THE EFFECTS OF ADDITION PALM SUGAR CONCENTRATION (*Arenga pinnata*) TOWARDS WADI BETOK QUALITY (*Anabas testudineus* Bloch)

by:
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Abstract

Wadi betok fish is a fermentation product which form is intermediate moisture whole fish, blackish colour, tough textured with specific odour and has a very salty taste. In principle, wadi is made by dry salting (*pickling*) with concentration of salt is 20%-50% w/w, at room temperature for seven days up to several months in a tightly covered container. High salt concentrations in the manufacturing process of wadi has caused salt content in products very high and made that taste become very salty. The one of alternative to overcome the flavor of one, besides salt also was added some palm sugar (*Arenga pinnata*). The research was conducted in South Kalimantan. The treatment of wadi from betok fish were added palm sugar with concentrations of 0%, 5%, 10% and 15% (w/w). The parameters tested including phsyco-chemical, organoleptic and microbiological. The main of research, the parameter phsyco-chemical included pH of flesh ranged between 30.770% – 36.348%, a_W ranged between 0.828% - 0.862%, salt content ranged between 3.995% - 5.443%, moisture content ranged from 30.770% - 36.348%, protein content ranged between 13.843% - 17.330%,fat content ranged from 1220% - 2.115%,ash content ranged from 0940% - 2.883%, and the TVB 4240 to 6.640. Microbiological parameters include TPC 1.575×10^{-6} – 2.475×10^{-6} CFU and LAB 0.160×10^{-6} - 2.900×10^{-6}.

Key words: Fermented fish, palm sugar, the quality of physicochemical, microbiological quality

1. INTRODUCTION

Fermentation process is a microbiological processes that break down complex compounds especially protein into simpler compounds, namely amino acids and peptides. Amino acids are further decomposed into component forming flavors typical fermentation products (Adawyah, 2007).

The product processing of fermented fish generally is always preceded by salting which aims to microbial selection. Generally, fermented fish occur in conditions where processing make favorable circumstances for activity and growth of microorganisms, so that during the fermentation of hydrolyzed fish protein into peptone, peptides and amino acids (Tanasupawat *et al*., 1998).

Betok fish (*Anabas testudineus* Bloch) is kind of fresh moisture fish which is widely available in public moistures in South Kalimantan and people familiar with the designation

of Papuyu fish. This fish are loved by the community and abundant only in the dry season (months from May to July) whereas in certain months (months from July to February) betok fish in fresh condition very hard to find so the price is relatively expensive. To overcome the scarcity of betok fish when famine and to preserve and enhance the value-added fish betok people usually do the processing traditionally. Traditional processing which is typically done is cooling, canning, salting, drying, curing, and fermentation (Winarno *et al*., 1984).

Since first, Kalimantan community have been made the preservation of betok fish by the method of fermentation and the fermentation result is known as wadi betok fish. Wadi is preservation of traditional fermented fish in 20-50% salt solution (Khairina, 2000). In Kalimantan, making of wadi is done by salting in a closed container and left a few days or even months until the time it can be consumed. But generally, the yield of wadi after 4 to 5 days storage is ready to consumed with salty taste, wadi's distinctive aroma and blackish color.

`The purpose of the addition salt in the fermentation process is to inhibit the growth of spoilage bacteria. The addition of salt during the making of wadi help bacteria that are halotoleran, halofilik, and halofobik can grow and develop (Rahayu, 2002). The addition of salt 25% from total weight of fish and length of storage for 14 days able to reduce levels of moisture and dissolved nitrogen but increasing levels of salt and ash (Khairina, 1998).

Palm sugar (*Arenga pinnata*) is a sugar that is produced from palm tree sap evaporation which in trading known as "java sugar" or "red sugar" and rich of carbohydrates and other minerals. Raw materials for manufacture of palm sugar is the sap leads the morning or evening. The nature of the newly tapped palm juice is fresh and sweet because it contains sugar that is high enough. According to Supardi and Sukamto (1999) sugar involved in the preservation of a variety food products. Some of them are usually find is jam, jell, concentrated fruit juice, fruits syrup, candy, etc. The use of palm sugar in the processing of food materials in high concentrations at least 40% dissolved solids, some moisture present in the material not available for microbial growth, so the moisture activity (a_w) of food to be low or declining.

Until today, written information about wadi betok fish products with addition of palm sugar is still rare so it needs to be investigated about the profile of wadi betok fish that created by modification with the addition of palm sugar by the people in South Kalimantan particularly Banjar Regency which is the central of wadi betok fish processing itself, so it would be useful efforts to provide information about the nutritional value from wadi betok fish and modification of wadi betok fish with the addition of palm sugar to processors and consumers.

2. METHODS

Time and Place Research

The research will be conducted at the Laboratory of THP Faculty of Fisheries and Marine Sciences Brawijaya University Malang, Laboratory of Fisheries Processing, Faculty of Fisheries Lambung Mangkurat University Banjarbaru South Kalimantan, and Laboratory of Microbiology, Medicine Faculty, Brawijaya University, Malang. The research will be conducted in December 2011.

Materials

Raw materials which is used in this study are betok fish (Anabas testudineus Bloch), salt and palm sugar (*Arenga pinnata*),and chemicals such as K_2SO_4, 4% H_3BO_3 solution, concentrated H_2SO_4, H_2O_2, distilled moisture, $CuSO_4$, 0.2 N HCl, and petroleum ether, distilled moisture, pH 4-7 buffer solution, 10 ml of 0.1 N $AgNO_3$ and 10 ml HNO_3, $AgCl_2$, and distilled moisture, 5 ml of ferric alum and 0.1 N KSCN.

Equipment which is used in this research are scales, fermentation container, ovens, desiccator, porcelain bowls, Kjeldahl heater, Kjeldahl flasks, distillator, erlenmeyer,

Whatman paper, electric heaters, Soxhlet extraction tools, conway dishes, petri dishes, incubators, pH meters, a_W meter and other glass tools.

Research Procedures

First step in this research is to make wadi betok fish experiment with addition of palm sugar. The concentration of salt that used is 15% of the weight of the fish whereas palm sugar that used for each is 0%, 5%, 10% and 15% with the fermentation during 7 days. Next experimental results of wadi with the addition of palm sugar conducted sensory tests (parameter flavor, color, texture, odour) to the Banjar Community, while samples for physico-chemical testing (moisture content, ash content, a_W, protein content, salt content, fat content, pH, TVB) conducted at the Laboratory of Chemistry, Faculty of Mathematics and Science, and for microbiological testing of products (TPC and BAL) conducted at the Laboratory of Microbiology Faculty of Medicine, Brawijaya University.

Physicochemical

Protein testing is done by using UV spectrophotometer fis using the Nessler reagent. Moisture testing conducted by the gravimetric method (AOAC, 2000:950.46). Fat content testing conducted by the method of soxhlet (AOAC, 1990). Salt content test conducted by Mohr Method. Testing of ash content conducted by Muffle Method (AOAC,1990). Determination of TVB (Total Volatile Base) conducted by the Conway dish method. Measurement of moisture activity (a_W) conducted by using a_W meter (Rotronic). PH testing is conducted by using a pH meter.

Microbiological testing

Sample preparation

Ten grams of sample (wadi betok fish), that have been mashed, inserted into a test tube (10^{-1} dilution) which is contain 0.9% solution of Na Physiological which then diluted to a dilution of 10^{-6}.

Total Plate Count (TPC)

Total Plate Count (TPC) testing determined by pouring the liquid sample of dilution into Plate Count Agar (PCA) media and then incubated in the incubator with room temperature (25-30 °C) for 24 hours.

Total Lactic Acid Bacteria (LAB)

Total Lactic Acid Bacteria (LAB) testing determined by pouring the liquid sample of dilution into MRSA (Demand Sharpe Ragosa order) media and then incubated in the incubator with room temperature (25-30 °C) for 48 hours.

Sensory

Panelists are asked to fill quisioner hedonic test, scoring test toward wadi betok fish parameters which is tested (colour, texture, flavor, odour), then asked to fill quisioner acceptance product test as a whole.

3. RESULTS AND DISCUSSION

The results of making of wadi betok fish with the addition concentrations of palm sugar each of 0%, 5%, 10% and 15% with the physico-chemical parameters (protein content, moisture content, salt content, protein content, fat content, ash content, TVB and pH of flesh); microbiology (BAL and TPC) and the organoleptic parameter (taste, color, odour, and texture).

4. ANALYSIS RESULTS

Protein's Level

Protein content in wadi betok fish with palm sugar addition with each treatment ranged between 13,843% - 17,330%. The highest protein levels in treatment C (palm sugar concentrations of 15%) of 17,330% and the lowest protein levels in the treatment K (palm sugar concentrations of 0%) of 13,843%. The best protein levels found in treatment C. The protein content test results of wadi betok fish can be seen in Table 1.

Table 1. Mean Levels of Protein Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	Protein Range (%)
			Mean ± SD
K	15%	0%	**13,843 ± 0.723**
A	15%	5%	**15,853 ± 0.670**
B	15%	10%	**16,260 ± 0,585**
C	15%	15%	**17,330 ± 0.615**

Increased levels of protein concentration of palm sugar which is more greater caused a decrease in moisture content take effect decrease the activity of microorganisms where the protein is one of the factors required for growth of microorganisms. So with the higher concentration of the moisture content of palm sugar caused the moisture content decreases consequently protein levels rise. According to Wibowo (1989), factors required Lactic Acid Bacteria and other bacteria are still available to grow, some of them come from the protein which will degraded into simpler compounds such as mono, di, tri and other nitrogen contribute to lowering levels of protein products.

Moisture Content

The moisture content in wadi betok fish with palm sugar addition of each treatment ranged between 30.770% - 36.348%. Highest moisture content in treatment K (0% concentration of palm sugar) of 36.348% and the lowest moisture levels in treatment C (concentration of palm sugar 15%) of 30 770%. The best moisture content founded in the treatment C. The moisture content test results of wadi betok fish can be seen in Table 2.

Table 2. Result of The Mean Moisture Levels in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	Moisture Content (%)
			Mean ± SD
K	15%	0%	36,348 ± 0.748
A	15%	5%	30,770 ± 1.430
B	15%	10%	31.163 ± 1.007
C	15%	15%	29.498 ± 1.272

The moisture content decreased because palm sugar had higroscopic characteristic thus able to absorb moisture from the fish's body, so the increasing numbers of palm sugar made moisture content in fish decreased. According to Satuhu (1994), sugar has hygroscopic properties which can draw moisture from comestibles.

Fat Contents

Fat contents in wadi betok fish with palm sugar addition of each treatment ranged between 1220% - 2.115%. Highest fat content in treatment C (15% concentration of palm sugar) of 2.115% and the lowest fat content on the treatment of K (0% concentration of palm sugar) of 1.220%.The best fat content was in treatment C. The results of fat content in wadi betok fish can be seen in in Table 3.

Table 3. Result of Fat Contents in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	Fat Content (%) Mean ± SD
K	15%	0%	1.220 ± 0.126
A	15%	5%	1.688 ± 0.051
B	15%	10%	1.870 ± 0.129
C	15%	15%	2.115 ± 0.070

Increased levels of fat content was supposed to be caused by decreased moisture content, which affected the decreasing microorganisms activity beside the protein is one of the factors required for the growth of microorganisms also acids content produced by lactic acid bacteria has not been high enough in this fermentation. So that the higher concentration of palm sugar made moisture content decreased consequently the fat content will increase. According to Winarno (1960), the presence of a high enough acid will coagulate the protein. Fat in fish body besides the form is free also bound to a protein called lipoprotein (Zaitsev *et al*., 1969),

Ash Content

The ash content in wadi betok fish with palm sugar addition of each treatment ranged between 0940% - 2.883%. The highest ash content was in treatment K (0% concentration of palm sugar) of 2.883% and the lowest ash content was in treatment C (palm sugar concentrations of 15%) of 0940%. The best ash content found on treatment C. The ash content test results of in wadi betok fish can be seen in Table 4.

Table 4. The Results of Mean Ash Content in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	Ash Content (%) Mean ± SD
K	15%	0%	2.883 ± 0.220
A	15%	5%	2.150 ± 0.287
B	15%	10%	1.380 ± 0.063
C	15%	15%	0.940 ± 0.110

The decrease of ash content was caused by mineral dissolution during the process of osmosis by palm sugar. According to Siahaan (2010), ash content related with a mineral material because if the ash content in the material is getting much the mineral content will be higher and vice versa. In this case, the kind of acid and acid concentration gave influence towards any kind of material.

Salt Content

Salt content in wadi betok fish with palm sugar addition of each treatment ranged between 3.995% - 5.443%. The highest salinity was in treatment C (15% concentration of palm sugar) of 5.433% and the lowest salt content was in treatment B (the concentration of palm sugar 10%) of 3995%. The best salt content treatment found on treatment B. The salt content test results of wadi betok fish can be seen in Table 5.

Table 5. The Results of Mean Salt Content in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	Salt Content (%) Mean ± SD
K	15%	0%	4.478 ± 0.791
A	15%	5%	4.503 ± 0.498
B	15%	10%	3.995 ± 1.020
C	15%	15%	5.433 ± 0.759

The decrease of salinity caused by addition of palm sugar which nature of the sweet palm sugar (sucrose) was able to reduce the salt concentration. The role of palm sugar in curing

was very important because it serves to maintain a sense of equilibrium which was caused by the influence of other preservatives such as salt and other ingredients (Putra, 2001).

Water Activitiy (a_W)

a_W levels in wadi betok fish with palm sugar addition of each treatment ranged between 0828% - 0862%. Highest a_W levels in treatment A (the concentration of palm sugar 5%) of 0862% and the lowest a_W levels was in treatment K (palm sugar concentrations of 0%) of 0828%. The best a_W levels found on treatment K. The a_W test results of wadi betok fish can be seen in Table 6.

Tabel. The Results of Levels Mean a_W on Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	aW
			Mean ± SD
K	15%	0%	0.828 ± 0.055
A	15%	5%	0.862 ± 0.004
B	15%	10%	0.842 ± 0.040
C	15%	15%	0.840 ± 0.020

It caused by the hygroscopic nature of sugar, causing adsorption of moisture from the flesh of fish out which makes the availability of moisture for the life of microorganisms decreased. Edwards (2000) said that the addition of sugar to the product made the product hygroscopically (absorbs moisture easily from the surrounding environment).

TVB

TVB wadi betok fish with palm sugar addition of each treatment ranged between 424 to 6.640. Highest TVB was in treatment C (15% concentration of palm sugar) of 6.640 and the lowest TVB was in treatment K (0% concentration of palm sugar) of 4240. The best TVB was in treatment K. The TVB test results of wadi betok fish can be seen in Table 7.

Table 7. The Results of TVB Mean in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	TVB (mgN/100g)
			Mean ± SD
K	15%	0%	4.240 ± 2.653
A	15%	5%	5.240 ± 4.099
B	15%	10%	5.040 ± 2.191
C	15%	15%	6.640 ± 3.946

Increased levels of TVB was supposed to be caused by not optimal moisture absorbed by the sugar (osmosis), so that microorganisms can still grow. According to Untari (1974), because the functions of palm sugar is not different than the function of salt which it gives a sweet taste, formed a dense texture, control the growth of microorganisms, which stimulate the growth of spoilage and pathogenic microorganisms. So properties of palm sugar is almost same as the salt that serves as a humectant in the osmosis processing.

Flesh pH

pH of flesh from Wadi betok fish by addition of palm sugar with each treatment ranged between 5.950- 6.150. The highest flesh pH was in treatment B (the concentration of palm sugar 5%) of 6.150 and the lowest flesh pH was in K treatment (0% concentration of palm sugar) of 6150%. Flesh pH test results of wadi betok fish can be seen in Table 8.

Table 8. The Results of Flesh pH Mean in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	Flesh pH
			Mean ± SD
K	15%	0%	5.950 ± 0.129
A	15%	5%	6.100 ± 0.141
B	15%	10%	6.150 ± 0.129

C	15%	15%	6.075 ± 0.171

The increase in flesh pH levels expected because the metabolism from lactic acid bacteria has not been optimal, so that pH tended to lead to alkaline conditions. Added by Putra (2001), the coconut sugar which is carbohydrate saccharide group with toasted rice is a substrate for fermentation of lactic acid bacteria that produce lactic acid, thus lowering the pH of the wadi fish Sepat siam.

TPC

TPC in wadi betok fish with palm sugar addition of each treatment ranged between 1.575 x 10^{-6} – 2.475 x 10^{-6} CFU. The highest TPC was in treatment B (10% concentration of palm sugar) at 2.475 x 10^{-6} CFU and the lowest TPC at treatment K (0% concentration of palm sugar) at 1.575 x 10^{-6} CFU. The best TPC found in treatment B. The TPC test results in wadi betook fish can be seen in Table 9.

Table.9 The Results of TPC Mean in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	TPC 10^{-6} Mean ± St.Dev
K	15%	0%	1.575 ± 0.340
A	15%	5%	2.050 ± 0.191
B	15%	10%	2.475 ± 0.222
C	15%	15%	2.100 ± 0.163

This was caused the nature of palm sugar which served to absorb moisture in the fish that was hindering the growth of microorganisms. According to Winarno *et al*., (1984) that growth of microbes in food is very closely related to the amount of moisture content where the microbial growth never occurred in the absence of moisture.

LAB

LAB in wadi betok fish with palm sugar addition of each treatment ranged between 0160 x 10^{-6} - 2.900 x 10^{-6}. Highest LAB was in treatment C (0% concentration of palm sugar) of 2.900 x 10^{-6} and the lowest LAB was in treatment C (palm sugar concentrations of 15%) of 0160 x 10^{-6}. The best LAB was in treatment C. The LAB test results in wadi betok fish can be seen in Table 10.

Table 10. The Results of BAL in Wadi Betok Fish

Treatment Code	Salt Concentration	Palm Sugar Concentration	BAL 10^{-5} (Mean ± SD)
K	15%	0%	2.900 ± 0.141
A	15%	5%	1.530 ± 1.024
B	15%	10%	2.800 ± 0.163
C	15%	15%	0.160 ± 0.018

This was caused by a condition at wadi fermentation process which is acid so caused the growth of lactic acid bacteria increased. Described by Agustiana and Aisha (2000), that lactic acid bacteria in some cases contributed in the fermentation of fish like wadi. During the fermentation process, this bacteria will produced lactic acid ($C_3H_6O_3$) as the main product which can inhibit the growth of pathogenic and spoilage bacteria, in addition another antimicrobial components such as hydrogen, diasetil and bacteriocins.

Sensory Test Results

The sensory test results of the acceptance product of wadi betok fish, panelists were more likely to choose the wadi betok fish products which made by using palm sugar concentrations of 10% and 15% with a value of 4 (four) which means ‘much like’ and based on the hedonic test, from the parameter test, the value texture was 4 (neutral) for level of

palm sugar 10% and 3 (a bit not like) 15% for palm sugar level; parameter odour value was 5 (somewhat like) for palm sugar content 10% and 4 (neutral) 15% for level of palm sugar; taste parameter was 4 (neutral) palm sugar level 10% and 4 (neutral) level of palm sugar 15%; color parameter 4 (neutral) level of palm sugar and 10% and 4 (neutral) 15% for level of palm sugar. While based on ranking test, the nature of products which are made with this concentrations of 10% is to have a dense texture, less compact / neutral (score 4); have less wadi odour characteristic, quite fragrant palm sugar (value 6); has quite salty taste (score 5), and has a brownish black color of flesh (score 4). While for addition of palm sugar by 15% has a dense texture, less compact / neutral (score 4); have less wadi odour characteristic, less fragrant palm sugar (value 5); has a bit salty taste (score 6); and has brownish black color of flesh (score 4).

The changes color during the fermentation process occured due to non-enzymatic reaction (Maillard reaction) caused reduction and oxidation of sugar, such as aldehydes which can react with free amino acids that would be free to be very wide with the length of time of fermentation which produces melanoidine (Lopetcharat *et al*., 2002). While the change in the texture of the wadi betok fish influenced by concentration of salt, because salt can absorb moisture in the tissues of fish's flesh, so that the texture of fish's flesh turns into a bit harsh and also affect the sense of being salty because the salt goes into the muscles tissue of fish. The formation of aroma of fermented products is usually caused by the breakdown of macromolecular compounds into compounds that are very simple and volatile.

5. CONCLUSION

The results of manufacturing wadi betok fish, with palm sugar addition concentrations of each 0%, 5%, 10% and 15% indicated that the wadi betook fish with the palm sugar addition concentrations of 15% is the best concentration of palm sugar.

The results of analysis wadi with palm sugar concentrations of 15% based on physico-chemical parameters indicate that included flesh pH which ranged from 30.770 - 36.348, a_W which ranged between 0828 - 0862, salt content ranged between 3.995% - 5.443%, moisture content ranged from 30.770% - 36.348%, protein content ranged between 13 843% - 17.330%, fat content ranged from 1220% - 2.115%, ash content ranged from 0940% - 2.883%, and the value of TVB (mgN/100g) 4240 to 6.640.

The results of analysis wadi with palm sugar concentrations of 15%. Microbiological parameters includes TPC 1.575×10^{-6} - 2475×10^{-6} CFU and BAL 0160×10^{-6} - 2.900×10^{-6}. The results of analysis wadi with palm sugar concentrations of 15% based on organoleptic texture parameters showed wadi betok fish of 3.97%; wadi betok fish's odour parameter of 4.60%; wadi betok fish's color parameter of 4.57%; wadi betok fish's taste parameter of 4.70%.

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