

Microbiological and chemical characteristics of porkfish *inasua*, traditional fish fermented from Maluku, Indonesia

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Abstract. Mahulette F. 2023. Microbiological and chemical characteristics of porkfish *inasua*, traditional fish fermented from Maluku, Indonesia. *Biodiversitas* 25: 1-8. Porkfish *inasua* consisted of two types, i.e., with and without coconut sap. The research aimed to analyze the microbiological and chemical characteristics of two types of porkfish *inasua*. The microbiological characteristics are used to determine the role of microbes and product safety whereas the chemical characteristics, including proximate composition, amino acids, and fatty acids contents can determine the nutritional value for consumption. The sample of porkfish *inasua* was taken from traditional producers in Layeni, Teon, Nila, and Serua (TNS) Islands, Maluku, Indonesia. The microbiological analysis was done using the plate count method. From the measurement, the total number of halotolerant and coliform bacterial in porkfish *inasua* without sap were 6.2 log cfu/g and 6.1 log cfu/g, respectively. In comparison, the total of *Bacillus* and related genera in porkfish *inasua* with sap was 5.9 log cfu/g at the end of fermentation. The fat content of both types of *inasua* was around 17%. The total amino acids and fatty acids contents of porkfish *inasua* without sap at the end of fermentation were 15.31 and 57.08%, while porkfish *inasua* with sap were 15.85 and 63.81%, respectively. The dominant bacteria found in porkfish *inasua* without sap was *Staphylococcus saprophyticus*, while porkfish *inasua* with sap was *Bacillus cereus*. These two bacteria play a role in fermentation so porkfish *inasua* was safe for consumption. Generally, chemical characteristics of porkfish *inasua* with sap were better than those of porkfish *inasua* without sap, but statistically, it was not significantly different. This research can improve the quality of porkfish *inasua* as local fermented fish products in Maluku.

Keywords: Coconut sap, fish fermented, porkfish, proximate composition, wax ester

INTRODUCTION

Ruvettus tydemani Weber (porkfish) is an oilfish from the Gempylidae family found in tropical and sub-tropical seas (Jawad et al. 2018). Consumption of this fish causes oily diarrhea (keriorrhea), so the commercialization of porkfish is prohibited or requires warning labels in several countries (Pardo et al. 2016). On the contrary, porkfish can be processed into *inasua* by the Teon, Nila and Serua (TNS) Communities in Maluku (Mahulette and Kurnia 2021). Consumption of this fermented product does not cause keriorrhea. *Inasua* is a spontaneous fermentation product, where the fish is not dried in sunlight but immersed in a salt solution (Persulesy et al. 2020).

Spontaneous fish fermentation generally involves lactic acid bacteria. The role of these bacteria is stimulated by adding of carbohydrates to several fermented fish products (Ngasotter et al. 2020). Porkfish contains high levels of undigested fat, making it possible to find other bacteria that play a role in the fermentation process. Processing of *inasua* does not controlled because it uses techniques and traditional equipment. This is possible the presence of pathogenic and putrefactive bacteria was found in this fermented product. *Inasua* is a fermented fish product with added salt as a preservative. Still, some people also add coconut sap, so there are two types of *inasua*, i.e., *inasua* without sap and *inasua* with sap. Adding coconut sap aims to extend the shelf life and increase the sensory taste of this

fermented fish product (Persulesy et al. 2020). Besides simple sugars, coconut sap also contains several microbes that play a role in fermentation (Chinnamma et al. 2019). Adding of coconut sap influenced more to the total number of bacteria and protein content of *inasua*. The more coconut sap added causes the number of bacteria and protein levels of the *inasua* to decrease (Wattimena et al. 2021).

Spontaneous fermentation also causes changes in the chemical composition of fish, such as proximate composition, amino acid and fatty acid contents (Koesoemawardani et al. 2018). These changes are largely determined by enzymatic activity, especially proteolysis and lipolysis in the fish. Fish proteins are gradually hydrolyzed by protease from fish and from bacteria into peptides and amino acids (Chen et al. 2017). Apart from proteolysis, lipolysis is also an important process that releases free fatty acids (Lee et al. 2014). The activity of these two enzymes is also influenced by salt concentration, which causes slowing proteolysis and lipolysis activities in fish (Sakpetch et al. 2022). Its freshness value and sensory characteristics greatly influence the quality of a fermented fish product.

These two types of *inasua* have different freshness and sensory properties. Total volatile base nitrogen (TVBN) and Trimethylamine (TMA) contents in *inasua* without sap were higher than in *inasua* with sap. The higher the two compounds, the lower the freshness of a fermented fish product (Mahulette and Kurnia 2021). *Inasua* without sap has a hard texture, flavorless and very salty (Nendissa

2013), while *inasua* with sap has a soft texture, flavorful, and slightly salty (Mahulette et al. 2018b). The freshness and sensory characteristics are largely determined by microbiological and physicochemical characteristics (Khair et al. 2020). Microbiological characteristics are very important in the food safety aspect to determine suitability for consumption, while chemical characteristics determine the nutritional value of a product. Chemical characteristics such as amino and fatty acid composition also greatly influence the sensory perception of a fermented fish product (Calanche et al. 2019). The characteristics of brown strip red snapper (*Lutjanus vitta*) *inasua* without sap and with sap are different. This is greatly influenced by fermentation product's microbiological and chemical characteristics (Mahulette et al. 2018b). This research aimed to analyze the microbiological and chemical characteristics of two types of porkfish *inasua*.

MATERIALS AND METHODS

Preparation of *inasua*

The *inasua* used in this study was taken from traditional producer in Layeni village, TNS Islands consisting of *inasua* without sap (*inasua* NS) and *inasua* with sap (*inasua* S). The processing of *inasua* was done by the producer. A total of 2 kg of porkfish as raw material was put into two jars, and 200 g of table salt was added, respectively. *Inasua* S processing also adds 1 liter of coconut sap. The jar was closed, and porkfish was allowed to ferment at room temperature for 12 weeks to produce *inasua*. The research was conducted in the Laboratory of Biology Education at Universitas Pattimura.

Isolation and characterization of bacteria in *inasua*

A total of 25 g of *inasua* sample was mixed with 225 ml of sterile saline solution (0.85%) and homogenized using stomacher bags (Zeng et al. 2016). Samples were serially diluted to 10^{-5} using saline solution. The sample dilutions used were 10^{-4} and 10^{-5} . One ml of the homogenized and diluted samples was poured into Petri dishes, then de Man, Rogosa and Sharp Agar (MRSA) (KGaA, Germany) containing 1% CaCO_3 and 3% NaCl was poured on it and incubated at room temperature for 48 hours (Maulidayanti et al. 2019). For isolation of halotolerant, coliform and total bacteria, 100 μl from the homogenate samples were inoculated on Mannitol Salt Agar (MSA) (Siddegowda et al. 2016) and Eosin Methylene Blue Agar (EMBA) (KGaA, Germany) (Rehman and Gohar 2022), and Nutrient Agar (NA) 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, spore staining and catalase test. The most dominant isolate from the two samples was then identified molecularly.

Molecular identification of bacteria

DNA extraction was carried out following the procedure from the 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 a PCR machine with 63F (5' CAGGCC TAACACATGCAAGTC-3') and 1387R (5'-GGG CGGWGTGTACAAGGC-3') primers (Patel et al. 2020). The volume of PCR reaction used was 25 μL , consisting of 12.5 μL Go Taq Green Master Mix 2X (Promega, Madison, WI, 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. PCR 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 the GenBank database using Basic Local Alignment Search Total Nucleotide (BLASTN) software.

Chemical characterization of *inasua*

Chemical analysis of both *inasua* products was carried out at the beginning and after 12 weeks of fermentation. The proximate composition analysis was performed based on Faithong and Benjakul (2014). The fat and protein content was determined using the Soxhlet apparatus and micro Kjeldahl methods. The total of carbohydrates was determined using carbohydrate by difference method. The moisture and ash contents were measured using the gravimetric methods. Proximate composition analysis was conducted in the Integrated Laboratory of Institut Pertanian Bogor, Bogor.

Amino acids and fatty acids contents were analyzed using high-performance liquid chromatography (HPLC) (20A, Shimadzu, Japan) and gas chromatography (GC) (Fid 17A Shimadzu, Japan) (Houéssou et al. 2019). A total of 1 g of *inasua* sample was dissolved in 20 mL of distilled water, then crushed using a homogenizer, and centrifuged to obtain a supernatant. Amino acids analyzed were only 15 types. The 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 and then heated for 20 minutes. The solution formed was added 2 mL of 16% BF_3 and 5 mg/ml of the standard solution, then heated for 20 minutes. The solution was then added to 2 mL of saturated NaCl and 1 mL of hexane after being cooled. 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. The difference in chemical characteristics of the two types of *inasua* after the fermentation were analyzed by Student's t-test to compare the mean values (SPSS 29.0 for Windows).

RESULTS AND DISCUSSION

The total number of *inasua* NS was 6.7 log cfu/g, while *inasua* S was 7.0 log cfu/g at the beginning of fermentation. This number decreased at the end of fermentation; both *Inasua* NS and *Inasua*-S were 6.5 log cfu/g and 6.4 log cfu/g, respectively (Figure 1a). The number of bacteria in *inasua* was not much because this product was preserved using high salt levels, and not all bacteria can decompose wax esters in fish (Arunkumar et al. 2017). High salt contents can inhibit the growth of Gram-Negative spoilage microorganisms (Chuon et al. 2014).

The addition of coconut sap at the start of *inasua* N fermentation caused the number of *Bacillus* and related genera to be higher than *inasua* NS. Coconut sap is an additional carbohydrate source for microbes that play a role in fermentation. Besides containing carbohydrates, this liquid also contains microbes dominated by *Bacillus* (Hebbar et al. 2020). These bacteria decreased until the end of fermentation in *inasua* NS and *inasua* S were 5.7 log cfu/g and 5.9 log cfu/g, respectively (Figure 1b). This decrease was due to limited nutrition and the accumulation of toxic compounds during fermentation.

At the beginning of the fermentation, halotolerant bacteria in *inasua* NS reached 6.7 log cfu/g, while *inasua* S only reached 6.4 log cfu/g. Both types of products were almost the same at the end of the fermentation (Figure 1c). Adding of coconut sap to *inasua* S causes a slight decrease in salt content. Halotolerant bacteria grew well in an environment with a rather high salt content (Ojagh et al. 2020). The number of coliform bacteria was not much different during the fermentation process (Figure 1d). The number of these bacteria was still high at the end of the fermentation, around 6.0 log cfu/g, because some of the

bacteria in this group could degrade undigested fat in fish (Arunkumar et al. 2021). Coconut sap can naturally ferment to produce alcohol, lactic acid, and acetic acid (Pandiselvam et al. 2021). The low number of coliform bacteria in *inasua* S was caused by the accumulation of alcohol, lactic acid, and vinegar (acetic acid) due to fermented coconut sap. Lactic and acetic are organic compounds that act as antimicrobials (Özcelik et al. 2016).

A total of 7 isolates of bacteria were isolated in *inasua* NS, and 6 isolates in *inasua* S. Isolates found in *inasua* S were also found in *inasua* NS, except for one isolate which was only found in *inasua* NS (data not shown). The result of the characterization of microbes in *inasua* found two dominant isolates, i.e., INS 04 and IS 02, in *inasua* NS and *inasua* S, respectively (Figure 2). The isolate INS 04 grew well on MSA media, while IS 02 was on MRSA media. The two isolates were characterized molecularly based on the 16S rRNA gene. The Amplification of the 16S rRNA gene from both isolates showed length fragments of around 1500 bp DNA (Figure 3). Analysis of gene sequences encoding 16S rRNA from two selected isolates with GeneBank data using the BLAST-N program revealed that isolate INS-04 was closely related to *Staphylococcus saprophyticus* (similarity index: 99.56%), while isolate IS 02 was closely related to *Bacillus cereus* (similarity index: 99.86%) (Table 1).

Generally, bacteria that play an important role in *inasua* fermentation are lactic acid bacteria (Mahulette et al. 2018a). This bacteria was very difficult to find in fermented *inasua* from porkfish because this fish's content of undigested fat (wax ester) was very high. One group of bacteria that can decompose wax esters is the *Bacillus* genera (Arunkumar et al. 2017).

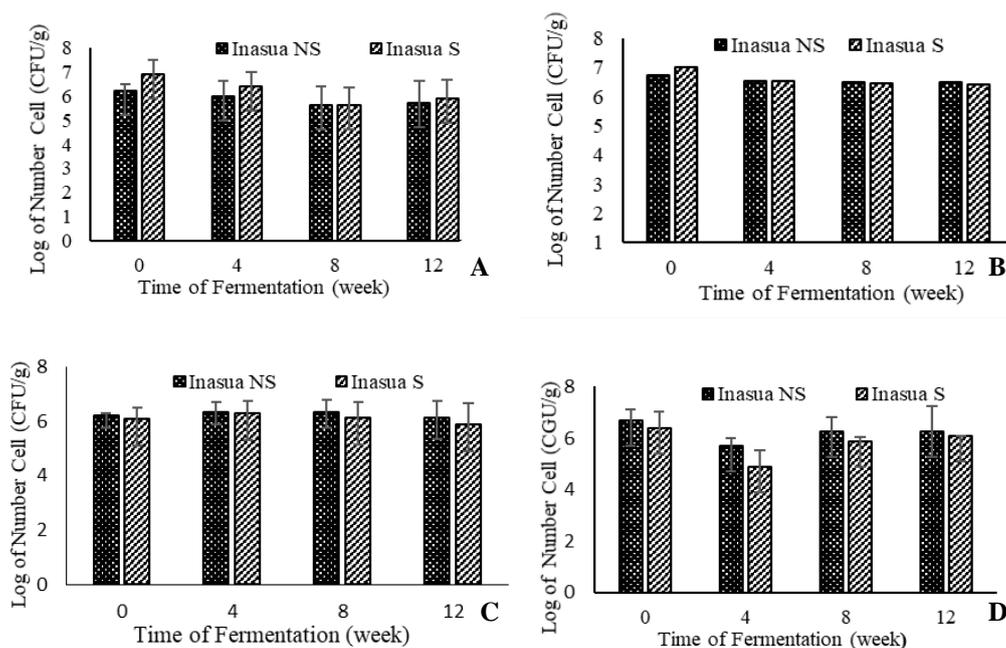


Figure 1. The changes in bacterial number in *inasua* fermentation. Total bacteria (A), *Bacillus* and related genera (B), Halotolerant bacteria (C), and Coliform bacteria (D)

Bacillus cereus is a widespread bacteria in nature found in several indigenous fermented fish products. Aside from soil, these bacteria are also found in the intestines of fish (Dan et al. 2017). *Bacillus cereus* was considered an opportunistic human pathogen because of its ability to produce toxins that cause diarrhea, a type of food-associated illness (Prihanto et al. 2021).

Bacillus cereus was found in ngari, hentak, and tungtap, traditional fermented fish products from India, and it's considered a pathogenic contaminant. These bacteria compete and suppress the growth of lactic acid bacteria which, play a role in fermentation (Thapa et al. 2004). However, other studies report that these bacteria play an important role in fish fermentation. *B. cereus* produced various extracellular enzymes such as protease, lipase, and phytase (Natarajan and Rajikkannu 2014). The high proteolytic ability causes this bacteria to be used as a co-starter with lactic acid bacteria in sausage fermentation. Lactic acid bacteria generally have low proteolytic capabilities (Shan et al. 2023). The phytase enzyme was used to break down phytic acid which binds proteins and other nutrients (Dan et al. 2017). In fish or shrimp paste fermentation, *B. cereus* produces the enzyme L-

asparaginase to decompose L-asparagine into L-aspartate (Prihanto et al. 2021).

Staphylococcus saprophyticus is a coagulase-negative Staphylococci (CNS) which is often found in spontaneously fermented products, such as ngari, fish sauce, and tai-pla (a fermented fish viscera product from Thailand). In ngari fermentation, these bacteria decompose fish amino acids into flavor compounds, lipase enzymes and produce antimicrobial compounds (Feng et al. 2021). *S. saprophyticus* has a high lipolytic potential (Daroopunt et al. 2018). These antimicrobial compounds was able to inhibit the growth of foodborne disease pathogens, including enteric bacteria (Khusro et al. 2020). The number of these bacteria was very difficult to predict during fermentation (Stavropoulou et al. 2018). In fish sauce fermentation, *S. saprophyticus* were more commonly found at the beginning of fermentation (Ma et al. 2021). *S. Saprophyticus* has been widely used as a starter culture in the processing of fermented products (Heo et al. 2020). Some bacterial strains were also considered pathogens because they colonize the human rectum and urogenital tract (Devi et al. 2015). The *S. saprophyticus* strain found in jeotgal (a fermented shrimp product from Korea) was not pathogenic (Jo et al. 2022).

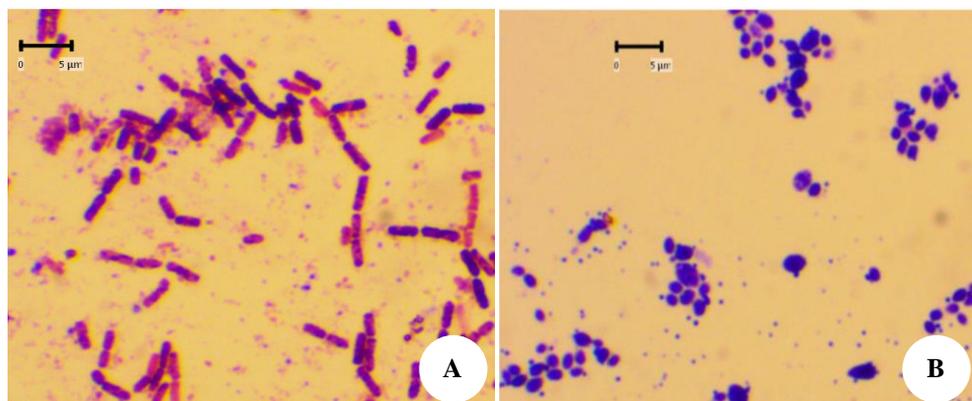


Figure 2. Microscopic characteristics of bacteria isolated from *inasua* on different media (Magnification 1000x). Isolates of INS 04 on MSA (A), and IS 02 on MRSA (B)

Table 1. Selected dominant bacteria isolates of both types *inasua*

Isolate	Description	Length of nucleotide (bp)	Identity (%)	Accession
INS 04	<i>Staphylococcus saprophyticus</i> ATCC 15305	1350	99.56	CP035294.1
	<i>Staphylococcus edaphicus</i> strain CCM 8730		99.56	NR_156818.1
	<i>Staphylococcus</i> sp. S04009		99.48	MH643903.1
IS 02	<i>Bacillus cereus</i> ATCC 14579	1426	99.86	CP034551.1
	<i>Bacillus wiedmannii</i> Strain FSL W8-0169		99.79	NR_152692.1
	<i>Bacillus proteolyticus</i> Strain MCCC 1A00365		99.79	NR_157735.1

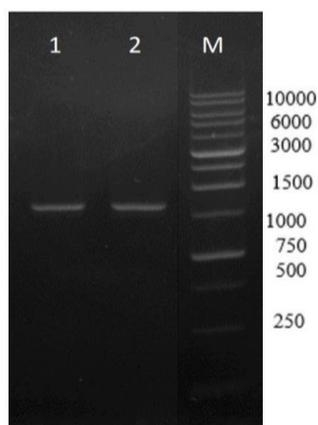


Figure 3. Electropherogram of 16S rRNA gene of bacteria isolates from *inasua* fermentation with a pair primer of 63F and 1387R (Marker 1 kb, 1: INS 04, and 2: IS 02 isolate)

Porkfish as the raw material for *inasua* has a high protein content (>18%) (Đorđević et al. 2016). The protein content of both types of *inasua* decreased during fermentation. The protein content of *inasua* NS and *inasua* S after fermentation for 12 weeks was 19.95 and 17.90%, respectively. The decrease in protein content was caused by proteolytic activity and protein degradation which was one of the important biochemical changes in fermented fish. Both fish endogenous and microbial enzymes decomposed the fish muscle protein to produce low molecular weight compounds such as peptides, free amino acids, and their derivatives during fish fermentation (Gao et al. 2016). These compounds greatly influence the final texture and flavor of the product (Nie et al. 2014).

Besides functioning as a source of protein for most of the world's population, fermented fish products are also a source of minerals needed in the human diet (Zang et al. 2020). The higher ash content indicates the higher the mineral content in the fermented product (Iskandar et al. 2019). The increase in ash content in fermented fish products comes from minerals in the salt used as a preservative. These minerals benefit human health (Khan et al. 2022). The longer the fermentation time causes the table salt to dissolve and diffuse into the fish.

The fat content of *inasua* was almost close to the protein content, i.e., 17.61 and 17.31 % in *inasua* NS and *inasua* S at the end of the fermentation. The fish used as the raw material contains a high-fat content. In contrast to other fish, the fat in porkfish was dominated by wax ester (esterified of long-chain fatty alcohol and long-chain fatty acid). Fat-degrading enzymes cannot break down this compound, so it does not function as a human food ingredient (Howard et al. 2017). Wax esters were naturally present in porkfish food, but these fish could not metabolize these compounds. Wax ester was stored in the fish's body to increase the buoyancy of porkfish at the surface (Đorđević et al. 2016). The decrease in fat content until the end of fermentation was not striking, indicating a lack of lipolytic

microbial activity that played a role during fermentation. Generally, microbes only produce lipase enzymes to break down triacylglycerol.

Generally, the water content of porkfish was not too high compared to other fish. This was due to the fat content of this fish which was close to 18% of body weight (Howard et al. 2017). During fermentation, the water content decreases due to evaporation and is used by microbes to hydrolyze complex molecules (Zhou et al. 2014). *inasua* S has a higher water content than *inasua* NS due to adding coconut sap at the beginning of fermentation. Apart from containing simple sugars, such as sucrose, glucose, and fructose (Femi et al. 2020), this liquid also contains microbes in fermentation. The presence of these simple sugars causes the carbohydrate content of *inasua* S to be higher than *inasua* NS at the beginning of fermentation. These simple sugars decrease at the end of fermentation because microbes rapidly utilize them for their growth. The carbohydrate content of *inasua* NS and *inasua* S after fermentation for 12 weeks was 2.35 and 1.12%, respectively (Figure 4).

The total amino acid contents of *inasua* NS and *inasua* S after fermentation for 12 weeks were 15.31 and 15.85%, respectively. The increase in amino acid content was caused by the proteolytic activity of microbes and protease enzymes in fish which convert several proteins and peptides into amino acids during fish fermentation (Xu et al. 2021). The dominant amino acids in *inasua* were non-essential, such as glutamate, aspartate and glycine. The glycine content in the two types of *inasua* has very striking differences. This amino acid reached 1.9% in *inasua* NS, while *inasua* S only reached 0.9% at the end of fermentation (Figure 5). Glutamic and aspartic acids were responsible for the stronger umami taste which causes porkfish to have good sensory properties (Đorđević et al. 2016).

The total fatty acid contents of *inasua* NS and *inasua* S after fermentation for 12 weeks were 57.08 and 63.81% (in fat content), respectively (Table 2). In general, the levels of fatty acids increase in line with the fermentation process. This indicates that there was hydrolysis of fat into fatty acids as the main precursors of volatile compounds in fish fermentation.

The fatty acid group that dominates *inasua* is monounsaturated fatty acids (MUFA), such as palmitic, oleic, eicosenoic, and nervoic acids; these fatty acids can lower cholesterol levels in the blood. The most dominant fatty acid was oleic acid. This fatty acid was the main constituent of wax esters in porkfish. The human digestive tract cannot absorb wax ester; this compound will decompose into fatty alcohol and fatty acids. The second mentioned molecule can be utilized as a nutrient for humans (Wiech et al. 2020). Apart from wax esters, porkfish fat also contains phospholipids composed of polyunsaturated fatty acids (PUFA) (Howard et al. 2017). Another group of fatty acids found in porkfish *inasua* is Saturated fatty acid (SUFA) which was dominated by palmitic acid. Statistical analysis of the chemical characteristics of the two types of *inasua* after fermentation for 12 weeks showed no significant differences (Table 3).

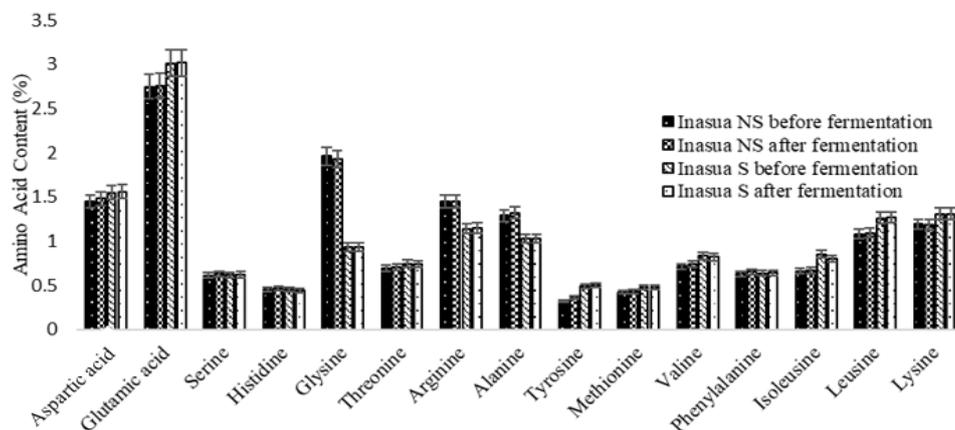


Figure 5. Characteristics of amino acids of both types *inasua* at the beginning and after 12-week fermentation

Table 2. Characteristics of fatty acids of both types *inasua* at the beginning and after 12-week fermentation

Parameter	Fatty acid content (% in fat content)			
	<i>Inasua</i> without sap		<i>Inasua</i> with sap	
	Time of fermentation (week)			
	1	12	1	12
Myristic acid	0.08±0.47	0.09±0.70	0.10±0.00	0.11±0.53
Palmitic Acid	0.77±0.35	0.79±0.07	1.06±0.14	1.08±0.07
Stearic acid	0.65±2.82	0.80±0.00	0.87±2.82	0.88±0.14
Other SUFAs	0.15±0.14	0.19±0.03	0.23±0.14	0.24±0.07
Total SUFAs	1.65±0.09	2.77±0.13	2.26±0.14	2.31±0.13
Palmitoleic acid	0.93±0.14	0.94±0.14	1.13±0.70	1.14±0.14
Oleic acid	37.41±0.07	38.18±0.14	41.52±0.07	43.05±0.70
Eicosenoic acid	8.21±0.01	8.22±0.28	9.41±0.14	9.43±3.53
Nervonic acid	1.28±0.35	1.28±0.01	1.70±0.14	1.71±0.07
Other MUFAs	0.76±0.02	0.94±0.07	1.05±0.14	1.14±0.13
Total MUFAs	48.59±0.18	49.56±0.08	54.81±0.07	56.47±0.13
Linoleic acid	1.05±0.14	1.06±0.07	1.20±0.07	1.20±0.07
Eicosapentaenoic acid (EPA)	0.57±0.01	0.57±0.14	0.81±0.70	0.83±0.07
Docosahexaenoic acid (DHA)	1.20±0.07	1.20±0.35	1.27±0.35	1.28±0.14
Other PUFAs	1.66±0.02	1.92±0.07	1.67±0.13	1.72±0.14
Total PUFAs	4.48±0.14	4.75±0.18	4.95±0.13	5.03±0.07
Total of Fatty Acids	54.72±0.09	57.08±0.14	62.02±0.10	63.81±0.18

Table 3. Difference analysis of chemical characteristics of two types of *inasua* after fermentation for 12 weeks

Sample	Chemical characteristics (%) (mean ± standard deviation)							
	Protein	Fat	Carbohydrate	Moisture	Ash	Salt	Amino acid	Fatty acid
<i>Inasua</i> NS	19.95 ±0.05	17.61 ±0.01	2.35 ±1.73	46.29±3.52	14.57 ±0.02	17.66 ±1.01	15.31 ±0.89	57.08 ±1.12
<i>Inasua</i> S	17.31 ±0.01	17.90 ±0.02	1.12 ±0.01	48.68±0.29	14.22 ±0.01	14.39 ±0.01	15.85 ±1.32	63.81 ±1.35

Note: Significance value: $0.124 > (\alpha) = 0.05$; No significant different

In conclusion, *inasua* without sap and *inasua* with sap have different microbiological and chemical characteristics. The total number of halotolerant and coliform bacterial was higher in *inasua* without sap, while Bacillus and related genera were higher in *inasua* with sap. The total amino and fatty acid contents of *inasua* with sap were higher than *inasua* without sap. The dominant amino and fatty acids in porkfish *inasua* were glutamic and oleic acid. The

dominant bacteria in *inasua* without sap and *inasua* with sap were *Staphylococcus saprophyticus* and *Bacillus cereus*, respectively. Generally, microbiological and chemical characteristics of *inasua* with sap were better than *inasua* without sap, but statistically, it was not significantly different. The results are a reference for further analyzing the sensory characteristics of porkfish *inasua*.

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REFERENCES

- Arunkumar N, Gulsar Banu J, Gopalakrishnan N, Prakash AH. 2017. Wax degrading bacteria: Scope and applications in agriculture. *Intl J Curr Microbiol Appl Sci* 6 (2): 649-664. DOI: 10.20546/ijcmas.2017.602.074.
- Arunkumar N, Banu JG, Gopalakrishnan N, Prakash AH. 2021. Efficacy of lipase-producing, wax-degrading bacteria against the *Solenopsis mealybug*, *Phenacoccus solenopsis* Tinsley and the striped mealybug, *Ferrisia virgata* Cockerell (Homoptera: Pseudococcidae) on cotton. *Pak J Zool* 54 (1): 1-10. DOI: 10.17582/journal.pjz/20191107111143
- Calanche J, Tomas A, Martinez S, Jover M, Alonso V, Roncalés P, Beltrán JA. 2019. Relation of quality and sensory perception with changes in free amino acids of thawed seabream (*Sparus aurata*). *Food Res Intl* 119: 126-134. DOI: 10.1016/j.foodres.2019.01.050.
- Chen Q, Kong B, Han Q, Xia X, Xu L. 2017. The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. *LWT* 77: 389-396. DOI: 10.1016/j.lwt.2016.11.075.
- Chinnamma M, Bhasker S, Binitha Hari M, Sreekumar D, Madhav H. 2019. Coconut neera—A vital health beverage from coconut palms: Harvesting, processing and quality analysis. *Beverages* 5 (1): 1-14. DOI: 10.3390/beverages5010022.
- Chuon MR, Shiimoto M, Koyanagi T, Sasaki T, Michihata T, Chan S, Enomoto T. 2014. Microbial and chemical properties of Cambodian traditional fermented fish products. *J Sci Food Agric* 94 (6): 1124-1131. DOI: 10.1002/jsfa.6379.
- Dan SK, Banerjee G, Nandi A, Ray AK. 2017. Nutritional evaluation of soybean meal after fermentation with two fish gut bacterial strains, *Bacillus cereus* LRF5 and *Staphylococcus caprae* CCF2 in formulated diets for *Labeo rohita* fingerlings. *J Fish* 5 (1): 445-454. DOI: 10.17017/jfish.v5i1.2017.165.
- Daroonpant R, Tanaka N, Uchino M, Tanasupawat S. 2018. Characterization and screening of lipolytic bacteria from Thai fermented fish. *Sains Malaysiana* 47 (1): 91-97. DOI: 10.17576/jsm-2018-4701-11.
- Devi KR, Deka M, Jeyaram K. 2015. Bacterial dynamics during yearlong spontaneous fermentation for production of ngari, a dry fermented fish product of Northeast India. *Intl J Food Microbiol* 199: 62-71. DOI: 10.1016/j.ijfoodmicro.2015.01.004.
- Đorđević Đ, Buchtova H, Borkovcova I. 2016. Estimation of amino acids profile and escolar fish consumption risks due to biogenic amines content fluctuations in vacuum skin packaging/VSP during cold storage. *LWT-Food Sci Technol* 66: 657-663. DOI: 10.1016/j.lwt.2015.11.020.
- Faithong N, Benjakul S. 2014. Changes in antioxidant activities and physicochemical properties of kapi, a fermented shrimp paste, during fermentation. *J Food Sci Technol* 51: 2463-2471. DOI: 10.1007/s13197-012-0762-4.
- Femi BMR, Malhija N, Mary TS, Kumaran T. 2020. Coconut (*Cocos nucifera*) inflorescence sap nutritional characteristics and health effects. *J Basic Appl Res Biomed* 6 (1): 50-52. DOI: 10.51152/jbarbiomed.v6i1.9.
- Feng L, Tang N, Liu R, Gong M, Wang Z, Guo Y, Chang M. 2021. The relationship between flavor formation, lipid metabolism, and microorganisms in fermented fish products. *Food Funct* 12 (13): 5685-5702. DOI: 10.1039/D1FO00692D.
- Gao P, Wang W, Jiang Q, Xu Y, Xia W. 2016. Effect of autochthonous starter cultures on the volatile flavour compounds of Chinese traditional fermented fish (Suan yu). *Intl J Food Sci Technol* 51 (7): 1630-1637. DOI: 10.1111/ijfs.13134.
- Hebbbar KB, Arivalagan M, Pavithra KC, Roy TK, Gopal M, Shivashankara KS, Chowdappa P. 2020. Nutritional profiling of coconut (*Cocos nucifera* L.) inflorescence sap collected using novel coco-sap chiller method and its value added products. *J Food Meas Characterization* 14: 2703-2712. DOI: 10.1007/s11694-020-00516-y.
- Heo S, Lee J-H, Jeong D-W. 2020. Food-derived coagulase-negative *Staphylococcus* as starter cultures for fermented foods. *Food Sci Biotechnol* 29: 1023-1035. DOI: 10.1007/s10068-020-00789-5.
- Houéssou MB, Yéouassi CAR, Zanmenou W, Mossi I, Suanon F, Yovo PD. 2019. Nutritional composition of fatty acids and amino acids of the fermented *Scomberomorus tritor* in Benin. *Sci J Chem* 7 (1): 19-25. DOI: 10.11648/j.sjc.20190701.14.
- Howard J, Yamauchi A, Nakano T, Yamaguchi T, Ochiai Y. 2017. Characterization of composition and lipid in various deep sea fish species from Suruga Bay. In: The JSFS 85th Anniversary-Commemorative International Symposium. The Japanese Society of Fisheries Science, 22-24 September 2017. [Japan]
- Iskandar, Andriani Y, Rostika R, Zidni I, Riyanti NA. 2019. Effect of using fermented Lemna sp. in fish feed on growth rate of Nile carp (*Osteochilus hasselti*). *World News Nat Sci* 26: 157-166.
- Jawad LA. 2018. Poisonous fishes. In: Jawad LA (eds). *Dangerous Fishes of the Eastern and Southern Arabian Peninsula*. Springer International Publishing, Switzerland. DOI: 10.1007/978-3-319-57926-9_5.
- Jo E, Hwang S, Cha J. 2022. Transcriptome analysis of halotolerant *Staphylococcus saprophyticus* isolated from Korean fermented shrimp. *Foods* 11 (4): 524. DOI: 10.3390/foods11040524.
- Khair T, Khan MM, Khan MNA, Reza MS. 2020. Physicochemical, microbiological and sensory characteristics of fish sauce from spined anchovy (*Stolephorus tri*). *Bangladesh J Fish* 32 (1): 125-134. DOI: 10.52168/bjf.2020.32.14.
- Khan M, Afrin M, Anjum M, Khatun A, Sabekunnahar M, Tannisa IJ, Islam MMA. 2022. Review of traditional fish processing methods practiced in Bangladesh. *Ecol J* 4 (1): 101-112.
- Khusro A, Aarti C, Salem AZM, Barbabosa-Pilego A. 2020. Technofunctional traits and safety aspects of coagulase-negative *Staphylococcus saprophyticus* isolated from traditional fermented food. *Food Biotechnol* 34 (1): 77-99. DOI: 10.1080/08905436.2019.1711114.
- Koesoemawardani D, Hidayati S, Subeki. 2018. Amino acid and fatty acid compositions of rusip from fermented anchovy fish (*Stolephorus* sp). In: The 3rd International Conference on Science, Technology, and Interdisciplinary Research (IC-STAR). IOP Publishing, 18-20 September 2017. DOI: 10.1088/1757-899X/344/1/012005. [Indonesia]
- Lee SH, Jung JY, Jeon CO. 2014. Effects of temperature on microbial succession and metabolite change during saeu-jeot fermentation. *Food Microbiol* 38: 16-25. DOI: 10.1016/j.fm.2013.08.004.
- Ma X, Bi J, Li X, Zhang G, Hao H, Hou H. 2021. Contribution of microorganisms to biogenic amine accumulation during fish sauce fermentation and screening of novel starters. *Foods* 10 (11): 2572. DOI: 10.3390/foods10112572.
- Mahulette F, Mubarik NR, Suwanto A, Widanarni W. 2018a. Diversity of lactic acid bacterial in *inasua* fermentation. *Iran J Microbiol* 10 (5): 314-323.
- Mahulette F, Mubarik NR, Suwanto A, Widanarni W. 2018b. Microbiological and physicochemical characteristics of *inasua* traditional fish fermented from Maluku Islands. *Biosaintifika: J Biol Biol Educ* 10 (2): 298-305. DOI: 10.15294/biosaintifika.v10i2.13537.
- Mahulette F, Kurnia TS. 2021. Karakteristik mikrobiologi dan kimia *inasua* ikan babi (*Ruvettus tydemani* Weber) dengan dan tanpa nira kelapa. *Jurnal Pengolahan Pangan* 6 (1): 1-6. DOI: 10.31970/pangan.v6i1.41. [Indonesian]
- Maulidayanti S, Mubarik NR, Suryani. 2019. Characterisation of a bacteriocin produced by *Lactobacillus rhamnosus* IN13 isolated from *inasua*, a fermented fish product from Central Maluku, Indonesia. *Intl Food Res J* 26 (5): 1557-1563.
- Natarajan N, Rajikkannu M. 2014. Antimicrobial activity of *Bacillus cereus* strain isolated from rohu (*Labeo rohita*). *Intl J Curr Microbiol Appl Sci* 3 (8): 474-480.
- Nendissa SJ. 2013. Pengaruh penambahan *Pediococcus acidilactici* F11 sebagai kultur starter terhadap kualitas ikan asin (*inasua*) bae (*Lutjanus malabaricus*). *Ekosains* 2 (1): 39-46. [Indonesian]
- Ngasotter S, Waikhom D, Mukherjee S, Devi MS, Singh AS. 2020. Diversity of Lactic Acid Bacteria (LAB) in fermented fish products:

- A review. *Intl J Curr Microbiol Appl Sci* 9 (5): 2238-2249. DOI: 10.20546/ijcmas.2020.905.255.
- Nie X, Lin S, Zhang Q. 2014. Proteolytic characterisation in grass carp sausage inoculated with *Lactobacillus plantarum* and *Pediococcus pentosaceus*. *Food Chem* 145: 840-844. DOI: 10.1016/j.foodchem.2013.08.096.
- Ojagh SMA, Fallah N, Nasernejad B. 2020. Biological treatment of organic compounds in produced water with use of halotolerant bacteria. *J Environ Chem Eng* 8 (6): 104412. DOI: 10.1016/j.jece.2020.104412.
- Özcelik S, Kuley E, Özogul F. 2016. Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria. *LWT* 73: 536-542. DOI: 10.1016/j.lwt.2016.06.066.
- Pandiselvam R, Manikantan MR, Binu SM, Ramesh SV, Beegum S, Gopal M, Shil S. 2021. Reaction kinetics of physico-chemical attributes in coconut inflorescence sap during fermentation. *J Food Sci Technol* 58: 3589-3597. DOI: 10.1007/s13197-021-05088-3.
- Patel P, Patel B, Amaresan N, Joshi B, Shah R, Krishnamurthy R. 2020. Isolation and characterization of *Lactococcus garvieae* from the fish gut for in vitro fermentation with carbohydrates from agro-industrial waste. *Biotechnol Rep* 28: e00555. DOI: 10.1016/j.btre.2020.e00555.
- Pardo MÁ, Jiménez E, Pérez-Villarreal B. 2016. Misdescription incidents in seafood sector. *Food Control* 62: 277-283. DOI: 10.1016/j.foodcont.2015.10.048.
- Persulesy CB, Kusdiyantini E, Ferniah RS, Agustini TW, Budiharjo A. 2020. *Inasua*: The traditional food fermentation from Teon Nila Serua, Central of Maluku, Indonesia. *J Ethn Foods* 7 (1): 1-7. DOI: 10.1186/s42779-020-00055-3.
- Prihanto A, Nurdiani R, Nursyam H, Muyasharoh H, Hayati Royani L. 2021. Terasi, an Indonesian fermented shrimp paste, a new reported source of L-Asparaginase-Producing bacteria. *Res J Biotechnol* 16 (1): 178-181.
- Rehman R, mazhar s, Gohar M. 2022. Evaluation of microbial contamination in meat and its control using preservatives. *Intl J Innov Sci Technol* 4 (2): 404-415. DOI: 10.33411/IJIST/2022040211.
- Sakpetch P, Benchama O, Masniyom P, Salaipeth L, Kanjan P. 2022. Physicochemical characteristics and flavor profiles of fermented fish sauce (budu) during fermentation in commercial manufacturing plant. *J Food Sci Technol* 59: 693-702. DOI: 10.1007/s13197-021-05064-x.
- Shan K, Yao Y, Wang J, Zhou T, Zeng X, Zhang M, Ke W, He H, Li C. 2023. Effect of probiotic *Bacillus cereus* DM423 on the flavor formation of fermented sausage. *Food Res Intl* 172: 113210. DOI: 10.1016/j.foodres.2023.113210.
- Siddegowda GS, Bhaskar N, Gopal S. 2016. Biochemical and bacteriological quality of rohu (*labeo rohita*) head sauce produced by enzymatic and fermentation method. *Fish Technol* 53 (3): 220-231.
- Stavropoulou DA, De Maere H, Berardo A, Janssens B, Filippou P, De Vuyst L, De Smet S, Leroy F. 2018. Species pervasiveness within the group of coagulase-negative staphylococci associated with meat fermentation is modulated by pH. *Front Microbiol* 9: 2232. DOI: 10.3389/fmicb.2018.02232.
- Thapa N, Pal J, Tamang JP. 2004. Microbial diversity in Ngari, Hentak and Tungtap, fermented fish products of North-East India. *World J Microbiol Biotechnol* 20: 599-607. DOI: 10.1023/B:WIBI.0000043171.91027.7e.
- Wattimena SC, Temartenan JS, Lesbatta KJ. 2021. The effect of coconut sap amount and salt texture on the protein content and total bacterial number in *inasua*. *J Phys Conf Ser* 1943 (1): 012064. DOI: 10.1088/1742-6596/1943/1/012064.
- Wiech M, Silva M, Meier S, Tibon J, Berntssen MH, Duinker A, Sanden M. 2020. Undesirables in mesopelagic species and implications for food and feed safety—insights from Norwegian Fjords. *Foods* 9 (9): 1162. DOI: 10.3390/foods9091162.
- Xu Y, Zang J, Regenstein JM, Xia W. 2021. Technological roles of microorganisms in fish fermentation: A review. *Crit Rev Food Sci Nutr* 61 (6): 1000-1012. DOI: 10.1080/10408398.2020.1750342.
- Zang J, Xu Y, Xia W, Regenstein JM. 2020. Quality, functionality, and microbiology of fermented fish: A review. *Crit Rev Food Sci Nutr* 60 (7): 1228-1242. DOI: 10.1080/10408398.2019.1565491.
- Zeng X, Chen X, Zhang W. 2016. Characterization of the microbial flora from Suan Yu, a Chinese traditional low-salt fermented fish. *J Food Process Preserv* 40 (5): 1093-1103. DOI: 10.1111/jfpp.12690.
- Zhou X-X, Zhao D-D, Liu J-H, Lu F, Ding Y-T. 2014. Physical, chemical and microbiological characteristics of fermented surimi with *Actinomyces elegans*. *LWT-Food Sci Technol* 59 (1): 335-341. DOI: 10.1016/j.lwt.2014.05.045.