Biosaintifika 10 (2) (2018) 298-305

Biosaintifika

Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Microbiological and Physicochemical Characteristics of Inasua Traditional Fish Fermented from Maluku Islands

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DOI: http://dx.doi.org/10.15294/biosaintifika.v10i2.13537

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History Article

Received 26 February 2018
Approved 12 June 2018
Published 30 August 2018

Keywords

Inasua; Lactic Acid Bacteria; Proximate Analysis; Total Number Bacteria

Abstract

Based on the raw materials, inasua consists of two types namely inasua with sap and inasua without sap. Research of inasua with sap has never been done and considered as the novelty of this research. The sensory characteristics and shelf life of two types of inasua were different. The research aims to analyze the microbiological and physicochemical characteristics of two types of inasua during fermentation. The microbiological analyzes include the total number of bacteria and lactic acid bacteria, while physicochemical analyzes include temperature, pH, water activity, proximate analysis, salt, alcohol, histamine, amino acids and fatty acids contents. The total number of bacteria and lactic acid bacteria has decreased during fermentation. At the end of the fermentation the total number of bacteria and lactic acid bacteria inasua with sap were 3.2×10^7 CFU/g and 3.0×10^7 CFU/g, while inasua without sap were 5.4×10^5CFU/g and 3.5×10^5 CFU/g, respectively. The moisture, protein, alcohol contents and water activity decreased, otherwise the salt, fat, ash, amino acids, and fatty acids contents increased during fermentation. Generally, microbiological and physicochemical characteristics of inasua with sap was better than inasua without sap. The results of this research to improve the quality of this fermentation product in the future.

How to Cite

Mahulette, F., Mubarik, N. R., Suwanto, A., & Widanarni. (2018). Microbiological and Physicochemical Characteristics of Inasua-Traditional Fish Fermented from Maluku Islands. *Biosaintifika: Journal of Biology & Biology Education*, 10(2), 298-305.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Inasua is a traditionally fermented fish produced by the communities in Teon, Nila and Serua (TNS) Islands, Maluku, Indonesia. This process is done to exploit the abundant fishery resources in the Banda Sea and as a food reserve to anticipate the lean time when fisherman could not go to sea (Mahulette *et al*., 2016). Inasua is a fish fermentation soaked in saline solution so often also called wet salt fish (Nendissa, 2013). The added salt ranges from 20 to 30%. Coconut sap is also added in the early of processing to extend the shelf life of inasua for more than a year. The coconut sap is a liquid that tapped from unopened inflorescence of coconut (*Cocos nucifera*). This liquid has a higher sugar content (Law *et al.*, 2011). Processing of inasua is not always use of coconut sap. When coconut sap is difficult to obtain, it is processed by adding salt only with known as inasua with sap (designated as inasua-S) and inasua without sap (designated as inasua-NS).

Both of these fermented products have different sensory characteristics. Inasua-S has a softly texture, slightly salty and flavorful, whereas inasua-NS has a hard texture, very salty, taste like sand and less flavorful (Nendissa, 2013). Fermentation of inasua-NS has similarities with the common fish fermentation, while fermentation of inasua-S is soaked in salt solution mixed with sap. This causes both of these fermented fish have different physicochemical characteristics, especially moisture, salt and carbohydrate contents. Although soaked in salt solution, the inasua-S have a longer shelf life than the inasua-NS.

The sensory characteristics and shelf life of a fish fermented product are very influenced by microbiological and physicochemical characteristics. The shelf life of a fermentation product is largely determined by the total number bacteria, especially lactic acid bacteria. These bacteria belong to the category of Generally Recognized as Safe (GRAS) so it is safe to be in a food product and act as probiotic. The spontaneous fermentation that causes the physicochemical characteristics of inasua is uncontrolled. The research aims to analyze the microbiological and physicochemical changes during spontaneous fermentation of inasua-S and inasua-NS obtained from local producer. The result of the research can be used to improve the quality of inasua.

METHODS

The inasua used in this research was taken from traditional producer in Layeni village, Sub district of Teon, Nila and Serua-Waipia, Ceram Island consisting of producer of inasua-S and inasua-NS. The processing of inasua was done by producers. The raw material of inasua was gurara fish (*Lutjanus vitta*) obtained from the sea around the Ceram Island. The materials used in this research were chemical materials for proximate analysis, salt, alcohol, histamine contents, amino and fatty acids

A total of 5 kg of gurara fish was split longitudinally, remove the gills and viscera, smeared with 20-30% salt of the weight of the fish then put in sacks and pressed with stones for 2-3 hours. Fish that has been pressed then put into a jar and added coconut sap until soaked. The jar closed and the fish allowed to fermentation at room temperature for 12 weeks to produce inasua-S. In the processing of inasua-NS not added coconut sap. Analysis of physicochemical characteristics were done in the first week and after fermentation 12 weeks, except for temperature and pH were measured during the fermentation process. The research was conducted in the Laboratory of Microbiology IPB, Integrated Laboratory of Bogor Agricultural University and Fish Inspection and Quality Control Laboratory.

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) media containing 1% $CaCO_3$ with 3%, 5% and 10% NaCl and nutrient agar (NA), respectively were poured on it and incubated at room temperature for 48 hours. All the isolates obtained were stained with Gram and spore staining, catalase test, and fermentation of carbohydrates (Saithong *et al.,* 2010; Fan *et al.* 2013).

The measurement of temperature, pH and water activity were use a thermometer, pH meter and *aw* meter. For temperature and pH determination, 10 g homogenized of sample were mixed with 90 ml of carbon dioxide free distilled water. Direct temperature and pH measurement were measured using a standard digital food thermometer (TP-300, China) and pH meter (CT-6022, Shenzhen Kedida Electronic, China). The temperature and pH were measured during fermentation. For water activity determination, 1 g of sample was inserted into aw meter (Rotronic, US) (Kopermsub & Yunchalard, 2008).

The proximate analysis and alcohol content based on AOAC (2012). The protein and lipid content were determined using Kjeldahl and Soxhlet methods, while moisture and ash content using desiccation method. The total of carbohydrate was determined using by difference method. The salt content was determined as the amount of sodium chloride using silver nitrate titration according Mohr method. For alcohol content determination, sample was diluted and injected to gas chromatography (Fid 17A, Schimdzu, Japan) and determined according retention time. Salt content was calculated from the volume of 0.1 M silver nitrate used to reach the end point of titration and reported as % sodium chloride (Kopermsub & Yunchalard, 2008). Histamine content determined use spectrofluorometer (Single BIM, Egilent Laboratories) (AOAC, 2012).

The analysis of amino acid was performed by filtering the hydrolyzed sample using millipore paper then adding borate potassium buffer and orthoptlaldehyde reagent. A total of 5 ml of sample injected into high performance liquid chromatography (HPLC) (20A, Shimadzu, Japan) column and left for 25 minutes. For analysis of fatty acid content. A total of 30 mg sample was added 1 ml NaOH 0.5 N then heated for 20 minutes. The formed solution added 2 ml of BF3 16% and heated for 20 minutes. After cooling, the solution was added 2 ml of saturated NaCl and 1 ml hexane. The formed hexane layer was injected into gas chromatography (GC) (Fid 17A, Schimdzu, Japan) (AOAC 2012). All measurement was reported as dry basis.

RESULT AND DISCUSSION

The Number of Microbes during Inasua Fermentation

The total number of bacteria in inasua-S was $2.2x10^8$ CFU/g higher than inasua-NS which only reached $5.3x10^6$ CFU/g at the beginning of fermentation. As well as the amount of lactic acid bacteria (LAB). The total number of LAB in inasua-S was $4.5x10^7$ CFU/g higher than inasua-NS which only reached $4.5x10^6$ CFU/g. This is caused by added of coconut sap as sources of carbohydrates and microbe, including LAB in inasua-S. The total number of bacteria were decreased sharply in the second week of fermentation. This decrease was due to high acid and alcohol accumulation at the beginning of fermentation. After fermentation for 4 weeks, the number of bacteria decreases more slowly until the end of fermentation. The total number of bacteria and LAB in inasua-S higher than inasua-NS almost 2 log cycles at the end of fermentation (Table 1).

Morphologically characterization of selected LAB isolates showed varying sizes and shape, white with slippery edges. The shape and size vary due because isolate of LAB use pour method. Several isolates embedded in the media showed different shapes and sizes than isolates on the surface MRSA media. White colony with slippery edge was characteristic of LAB colony. The results of Gram staining showed all isolates including Gram positive. At the beginning of fermentation found only rod-shaped bacteria, while at the end of fermentation found rod and coccus-shaped bacteria. The catalase test showed that all isolates were negative catalase, except for the last 3 isolates that found in inasua-NS fermentation which were *Staphylococcus* (Table 2).

The Changes of Temperature and pH during Inasua Fermentation

The temperature of inasua-S and inasua-NS were increases until the end of fermentation. After 4 weeks fermentation, the temperature of the inasua-NS was 26.0°C lower than inasua-S that reaches 29.6°C but after 8 weeks until the end of fermentation there was no noticeable dif-

Table 1. The total number of bacteria and LAB during inasua fermentation

Samples	Time of fermentation (week)	Total number of bacteria (CFU/g)	Total number of LAB (CFU/g)
Inasua-S	1	$2.2x10^8$	$4.5x10^7$
	2	$4.4x10^7$	$4.1x10^7$
	4	$3.5x10^7$	$3.3x10^7$
	8	$3.4x10^7$	$3.2x10^7$
	12	$3.2x10^7$	$3.0x10^7$
Inasua-NS	1	$5.3x10^6$	$4.5x10^6$
	2	$3.5x10^6$	$1.6x10^6$
	4	$3.3x10^6$	$1.3x10^6$
	8	$9.6x10^5$	$9.0x10^5$
	12	$5.4X10^5$	$3.5X10^5$

ference. At the end of the fermentation the temperature of the inasua-S was 28.2°C, whereas inasua-S was 29.8°C (Figure 1).

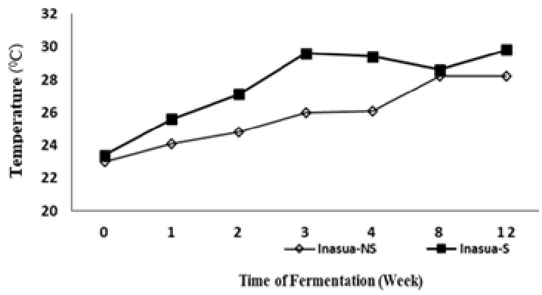

Figure 1. The changes of temperature during inasua fermentation

The increasing temperature of both types of inasua during fermentation were caused the microbial activity that plays a role in fermentation. The optimum temperature for bacterial activity and proliferation in fish fermentation is 30°C (Kopermsub & Yunchalard, 2008). Higher temperatures also increase the rate of bacterial metabolism, especially, an enzymatic fermentation process.

The pH of both types of inasua were showed noticeable changes as the increases of fermentation time. At the beginning of fermentation pH of inasua-NS and inasua-S were 6.36 and 6.67, respectively. After first week fermentation, pH of inasua-S sharply decreased to reach 4.57. After 3 weeks fermentation, the pH of both types of inasua decreased to 5.52 in inasua-NS and 4.45 in inasua-S, then increased until the end of fermentation. The pH of the inasua-NS and inasua-S at the end of fermentation were 6.27 and 4.91, respectively (Figure 2).

The pH values of both types of inasua decreased during fermentation. Generally the life fish has a pH of about 7, but after death, the residual of glycogen in the fish meat is degraded

Table 2. Morphologically characteristic of selected LAB isolated on MRSA added NaCl and $CaCO_3$.

Isolates	Gram	Characteristic of Colony					Catalase Test
		Forms	Sizes	Pigments	Margins	Elevations	
IS-01	+	Circular	Medium	White milk	Entire	Raised	-
IS-02	+	Circular	Medium	White milk	Entire	Unclear*	-
IS-04	+	Oval	Medium	White milk	Entire	Unclear*	-
IS-05	+	Elongated	Medium	White milk	Entire	Raised	-
IS-06	+	Elongated	Medium	White milk	Entire	Unclear*	-
IS-07	+	Circular	Small	White milk	Entire	Raised	-
IS-12	+	Circular	Medium	White milk	Entire	Raised	-
IS-13	+	Circular	Medium	White milk	Entire	Unclear*	-
IS-15	+	Circular	Small	White milk	Entire	Convex	-
IS-17	+	Elongated	Medium	White milk	Entire	Unclear*	-
IS-19	+	Circular	Medium	White milk	Entire	Raised	-
IS-27	+	Elongated	Medium	White milk	Entire	Unclear*	-
INS-03	+	Circular	Small	White milk	Entire	Raised	-
INS-05	+	Elongated	Medium	White milk	Entire	Unclear*	-
INS-06	+	Circular	Small	White milk	Entire	Raised	-
INS-12	+	Circular	Medium	White milk	Entire	Raised	-
INS-13	+	Elongated	Medium	White milk	Entire	Unclear*	-
INS-17	+	Circular	Medium	White milk	Entire	Unclear*	-
INS-23	+	Circular	Medium	White milk	Entire	Raised	+
INS-24	+	Circular	Medium	White milk	Entire	Raised	+
INS-25	+	Circular	Medium	White milk	Entire	Raised	+

IS = Inasua-S; INS = Inasua-NS; * = Embedded in the medium into acidic compounds so that decreasing the pH value. This phase is called rigor mortis. The pH value of fish is strongly influenced by the amount of glycogen and buffering capacity in fish meat. Buffering capacity is determined by the composition of histamine related compound and inorganic phosphate (Abe, 2000).

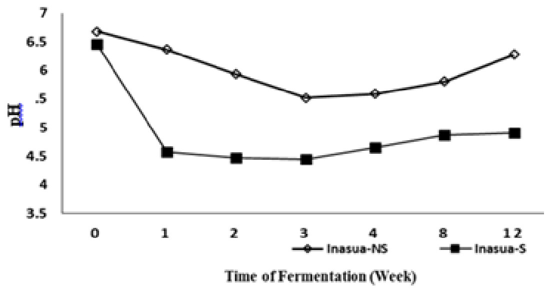

Figure 2. The shift of pH during inasua fermentation

The Changes of Physicochemical Characteristics in Inasua Fermentation

The proximate analysis of both types of inasua were showed that lipid and ash contents increased, otherwise the protein content and moisture decreased until the end of fermentation. At the beginning of fermentation, the carbohydrate content of inasua-S was 25.64% higher than inasua-NS that only reaches 16.32%, but the carbohydrate content of inasua with sap decreased to 7.14% at the end of fermentation, otherwise carbohydrate content of inasua-NS has increased up to 19.69%. At the end of fermentation, the moisture, protein and fat contents of inasua-S were higher than inasua-NS. The protein and fat content of inasua-S were 22.18% and 23.74% higher than inasua-NS which only reaches 18.3% and 10.5%, respectively.

Water activity of both types of inasua decreased during fermentation, although in inasua-S decrease was not noticeable. At the beginning of fermentation, water activity of inasua-NS was 0.83 and than decrease to 0.74, whereas in inasua-S, it decrease from 0.93 to 0.92 at the end of fermentation. The salt content of inasua-NS was higher than inasua-S. The salt content of inasua-NS was 39.89%, whereas inasua-S only reaches 13.26% at the end of fermentation. At the beginning of fermentation, the alcohol content of inasua-S was 13.21% and then decrease to 8.66% at the end of fermentation, whereas alcohol content of inasua-NS was almost undetectable. The histamine content of inasua-S was 1.63 mg/kg, while in inasua-NS was undetectable (Table 3).

The addition of coconut sap containing simple sugars at the beginning of fermentation is utilized by acid producing microbes to form acidic compounds so that the pH decreases very rapidly after 3 weeks fermentation. The sharply decrease of pH is also influenced by the buffering capacity of fish meat. Fish that used as the raw materials of inasua processing is reef fish. These fish have lower buffering capacity than pelagic fish (Abe, 2000). Fish that have a low buffering capacity is better used as a raw materials of fish fermentation because it has a longer shelf life. The changes of pH during fermentation of inasua-NS not notice-

Table 3. Physicochemical characteristics in Inasua Fermentation (dry basis)

Parameter	Time of fermentation (week)			
	Inasua-NS		Inasua-S	
	1	12	1	12
Moisture (%)	137.13	90.45	259.19	253.83
Carbohydrate content (%)	16.32	19.69	25.64	7.14
Protein content (%)	45.26	18.03	43.17	22.18
Fat content (%)	3.84	10.05	4.13	23.74
Ash content (%)	37.9	49.28	28.73	44.83
Salt content (%)	30.02	35.89	10.20	13.26
Alcohol content (%)	□0.03	□0.03	13.21	8.66
Histamine content (mg/kg)	-	nd	-	1.63
Water activity	0.83	0.74	0.93	0.92
Amino acid (%)	42.32	39.91	33.90	54.17
Fatty acid (% in fat content)	112.59	111.00	260.73	309.55

nd = not detected

able because the salt content of this type inasua is higher than inasua-S. The addition of high salt content in fish fermentation is caused decrease of pH to be slow, although fermentation for a long time (Owens & Mendoza, 1985).

In the middle of fermentation, the pH of both types of inasua were increased. Besides being caused by the degradation of amino acids to form nitrogen containing compounds, some lactic acid bacteria also utilize acidic compounds from the degradation of carbohydrates as substrates for growth (Unden & Zaunmuller, 2009). The utilization of amino acids and acidic compounds as substrate by microbes takes place in the middle of fermentation because it is repressed by high sugar content at the beginning of fermentation (Jonsson *et al.*, 1983).

The moisture is essential for microbial growth and enzyme activity that plays a role in fermentation (Anggo *et al.*, 2015). The moisture in a fish fermented is correlated with the water activity (*aw*). Water activity describes the amount of free water in food products that microbial can use for growth. Generally the *aw* values below 0.96 can inhibit the growth of most Gram negative bacteria. Lactic acid bacteria such as *Lactobacillus* is to tolerate low *aw* and may act as competitor microbe during fish fermentation process (Francoise, 2010).

The increased of salt content indicate the molecular penetration is more perfect in the meat of fish during fermentation. At the beginning of fermentation, the salt granules surround the fish flesh. As the fermentation time increases, the salt grains become dissolved because the high surface moisture of fish meat. When solubility reaches saturation point, the salt penetrates into and rejects the water molecule out of the fish meat. Salt can reduce the water holding capacity of fish meat so that the shelf life of a fermented fish product is longer (Jittrepotch *et al.*, 2015). Besides being hygroscopic, salt is also acts as a bacteriostatic agent to against pathogenic and spoilage bacteria in fish meat.

The high salt content of inasua-NS cause a few microbes that can grow and use carbohydrates as a source of energy in fermentation. In contrast to the inasua-S fermentation. After the first week of fermentation, these carbohydrate content was higher than inasua-NS. This indicates still many carbohydrates that have not been degraded by microbes. The high content of carbohydrates in inasua with sap caused the addition of coconut sap at the beginning of fermentation, but the carbohydrate content decrease rapidly at the end of fermentation because most of the carbohydrate, especially those from coconut sap have been degraded into organic acids and alcohols by microbes that play a role in fermentation. The composition of coconut sap that containing simple sugars is more easily degraded by microbes than complex carbohydrates.

The protein content of a fermented fish product is strongly influenced by the availability of proteolytic enzymes (Chadong *et al.*, 2015) and salting. High salting may inhibit the activity of some proteolytic enzymes (Xu *et al.*, 2008). The predominant salt tolerant bacteria in inasua without sap has a high proteolytic ability in fish fermentation. Tolerance to high levels of salt causes fish proteins to be rapidly degraded by these bacteria. In contrast to the fermentation inasua with sap. Lactic acid bacteria as the dominant microbes in these type of inasua has low proteolytic activity (Barrangou *et al.*, 2012). Nevertheless, the accumulation of acidic compounds produced by these bacteria and the low salt content of the inasua with sap can accelerate the rate of protein degradation because proteolytic enzymes work optimally under acidic conditions with low salinity (Klomklao, 2008).

Fish used as the raw materials in the processing of inasua is gurara fish. These fish are classified as lean fish because they have low fat content (Murillo *et al.*, 2014). The fat content of both types of inasua were similar in the first week of fermentation, but at the end of fermentation the fat content of the inasua-S was higher than inasua-NS. The difference of fat content in both types of inasua because salt tolerant bacteria in inasua-NS has high lipolytic ability, compared to lactic acid bacteria in inasua with sap (Boulares *et al.*, 2012)

The ash content consists of a number of minerals in inasua. The ash content was mostly sourced from the addition of salt in fish fermentation. The salt used in the processing of inasua is table salt. Beside being containing sodium and chloride (NaCl), the salt also contains trace elements, such as iron, magnesium, sulfur and iodine (Nafees *et al.*, 2013). The high salt content of inasua-NS correlates with the high ash content of this fish fermented than inasua-S.

The high levels of alcohol in inasua-S caused the addition of coconut sap that has low alcohol content and simple sugars that can be utilized rapidly by microbe, especially yeast as the main alcohol producer in fermentation. The decrease of alcohol content at the end of fermentation of inasua-S was not noticeable because some lactic acid bacteria are able to produce alcohol through hetero-fermentative pathways. The alcohol content of inasua-NS almost was not detected during fermentation. High salt content cause the microbes could not produce alcohol.

The histamine content of inasua-S was 1.63 mg/kg, whereas inasua-NS was not detected. This is because a number of lactic acid bacteria, especially *Lactobacillus* were able to produce histamine (Fernandez & Zuniga, 2006). Nevertheless, the content very well below the threshold by Food and Drug Administration that 50 mg/kg (FDA, 2011). The content of histamine was not detected in inasua-NS because the high salt content of the fermented end product. The amino and fatty acid content of both types of inasua were increased because degradation of protein and fat during fermentation. At the end of fermentation, the amino acid content of inasua-S was 54.17% higher than inasua-NS that only reached 39.91%, while the fatty acid content of inasua-S was 309.55% higher than inasua-NS that only reaches 111.00% (in fat content). Addition of coconut sap that contains bacteria and yeast in inasua-S cause the microbe that play a role in the fermentation is more complex. The fermentation involve bacteria and yeast cause degradation of protein and fat to take place more rapidly (Hidayat *et al.*, 2015).

Inasua is a local fermented product that has not been widely studied. Research of inasua-S has never been done. This is considered as the novelty of this research. The results of this research are expected to information about microbiological and physicochemical characteristics to improve the quality of this fermentation product in the future.

CONCLUSION

Inasua with sap and inasua without sap have different microbiological and physicochemical characteristics. The total number of bacteria and lactic acid bacteria has decreased during fermentation. The moisture, protein, alcohol contents and water activity decreased, otherwise the salt, fat, ash, amino acids, and fatty acids contents increased during fermentation. The carbohydrate content of inasua without sap was increased, while at inasua with sap decreased. Generally, microbiological and physicochemical characteristics of inasua with sap was better than inasua without sap.

ACKNOWLEDGEMENTS

The Research was funded by the Ministry of Research, Technology and Higher Education through Excelent Strategic Research Scheme on 2017 to second author and Domestic Scholarship for Graduate Education 2014 to first author.

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