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CHANGES OF AMINO AND FATTY ACIDS IN ANCHOVY (*Stolephorus* sp) FERMENTED FISH PASTE WITH DIFFERENT FERMENTATION PERIODS

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Abstract

Fish paste is popular condiment that possessing a characteristic appetite-stimulating aroma. Nowadays many fermented fish paste prefer to use lower salt to produce fish paste. Fermented fish paste from dried anchovy fish was prepared with 2% NaCl (w/w), fermented at room temperature for 8 and 32 days of fermentation. The dried anchovy fish obtain from Rejomulyo Market, Central Java, with ± 7 cm of size. The method of research was experimental field. Biochemical changes during fermentation were investigated. Result revealed that during fermentation, pH and water content value were increased. Amino acid composition showed that the contents of glutamic acid, aspartic acid, lysine, leucin and arginin were higher than other amino acids. Free amino acid content of fermented fish paste was fluctuated during the fermentation period but most of free amino acids were increased. Almost all fatty acids decreased during fermentation except stearic acid, EPA and DHA. The essential fatty acid found was linolenic acid which was higher in initial period. Content of amino and fatty acids may contribute to the taste and flavor of fermented fish paste. From the nutritional view, the use of low salt concentration (2%) in producing fish paste was still able to contribute the fermentation process, avoid putrefaction and result in protein, some amino acid and fatty acid increase during early fermentation. But allegedly the shelf life of this paste is shorter because in the 32 days fermentation, the pH start to increase which means the volatile base compound formed already high.

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Keywords: Anchovy; fish paste; fermentation period; amino acids; lipid acids

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1. Introduction

Fish paste is popular condiment that possessing a characteristic appetite-stimulating aroma. Fish paste is obtained through fermentation of fish or shrimp with 20-25% salt under ambient conditions (1). Fish fermentation is one way to preserve food products, enhance nutritive value, destroy undesirable factors, improve the appearance and taste of food, reduce the energy required for cooking, and make a safer product (2), have a high nutrition, unique flavor and long storage capability (3). Microorganisms and endogenous enzymes activities can convert organic substances into simple compounds such as peptides, amino acids, other nitrogenous compounds and fatty acid (4). Antioxidant activity has been found in a number of fermented fishery products such as fermented blue mussels (5), fish sauces (6) and fermented shrimp paste (7). Fish is generally good sources of protein and PUFA (8) but could be damaged during severe fermentation conditions.

Salt fermented shrimp paste exhibited antioxidant activity. Increasing in total antioxidant activity can be done by prolonging the fermentation period. Studies have shown that amino compound such as amino acids and peptides that can perform function as a primary antioxidant (1). The function of salt addition in making of fish paste is to create the specific condition for fermentation process, without salt the fish will deteriorate. Nowadays many of fish paste producer in Indonesia prefer to use lower salt to produce fish paste. With lower salt, the fermentation process could be disturbed by putrefaction, which may reduce the nutritional value of the product. This study was aimed to investigate the changes of amino acid and fatty acid during early stage of fermentation periods (8 and 32 days fermentation) of Anchovy fish paste with low salt concentration (2%). Anchovy fish paste has a specific morphology (9).

2. Materials and methods

2.1. Fish paste preparation

Fish as raw material and solar salt obtain from local market in Semarang. Three batches of fish paste were prepared. Fish (98%) and solar salt (2%) were thoroughly mixed and grinded. Minced fish and salt was dried in the sun until it perform fine non sticky and then grinded again. Semi dried paste was fermented for two days at ambient temperature. Semi dried paste was formed into tube like with 3cm diameter, 10 cm length and dried in the sun two days and then wrapped tightly in banana leaves and left for fermentation process. Samples were subjected for sensory and chemical analyses (amino and fatty acid) on 8 days and 32 days fermentation.

2.2. Sensory

The fish paste samples after 8 and 32 days of fermentation were evaluated by 30 semi-trained panellist panel using shrimp paste score sheet from Indonesian National Standard (10).

2.3. pH

The pH of fish paste was measured as described by (11). Sample was homogenized with 10 volumes of aquadest and the pH was measured using a pH meter.

2.4. Water content (Moisture Analyzer MB45 Ohaus)

Water content determined by moisture analyzer instrument (Ohaus MB45).

2.5. Amino acid profile

Amino acid compositions were determined by HPLC (waters corporation, USA). Amino acid standard solution used for calibration from Thermo Scientific, AccqTag column (3.9x150 mm), 37°C temperature, mobile phase acetonitril 60%-AccqTag Eluent A. Flow rate 1,0 mL per min with fluorescence detector. Volume injected for each sample was 5 µL.

2.6. Fatty acid profile

Fatty acid was determined to follow AOAC Procedure (12). Total lipid was extracted from the homogenate of fish paste with chloroform and methanol mixture. Fatty acid constituents in the total lipid were converted to fatty acid methyl esters using 5% hydrochloric methanol. The sample was then analyzed by gas-liquid chromatography (Shimadzu Co., Kyoto, Japan) with flame ionization detector. The carrier gas was helium at a flow rate of 1.6 mL/min and the split ratio was 50 to 1. The injection and detection port were set to 250 to 260°C with an oven temperature program of 180-230°C at 1°C/min. The fatty acid methyl esters were identified by comparing the retention times with authentic ones.

2.7. Statistical analyses

The data were expressed as mean ± SD. Data was subjected to analysis of variance (ANOVA). Mean comparison was carried out by honestly significant difference of means *p* value <0.05 were regarded as significant (13).

3. Result and discussion

pH is a factor that determine organism species dominated in the fermentation. Result of pH of fish paste with different fermentation time is displayed in Table 1. Longer fermentation time would increase pH of the product. The early step of fish paste processing, pH of product was approximately 6. During the fermentation process, pH would increased to 6.5 and in the end of the process pH would decline to 4.5 (14). But when the fermentation proceeds, there would be increased in pH due to the formation of ammonia. The research result of (15), reported that Chinese traditional low salt fermented whole fish product had a high acidifying activities and were able to reduce the pH to lower than 4.5.

Table 1. Changes in pH content of fish paste during 8 and 32 days of fermentation time.

Fermentation time (days)	pH of fish paste		Water content of fish paste (%)	
	Average	± SD	Average	± SD
8	6.42	± 0.03[a]	28.81	± 1.49[a]
32	6.90	± 0.08[b]	33.85	± 1.10[b]

In the 8 days fermentation pH was 6.42 and in the 32 days the pH was 6.90, and it was almost reached pH 7. It showed that there was higher base substances produced during fermentation so the pH increased. The high pH of product probably due to the lower salt concentration used in the experiment. One of salt functions is to inhibit the microorganism activity. By Using lower salt concentration, the activity of microorganism include the putrefaction bacteria would be higher and the base volatile substances would be increased and would made the increased of the pH product.

Water content was the largest compound in the fresh fish. The water content from the experimental is lower than research of (14). Fish paste has water content approximately 35-50% (14). Water content has significantly importance for a number reason. The determination of water content is therefore the most frequent general analysis performed on foodstuffs. The amount of water in food often determines its nutritive value and taste. The stability and shelf life of foods are highly dependent on water content, since it is crucial for microbiological growth and most enzymatic activities (16). The mass loss is not only caused by loss of water but by the loss of all volatile substances under the drying conditions, comprising those already contained in the original sample and those produce by the heating process.

The change of moisture content in fish paste during fermentation was shown in Table 1. The longer fermentation period gives higher water content. It was probably due to the absorption from the environment and reaction during fermentation which liberate some volatile compound for example trimethylamin (TMA), ammonia etc. The research of (17) reported that there was increased in water content of seasoning powder made from fish paste during storage in aluminium foil, plastic polypropylene and oil paper packaging. The lower permeability of packaging material the more difficult gas and moisture can be passed through the packaging. The product of this research was packed with banana leaves that still allow the gas and moisture passed easily through the packaging so the water content would be higher with prolonging in fermentation time.

Table 2, presents the amino content of fish paste at 2 different fermentation periods. The total free amino acid content was 32.32 mg/100 g of sample in 8 days fermentation time (wet base) and the value increased to 37.15 at 32 days fermentation. The major amino acids present in the sample were glutamic acid, aspartid acid and arginin.

Although the content of some individual free amino acids was decreased, most free amino acids were increased.

Table 2. Changes in amino acid content of fish paste during 8 and 32 days of fermentation time.

No	Amino acid	8 days fermentation time		32 days fermentation time	
		Average	± SD (%)	Average	± SD (%)
1	L-aspartic acid	**3.08**	**± 0.62**	**3.69**	**± 0.16**
2	L-serine	1.14	± 0.26	1.32	± 0.03
3	L-glutamic acid	**4.61**	**± 1.00**	**5.52**	**± 0.13**
4	Glycine	1.98	± 0.10	2.36	± 0.32
5	L-histidine*	0.97	± 0.20	1.26	± 0.18
6	L-agrinine*	2.02	± 0.36	2.49	± 0.20
7	L-threonine*	1.61	± 0.09	1.85	± 0.22
8	L-alanine	1.97	± 0.22	2.38	± 0.17
9	L-proline	1.37	± 0.13	1.71	± 0.10
10	L-Cystine	0.37	± 0.01	0.34	± 0.08
11	L-Tyrosine	1.30	± 0.27	1.30	± 0.22
12	L-Valine*	1.87	± 0.29	2.21	± 0.12
13	L-Metheonine*	1.15	± 0.02	1.29	± 0.09
14	L-LysineHCL*	2.87	± 0.59	3.12	± 0.00
15	L-isoleucine*	1.61	± 0.18	1.79	± 0.06
16	L-Leucine*	2.56	± 0.33	2.80	± 0.01
17	L-Phenylalanine*	1.84	± 0.44	1.71	± 0.37
	Total	32.32	± 3.49	37.15	± 2.43

Note: *: essensial amino acid.

Fish fermentation is the transformation of organic substances into simple compounds such as peptides, amino acids, and other nitrogenous compounds either by the action of microorganisms or endogenous enzymes (1). The research of (18), reported that the content of some individual free amino acids in fermented oyster sauce fluctuated during the fermentation period. These compound are released by microbial action (mainly by microbial enzymes), through the biochemical reactions taken place during fermentation. Fish paste also had high contents of glutamic acid which are regarded as important contributors to the flavour and taste of fish paste (19).

The research of (1) reported that shrimp paste trough prolonged fermentation give result total free amino acid content increase dramatically in the beginning fermentation time, stable in the mid fermentation time and then decreased along with the fermentation time. The decline in the free amino acid content could be due to its degradation to amines, volatile acids, and other nitrogenous substances as by-products of bacterial metabolism or enzymatic decomposition. Ammonia, an index of the degradation has distinctly increased during prolonged fermentation and manifested by the increase of odour. The observed decline in amino acids would be also responsible for the formation of MRPs (Maillard Reaction Products) and should be manifested by the increase in brown colour and fluorescence intensity.

Free radicals are generally reactive and attack molecules such as sugars, protein and lipids. This may result in damage from oxidation such as deterioration of foods, protein modification and enzyme inactivation. Antioxidant substances can prevent these damages by inhibiting the production of primary catalyst of lipid peroxidation (20). Maillard Reaction Products (MRPs) that could formed during fermentation was responsible for the increase in antioxidant activity of the salt-fermented shrimp paste during prolonged fermentation salt fermented shrimp was found to exhibit antioxidant activity, and the composition of fatty acid (%) did not change during fermentation for 36 days (use 25% w/w salt) (1).

Table 3. Changes in fatty acid content of fish paste during 8 and 32 days of fermentation time.

No	Fatty acid	Molecule formula	8 Days fermentation time		32 Days fermentation time	
			average	± SD	average	± SD
1	Caprylic acid	C8:0	1.30	± 1.26	0.58	± 0.74
2	Capric acid	C10:0	0.35	± 0.26	0.43	± 0.21
3	Lauric acid	C12: 0	2.36	± 0.36	1.66	± 0.77
4	Myristic acid	C14:0	2.83	± 1.18	2.45	± 1.19
5	Palmitic acid	C16:0	10.66	± 3.79	9.88	± 3.49
6	Palmitoleic acid	C16:1	2.51	± 0.02	2.20	± 1.04
7	Stearic acid	C18:0	6.09	± 7.67	16.57	± 8.64
8	Oleic acid	C18:1	7.72	0	5.75	± 0.75
9	Linoleic acid*	C18:2	nd	-	nd	-
10	Linolenic acid*	C18:3	2.53	± 2.83	0.35	± 0.42
11	Arachidic acid	C20:0	nd	-	nd	-
12	EPA:	C20:5	1.18	± 0.91	1.92	± 0.87
13	DHA:	C22:6	3.04	± 0.50	3.91	± 1.96
	Total		40.57	± 15.30	45.70	± 1.32

The result of the research showed the major fatty acids in the 8 days fermentation were palmitic acid, oleic acid, stearic and DHA while in the 32 days fermentation were stearic acid, palmitic acid, oleic acid and DHA (Table 3). Fish paste contain large amount of PUFAs and important fatty acid (EPA and DHA) did not damage during the beginning of the fermentation (Table 5).

The change of amount fatty acid in the fish paste during fermentation time was not similar for each fatty acid. Some of fatty acid was decreased during fermentation (caprylic acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, oleic acid and linolenic acid), while others were increased (capric acid, stearic acid, EPA and DHA).

Table 4. Organoleptic scores of fish paste sample during 8 and 32 days of fermentation time

Indicator	8 days fermentation time	32 days fermentation time
Appearance	7.7	7.7
odor	7.5	7.7
taste	7.3	7.2
Texture	7.9	7.9
Mold	9.0	9.0
Average	7.88	7.9

Different with the result of (1) that salt-fermented shrimp paste, could increase in antioxidant activity and PUFA (poly unsaturated fatty acid) almost remained. The formation of MRPs was responsible for the increase in antioxidant activity of the salt-fermented shrimp paste during prolonged fermentation. Our result showed that use low salt (2% w/w) some of fatty acid decreased during early fermentation (32 days). This difference probably due to the different concentration salts that use 25% salt. With higher salt concentration (25%), it could inhibit the further reaction of protein to become volatile base substances (the action of microorganism) and Maillard reaction could take place and produce antioxidant (MRPs). With the decreased of MRPs or antioxidant, the oxidation of lipid could be increased so the fatty acid will be reduced.

Fish paste was analyzed for sensory based on appearance, odor, taste, texture and mold (10). The results obtained are served in Table 4. Organoleptic of fish paste in 8 days and 32 days fermentation period did not show any difference. The appearance characteristic fish paste was bright and clean. The flavor was specific fish paste flavor, the texture was solid and compact. No mold found in the product.

4. Conclusion

Fish paste with low concentration salt (2%) which is normally processed with about 25% salt, could increased in nutrition value especially total amino acid, some of fatty acid during 8 and 32 days fermentation time. But the pH and moisture content continually increases that indicate the beginning of deterioration process, thus the shelf life of the product is very short.

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