation

DNA extraction was carried out following procedure from Presto TM Mini GDNA Kit (Geneaid). The result of DNA extraction was used to amplify 16S rRNA gene. The 16S rRNA gene was amplified using PCR machine with 63F (5’ CAGGCC TAACACATGCAAGTC-3') and 1387R (5'-GGG CGGWGTGTACAAGGC-3') primers (Marchesi et al. 1998). The volume of PCR reaction used was 25 µL, consisting of12.5 µL Go Taq Green Master Mix 2X (Promega, Madison, W1, USA); 2.5 µL 63F and 1387R primers each (10 pmol); 6.5 µL Nuclease Free Water and 1 µg DNA genome as template. The reaction was amplified in 30 cycles and each PCR comprised pre-denaturation at 95°C for 5 minutes, annealing at 55°C for 1 min, elongation at 72°C for 1.5 min, and extension at 72°C for 10 minutes. PRC product was visualized using an electrophoresis machine at 80 volts for 45 minutes and stained with ethidium bromide. The amplified DNA was further sequenced and analyzed using ChromasPro software (Technelysium, AU) for sequence coupling, The sequences were then compared with GenBank database using Basic Local Alignment Search Total Nucleotide (BLASTN) software.

Physicochemical measurements

Amino acids and fatty acids contents were analyzed based on AOAC (2012). A total of 1 g of sample was dissolved in 20 mL of distilled water and then crushed using a homogenizer and centrifuged to obtain a supernatant. Amino acids were analyzed using high-performance liquid chromatography (HPLC) (20A, Shimadzu, Japan). Amino acids analyzed were only 15 types. Percentage of amino acid content (wet weight) was determined from protein content. For fatty acid contents analysis, a total of 30 mg of sample (homogenized) was added with 1 mL of 0.5 N NaOH then heated for 20 minutes. The solution formed was added 2 mL of 16% BF3 and 5 mg/ml of standard solution then heated for 20 minutes. The solution was then added 2 mL of saturated NaCl after being cooled, and 1 mL of hexane. The hexane layer formed was separated and injected into the gas chromatography (GC) (Fid 17A Shimadzu, Japan). The percentage of fatty acids content (wet weight) was determined in the fat content.

RESULTS AND DISCUSSION

The number of bacteria in *bakasang laor* fermentation

The total number of *bakasang laor* NV was 6.7 log CFU/g while *bakasang laor* V was 6.6 log CFU/g at the beginning of fermentation. This number decreased at the end of fermentation both *bakasang laor* NV and *bakasang laor* V was 6.5 log CFU/g and 5.5 log CFU/g, respectively (Figure 1A). The number of bacteria in *bakasang laor* does not high because preservation of this product uses high content of salt. In addition, laor also contains antibacterial compounds that can inhibit halotolerant and coliform bacterial (Jekti et al. 2008). Processing *bakasang laor* V using double preservation of salt and vinegar causes the number of bacteria of this product was less than *bakasang laor* NV. The non-salt-tolerant bacteria were easily inhibited by the presence of salt contains more than 7% in fishery fermentation (Yuen et al. 2015).

The number of lactic acid bacteria of the two types of products was almost the same at the beginning of the fermentation then decreased to log 4.5 CFU/g for *bakasang laor* NV and 6.6 log CFU/g for *bakasang laor* V at the end of fermentation (Figure 1B). Lactic acid bacteria were found in both types of *bakasang laor* only cocci shaped bacteria.

The number of lactic acid bacteria in *bakasang laor* V was still high at the end of fermentation. The addition of vinegar to the fermentation of this product makes it more acidic to support the growth of lactic acid bacteria. Lactic acid bacteria also produce bacteriocin which inhibits the growth of spoilage and pathogenic bacteria, include halotolerant and coliform bacterial (Fatma and Benmechernene 2013). The types of lactic acid bacteria found were not diverse because *bakasang laor* fermentation does not add carbohydrates as a source of additional nutrients for these bacteria.

Halotolerant bacteria of the two types of products were almost the same at the beginning of the fermentation then decreased to log 6.2 CFU/g for *bakasang laor* NV and 5.7 log CFU/g for *bakasang laor* V at the end of fermentation (Figure 1C). The number of coliform bacteria was significantly different at the end of the fermentation of the two types of *bakasang laor* i.e .6.5 log CFU /g for *bakasang laor* NV and 4.7 log CFU/g for *bakasang laor* V (Figure 1D). Processing with high salt content causes halotolerant bacteria to be found more in *bakasang laor* NV, while the number of these bacteria was less in *bakasang laor* V due to double preservation. Vinegar was a weak acid that can inhibit the growth of halotolerant and coliform bacteria (Chen et al. 2016). The lower number of halotolerant and coliform bacteria in the fishery fermentation products may also be due to the accumulation of lactic acid produced by lactic acid bacteria. Vinegar (acetic acid) and lactic acid were antimicrobial compounds.

The bacterial diversity of *bakasang laor*

A total of 7 isolates of bacteria were isolated in *bakasang laor* NV, and 6 isolates in *bakasang laor* V. Isolates found in *bakasang laor* without vinegar were also found in *bakasang laor* with vinegar, except on EMBA media. The six dominant isolates (2 isolates in each media) were characterized molecularly base on 16S rRNA gene. The result of amplification of 16S rRNA gene from these isolates produced length fragments around 1500 bp DNA (Figure 2). Analysis of gene sequences encoding 16S rRNA from 6 selected isolates with GeneBank data using the BLAST-N program revealed showed that one isolate (BK-01) was closely related with *Bacillus*, one isolate (BK-02) with *Leuconostoc*, 2 isolates with *Staphylococcus* and two other isolates were *Escherichia* and *Enterobacter* (Table 1). The phylogenetic tree showed the six selected isolates were closely related to *Bacillus, Leuconostoc, Staphylococcus*, and *Enterobacter* (Figure 3).

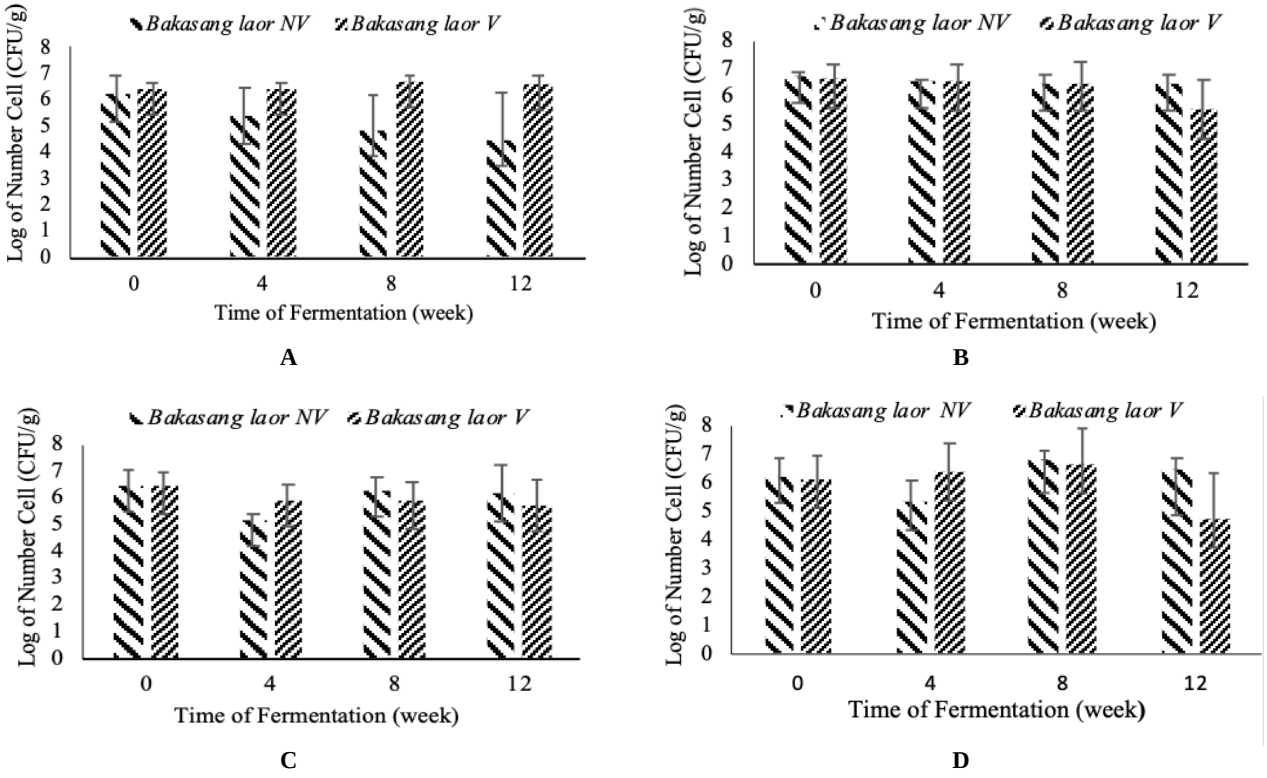

Figure 1. The changes in bacterial number in *bakasang laor* fermentation. A. Total bacteria, B. Lactic acid bacteria, C. Halotoleran bacteria, and D. Coliform bacteria

Table 1. Bacterial diversity of both types *bakasang laor* fermentation

Isolate	Media	Description	Length of nucleotide (bp)	Identity	Accession	Isolate source	References
Bakasang laor NV and V							
BK-01	MRSA	*Bacillus subtilis*	1416	98	CP015222.1	Doenjang	Kim (2017)*
BK-02	MRSA	*Leuconostoc mesenteroides*	1339	99	NR074957.1	Culture Collection	Makarova et al. (2006)
BK-03	MSA	*Staphylococcus arlettae*	1427	100	AP019698.1	Floor surface of biological laboratory	Kitahara and Uesaka (2019)*
BK-05	MSA	*Staphylococcus petrasii*	1000	99	MH753603.1	Camalti Saltern	Caglayan (2018)
Bakasang laor NV							
BK-06	EMBA	*Escherichia coli*	1407	98	LC056477.1	Culture collection	Akiba et al. (2016)
Bakasang laor V							
BK-08	EMBA	*Enterobacter cloacae*	1415	94	KU747082.1	Culture Collection	Najib and Yunus (2016)*

Note: * Unpublished

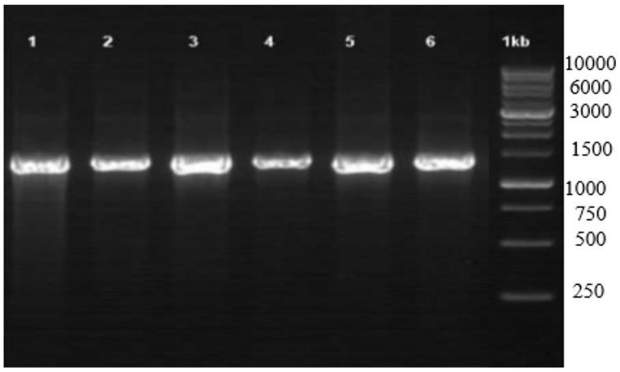

Figure 2. Electropherogram of 16S rRNA gene of bacteria isolates from *bakasang laor* fermentation with a pair primer of 63F and 1387R (Marker 1 kb, 1: BK-01, 2: BK-02, 3: BK-03, 4: BK-05, 6: BK: 06, and 6: BK-08 isolate)

Spontaneous and uncontrolled fermentation causes many bacteria to be involved in *bakasang laor* fermentation. *Bacillus subtilis* was often found in uncontrolled fermentation. The ability to spore formation that was tolerant of high salt content and high proteolytic activity causes this bacteria was dominant during the whole periods of fish sauce (Faisal et al. 2015) and terasi fermentation (Prihanto et al. 2016). Although *B. subtilis* not traditionally considered to be human pathogen, some strain of this species may occasionally cause food poisoning (Fernandez-No et al. 2011).

The lactic acid bacteria found in the *bakasang laor* fermentation was *Leuconostoc mesenteroides*. The characteristics of bacteria were cocci shape, Gram positive, and heterofermentative, so it was capable of degradation of glucose molecules to lactic acid, ethanol, and carbon dioxide. These bacteria play a role in the fish fermentation, such as *inasua*, fermented fish products from Maluku (Mahulette et al. 2018). Other lactic acid bacteria were not found because fermentation of *bakasang laor* was not added carbohydrates as an energy source for the growth of these bacteria. Fermentation of *bakasang laor* without the addition of carbohydrates causes the decrease of pH value during of fermentation was not significant (Mahulette and Kurnia 2020). *L. mesenteroides* usually plays a role in the fermentation of food products that have a rather high pH value.

The halotolerant bacterial found in the *bakasang laor* fermentation were *Staphylococcus arlettae* and *S. petrasii*. *S. arlettae* was emerging opportunistic pathogen. These bacteria play a role in the fish fermentation, such as *budu* and *Pa-som*, fermented fish products from Malaysia and Laos, respectively. In the fermentation of these two fermented products, *S. arlettae* plays a role in breaking down fish protein into simpler compounds (Yuen 2009; Marui et al. 2014). The presence of *S. arlettae* in *bakasang laor* fermentation due to the high salt content of this product. *S. petrasii* has no play a role in fermentation. The presence of these bacteria may be due to the lack of hygienic processing of *bakasang laor*. *S. arlettae* and *S. petrasii* were commensal species that commonly found on human skin, but they were opportunistic pathogens can also be associated with different types of infection (Lavecchia et al. 2019).

Another group of bacteria found in fermentation of *bakasang laor* was coliform. The presence of *Escherichia coli* in fermented fishery products might be attributed to poor handling practices and fecal contamination during processing and storage (Kakati and Goswami 2013). This bacteria was often non-pathogenic, but some strains may cause disease in gastrointestinal (Costa 2013). *E. coli* was a common indicator of sanitation and microbial safety of food products (Ananchaipattana et al. 2012). The growth of the bacteria can be inhibited by vinegar (Chen et al. 2016). This might causes *E. coli* not found in the fermentation of *bakasang laor* V. Another coliform bacteria found in the fermentation of *bakasang laor* V was *Enterobacter cloacae*. This bacteria was histamin producer in fishery fermentation (Kuley et al. 2019).

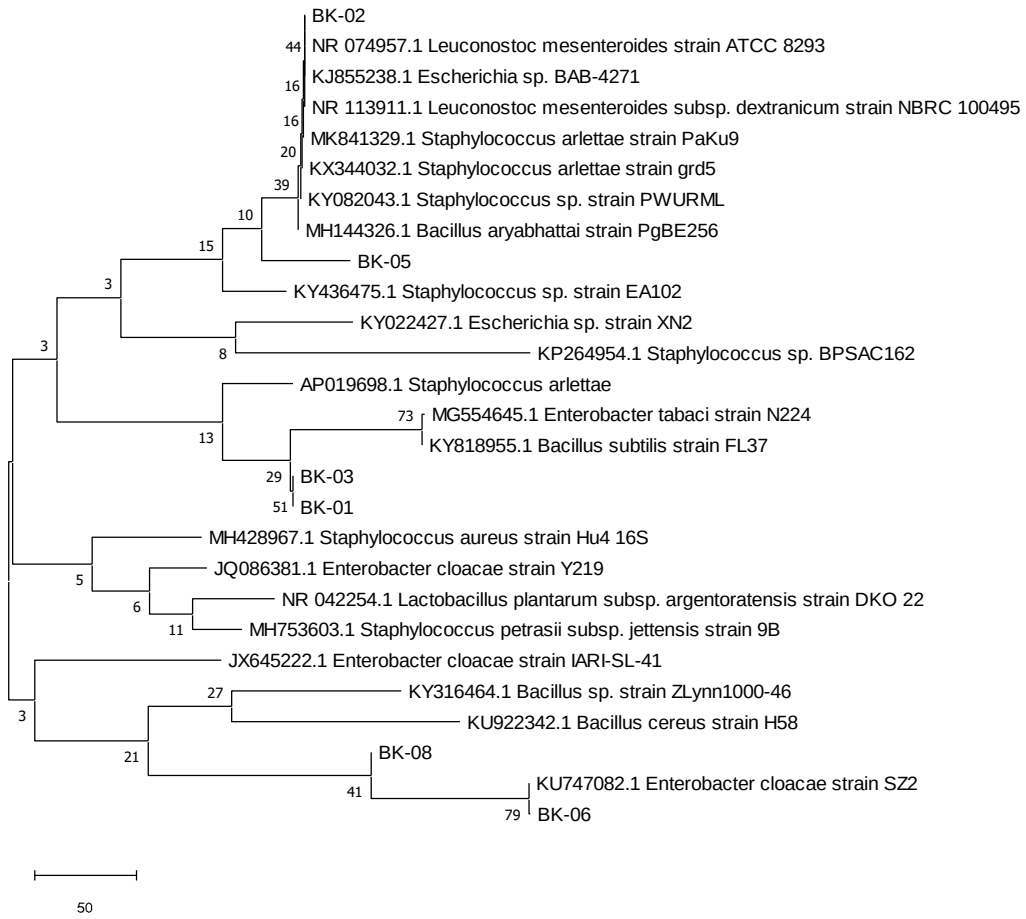

Figure 3. Phylogenetic tree of isolates bacteria from *bakasang laor* using the maximum likelihood method (bootstrap: 1000)

Characteristics of amino acid in *bakasang laor* fermentation

The total amino acid content of *bakasang laor* NV was higher than *bakasang laor* V. The total amino acid content of *bakasang laor* NV sharply decreased from 22.02% at the beginning to 11.25% at the end of fermentation, whereas in *bakasang laor* V only decreased from 9.94% to 9.38%. The dominant amino acid in both types of *bakasang laor* was glutamic acid, arginine, and glycine (Table 2). The high content of amino acids in *bakasang laor* NV because vinegar was not added and more bacterial that have high proteolytic ability play a role in the fermentation of this product. Halotolerant and coliform bacterial usually have high proteolytic activities in fishery fermentation (Yuen 2009).

The highest of glutamic acid in *bakasang laor* was not fermented, but from laor worms which were used as a raw material (Latumahina 2011). The highest content of glutamic acid was regarded as important contributors to flavor and taste of fishery fermented products (Anggo et al. 2015). This amino acid can produce savory (*umami*) flavor, one of the basic flavors used in food sensory testing. Glutamic acid also gives meaty flavor in fishery fermented products (Koesoemawardani et al. 2018). The highest of amino acid content in *bakasang laor* V was arginine. This amino acid was essential which can not be synthesized by the human body.

The sharply decrease of amino acid content of *bakasang laor* NV could be due to it's degradation to amines, volatile acids, and other nitrogenous substances as byproducts of bacterial metabolism or enzymatic decomposition. Decreased amino acids content also responsible for the formation of Maillard Reaction Products (MRPs) (Anggo et al. 2015). The MRP can increase of brown color intensity in *bakasang laor* NV. The degradation of amino acids almost does not occur in *bakasang laor* V, so this product has a solid texture with specific flavor.

Characteristics of fatty acid in *bakasang laor* fermentation

The total fatty acid content of *bakasang laor* V was higher than *bakasang laor* NV at the end of fermentation. The total amino acid content of *bakasang laor* NV sharply decreased from 35.34% at the beginning to 32.25% at the end of fermentation, whereas in *bakasang laor* V increased from 30.12% to 32.12%. The dominant fatty acid in both types of *bakasang laor* was palmitic acid, myristic acid, and stearic acid (Table 3). The highest of palmitic acid in *bakasang laor* was not fermented, but from *laor* worms

which were used as a raw material (Mapanawang et al. 2018). The essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of *bakasang laor* V were higher than *bakasang laor* NV. Both of these fatty acids were needed for intelligence and prevent heart attack (Houessou et al. 2019). EPA and DHA were usually found in fishery product which can not be synthesized by the human body.

Table 2. Characteristics of amino acids of both types *bakasang laor* at the beginning and after 12-week fermentation

Parameter	Amino acid content (% Protein content)			
	Bakasang Laor NV		*Bakasang laor* V	
	Time of fermentation (week)			
	0	12	0	12
Aspartic acid	1.92±0.07	1.02±0.14	0.83±0.07	0.74±0.07
Threonine	1.07±0.14	0.53±0.70	0.46±0.14	0.42±2.82
Serine	0.78±3.53	0.44±0.70	0.37±0.07	0.26±0,01
Glutamic acid	3.43±0.14	1.63±0.70	1.46±2.82	1.48±0.14
Glycine	2.89±0.07	1.33±0.28	1.26±4.24	1.07±0.14
Alanine	1.61±0.07	0.79±3.53	0.70±0.01	0.63±0.71
Valine	0.18±2.12	0.58±0.07	0.48±0.28	0.43±2,82
Methionine	0.51±0,00	0.25±0.70	0.19±0.01	0.18±2.12
Isoleucine	1.19±0.07	0.64±0.14	0.53±0.35	0.48±0.70
Leucine	1.80±0.70	0.91±0.14	0.78±0.07	0.71±0.70
Tyrosine	0.73±0.14	0.37±0.14	0.33±0.07	0.27±2.12
Phenylalanine	1.01±0.00	0.50±2.82	0.43±0.70	0.39±0.14
Histidine	0.56±0.28	0.29±0.71	0.27±0.07	0.34±0.28
Lysine	1.28±0.07	0.58±0.07	0.57±0.14	0.46±0.07
Arginine	2.06±0.14	1.38±0.00	1.30±0.07	1.52±0.07
Total of Amino Acids	22.02±0.28	11.25±0.14	9.94±0.07	9.38±0.07

Table 3. Characteristics of fatty acids of both types *bakasang laor* at the beginning and after 12-week fermentation

Parameter	Fatty acid content (% in fat content)			
	Bakasang laor NV		*Bakasang laor* V	
	Time of fermentation (week)			
	1	12	1	12
Lauric acid	0.01±0.07	0.06±0.14	0.04±0.84	0.04±0.07
Myristic acid	3.74±0.01	2.54±0.70	4.20±0.00	3.77±0.07
Myristoleic acid	0.02±0.71	0.03±0.14	0.03±0.14	0.03±0.00
Asam pentadekanoat	0.41±0.28	0.29±0.07	0.35±0.07	0.36±0.82
Palmitic Acid	14.91±0.35	11.76±0.07	11.72±0.14	11.56±0.07
Palmitoleic acid	1.88±0.14	1.30±0.14	1.30.±0.70	1.50±0.07
Heptadecanoic acid	0.90±0.84	0.58±2.82	0.64±2.12	0.66±0.14
Cis-10-Heptadecanoic acid	0.11±4.24	0.09±0.14	0.10±3.53	0.13±0.82
Stearic acid	4.19±2.82	3.23±0.00	3.22±2.82	3.31±0.14
Elaidic acid	0.63±0.07	0.45±0.35	0.32±2.12	0.41±0.14
Oleic acid	1.62±0.07	3.19±0.14	1.39±0.07	1.51±0.70
Linoleic acid	0.58±0.14	1.32±0.07	0.53±0.07	0.74±0.07
Arachidic acid	0.25±0.70	0.31±0.07	0.22±0.84	0.28±0.28
Cis-11-Eicosenoic acid	1.99±0.01	2.12±0.28	1.68±0.70	1.96±3.53
Linoleic acid	0.55±2.82	0.84±0.00	0.52±0.14	0.77±0.82
Heneicosanoic acid	0.08±0.84	0.08±0.84	0.08±0.00	0.09±0.35
Cis-11,14 Eicosedinoic acid	1.06±0.70	1.09±3.53	1.00±4.24	1.26±2.82
Behenic acid	0.17±0.70	0.18±0.07	0.19±0.28	0.21±0.00
Cis-8,11 Eicosedinoic acid	0.18±0.14	0.21±3.53	0.20±0.70	0.28±0.14
Erucic acid	0.06±0.35	0.09±0.84	0.06±0.14	0.08±0.01
Cis-8,11,14 Eicosetrienoic acid	0.10±0.01	0.11±0.14	0.13±0.70	0.20±0.35
Arachidonic acid	0.71±0.07	1.00±0.70	0.92±0.07	1.57±3.35
Tricosanoic acid	0.09±4.24	0.09±0.01	0.09±0.07	0.11±0.84
Cis-3,16 Docosadienoic acid	0.04±2.82	0.06±0.14	0.02±0.00	0.03±2.82
Lignoceric acid	0.89±0.71	0.07±0.70	0.08±0.14	0.11±0.14
Cis-5,8,11,14,17 Eicosapentaenoic acid	0.05±0.01	0.95±0.70	0.89±0.70	1.57±0.07
Nervonic acid	0.07±0.35	0.06±0.01	0.06±0.14	0.11±0.07
Cis-4,7,13,16,19 Decosahexaenoic acid	0.04±0.07	0.05±0.35	0.04±0.14	0.09±0.14
Total of Fatty Acids	35.34±0.14	32.25±0.35	30.12±0.14	32.72±0.07

In conclusion, *bakasang laor* without vinegar and vinegar have different microbiological and physicochemical characteristics. The total number of halotolerant and coliform bacterial were higher in *bakasang laor* without vinegar, while the total number of lactic acid bacteria was higher in *bakasang laor* with vinegar. The total amino acid content of *bakasang laor* NV was higher than *bakasang laor V*. In contrast, the total fatty acid content of *bakasang laor* V was higher than *bakasang laor* NV. The dominant amino acid and fatty acid in *bakasang laor* were glutamic acid and palmitic acid, respectively. The bacteria found in *bakasang laor* were *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Staphylococcus arlettae*, *Staphylococcus petrasii*, *Escherichia coli*, and *Enterobacter cloacae*. Generally, microbiological and physicochemical characteristics of *bakasang laor* with vinegar were better than *bakasang laor* without vinegar.

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