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Change of Fatty Acids Compositions (Omega 3, 6, 9) from Milkfish *(Chanos chanos* Forsk*)* Bekasam that Fermented with Different Carbohydrates Sources

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ABSTRACT

Bekasam is one of fermentation products made from Milkfish (*Chanos chanos* Forsk). The aim of this study was to determine the effect of different sources of carbohydrates on fermented waste to fatty acid composition. The method used was experimental laboratories using Completely Randomized Design. The treatment applied was the use of different carbohydrate sources of rice, brown sugar and garlic in the milkfish bekasam production using 3 repetitions. The data were analyzed using Analysis of Variance and continued by Honestly Significant Difference test if there was any effect on the treatment given. The results showed that the difference of carbohydrate sources during fermentation process could affect the composition of fatty acids. Lactic acid bacteria use carbohydrate sources as energy to grow and then hydrolyze fat into fatty acids. The best result was bekasam with the sources of carbohydrate of rice with total value of Lactic Acid Bacteria 7,888 log cfu/g, Total Volatile Base Nitrogen value 34,796 mgN/100ml, lipid content 0,055% and the highest fatty acids compositions. The results of the study showed that the use of different sources of carbohydrate on bekasam had significant effect (P<5%) on the fatty acid composition on bekasam.

Keywords: *milkfish, fermentation, bekasam, carbohydrates sources, fatty acid*

1. Introduction

Milkfish (*Chanos chanos* Forsk) is nutritious and cheap that a lot of people consume it. Milkfish can be used as the raw material for producing bekasam. Bekasam is an Indonesian traditional fermented fish. Fermentation is one of methods to preserve and improve the nutritive aspect of fish. The aims of fermentation are to make new product, improve physical characteristic and to extend the self life (Sastra, 2008). According to Aziza et al. (2015), Milkfish consists of 20,53% protein and 6,73% lipid. Lipid contained in fish is a source of PUFA omega-3 19,56%, omega-6 7,47%, and omega-9 19,24%.

Bekasam production requires rice and salt. Rice or “kerak” has a role to stimulate the growth of lactic acid bacteria (LAB). LAB will degrade starch into simple compound like lactic acid, acetic acid, propionic acid and ethyl alcohol. These compounds not only give flavor but also act as preservative in bekasam (Nuraini et al., 2014).

Bekasam can be made by using different sources of carbohydrate to increase it’s food consumption value. Bekasam is not famous in young people because it’s taste. To overcome these problems several study had been conducted to improve the sensory score by modifying source of carbohydrate. Yulianni (2014), reported that bekasam with the sources of rice and brown sugar was better quality than other treatment. While Widayanti et al. (2015), said that the addition of rice, brown sugar, and garlic gave the best sensory.

When sensory aspect is increase by modification production method. There is no information abou the nutrition changes with the chnges of production method. The study of nutritional fact in the modified method of bekasam production has not been conducted. Lipid is one of the important nutrition of fish which has high number of PUFA that has a great benefit for human.

The aim of this study was to discover the differences of lipid content and omega-3, omega-6, and omega-9 contents from the different sources of carbohydrate used in bekasam production.

2. Materilas and Methods

2.1. Chemicals

All solutions have been conducted with pro analysis (p.a). MRS broth (Merck), PCA 6%, Fenolftalein indicator, anti-foaming silicon, NaOH 20%, H_3BO_4 3%, Tashiro indicator, HCL 0,02 N. Whatman 42.

2.2. Method of production bekasam

Milkfish was cleaned by removing the head, stomach contents, scales, fins, and gills. Then it was cut into 3 pieces, then was washed with clean water and drained. The weight of each fish sample ± 170 gr, fish was added with salt 17 gr (10% w/w), then mixed evenly, and was being let stand for 1 hour. Then the source of carbohydrate for each of the bekasam was being varied. Bekasam K used the rice 40% (w/w) as the control, bekasam A used the best concentration of the research Nuraini et al. (2014), using rice 40% (w/w) and brown sugar 3% (w/w) and bekasam B takes the best concentration from Widayanti et al. (2015), using rice 40% (w/w), brown sugar 3% (w/w) and addition of 6% garlic (w/w) The prepared ingredients are then mixed evenly and then put into the jar and closed tightly, the sample fermented for 7 days at room temperature.

2.3. Research methodology

The parameters observed are fatty acid levels (AOAC, 2005), water content (BSN, 2006), total lactic acid bacteria (Fardiaz, 1993), Total Volatile Base Nitrogen (TVBN) (BSN, 2009), lipid content (BSN, 2006), and hedonic.

3. Results and Discussion

3.1. Water content of milkfish bekasam

The bekasam having the lowest water content was bekasam A 66,097%. This phenomenon was caused by the addition of brown sugar which had the ability to absorb water out of the fish flesh. The result showed that water content in bekasam decreased compared to the control (only rice). According to Kartika and Fithri (2015), water content would decrease along with the increasing sugar concentration in osmotic solution. The decreasing water content had correlation with higher sucrose content. Sugar (sucrose) had hygroscopic characteristic, which means that it has the ability to absorb water. The higher sugar concentration would give the higher water absorption, so that the water content of the product would decrease.

Bekasam B had the highest water content 68,793%. The treatment was conducted by adding rice, brown sugar and garlic. The water content was high because sugar absorbed water from garlic so water in bekasam B not much been absorbed by sugar. While those results have been shown at **Table I.**

Table 1. Average value of water content (%) in bekasam milkfish with different carbohydrate source

Bekasam	Value
K	67,307 ± 0,020 [b]
A	66,097 ± 0,065 [a]
B	68,793 ± 0,080 [c]

Information :
• Data is the average of three times ± standard deviation
• Data followed by different superscript showed significant differences (P <0.05)

3.2. Total lactic acid bacteria of milkfish bekasam

Table 2. Average value of total lactic acis bacteria (log cfu/g) in milkfish bekasam with different carbohydrate source

Bekasam	Value
K	67,307 ± 0,020 [b]
A	66,097 ± 0,065 [a]
B	68,793 ± 0,080 [c]

Information :
• Data is the average of three times ± standard deviation
• Data followed by different superscript showed significant differences (P <0.05)

Bekasam K had 7,888 log cfu/g, the lowest total plate count. Rice was the only source of carbohydrate so the nutrition for the microorganism was not much. Bekasam A had the highest total plate count 8,124 log cfu/g. It was because of the nutrition that also come from the brown sugar, so that microorganisms can grow better than using the other treatments. Anwar et al. (2014), explained that the increasing glucose concentration in the fermentation would make LAB and halophilic also increase. LAB and halophylic would utilize glucose or carbon source for their growth.

Bekasam B had total plate count between K and A. This phenomenon was resulted by garlic additions as carbon source. According to Muller et al. (2001), garlic addition in somfug, a fermented fish product had a function as carbohydrate source and stimulate the growth of Lactobacillus plantarum. However garlic also had antimicrobial activity. Prihandani et al. (2015), explained that bioactive compound from garlic is allicin with sulphur. It had an antibacterial activity. Another bioactive compound were dialyldisulfide and dialyltrisulfide that also had antibacterial activity. Bernbom et al. (2009), reported that garlic is spices that often be used in fish fermentation and has antibacterial activity.

3.3. Value of *total Volatile Base Nitrogen* (TVBN) milkfish bekasam

Table 3. Average value of TVBN (mgN/100ml) in milkfish bekasam with different carbohydrate Source

Bekasam	Value
K	34,796 ± 0,724 [a]
A	37,966 ± 0,162 [c]
B	36,483 ± 0,341 [b]

Information :
• Data is the average of three times ± standard deviation
• Data followed by different superscript showed significant differences (P <0.05)

Bekasam K had the lowest TVBN value. In the bekasam K the source of carbohydrate was only from rice. Bekasam K also had the lowest total plate count value. It means that number of microorganism in bekasam K was less than in the other treaments and the decomposition process of macromolecule was also lower. The decomposition nutrient by several types of microorganism would generate bases compound. The higher bases concentration (TVBN) indicated the higher deterioration in food.

The highest value of TVBN was bekasam A. This phenomenan was caused by the using of rice and brown sugar as the source of carbohydrate in bekasam A, so the total plate count value was higher than the other treatments and gave the highest TVBN value. Nuraini et al. (2014), explained that the increasing TVBN value during fermentation probably because the protein decomposition by bacteria into nitrogen compound which is more simple, for example trimetylamine, dimethylamine, and ammonia. Ratnasari et al. (2014), reported enzym from fish and bacteria could degrade the protein into amino acid or some volatile bases. Koesoemawardani and Yuliana (2009), added that volatile compounds from protein degradation by microorganism activity were ammonia, monoamine, diamond and cadaverine.

TVBN value of bekasam B was in between bekasam A and bekasam K. It was because the garlic addition could stimulate the growth of bacteria. Accoding Sunanti (2007), Garlic also had a antimicrobial compound namely allcin.

Some types of bacteria including lactic acid bacteria can use garlic as carbon source thus produce lactic acid. Some other bacteria are not tolerant to garlic and is sensitive to antimicrobial activity of garlic so they was unable to grow in bekasam B. Because of several factors in garlic, the total plate count value of bekasam B was in between bekasam K and bekasam A and so did the TVBN value.

Addition of garlic could stimulate the growth of lactic acid bacteria and it would increase the lactic acid so the pH would drop. In low pH a lot of bacteria did not survive. According to Widayanti et al. (2015), concentration of garlic would decrease the TVBN in bekasam.

3.4. Lipid content in milkfish bekasam

Lipid content was vary from 0,046% - 0,055% (db). Bekasam B had the lowest lipid content. Garlic had the ability to decrease lipid (hidajati, 2005). Bekasam K had the highest lipid content. Bekasam K did not use brown sugar. Brown sugar could conduct plasmolysis, so that water and lipid could go out from fish cell.

According to Kartika and Fithri (2015), when sugar concentration increased in the

osmotic solution, the membrane permeability would plasmolyze because of the different osmotic pressure. Restu (2014), explained that with the increasing of palm sugar concentration in Patin fish the lipid content would decrease. While those results have shown at **Table 4.**

Table 4. Average value of lipid content (% db) in milkfish bekasam with different carbohydrate source

Bekasam	Value
K	0,055 ± 0,078 [c]
A	0,047 ± 0,020 [b]
B	0,046 ± 0,045 [a]

Information :
- Data is the average of three times ± standard deviation
- Data followed by different superscript showed significant differences (P <0.05)
- Db : dry base

Bekasam K had 0,055% lipid content. The total plate count in bekasam K was the lowest so the lipid was not much been degraded by bacteria. Increasing or decreasing lipid content on fermentation process of bekasam probably due to the microorganism activity and also the enzimatic reaction in fish that degraded lipid. According to Yuliana (2007), the decreasing lipid content in the rusip fermentation was due to lipid degradation from microbes activity and enzymatic reaction in the fish. The hydrolytic process of microbes was catalyzed by lipase and lipoxidase.

3.5. Fatty acids content in milkfish bekasam

The fatty acid content was different between each treatment. Unsaturated fatty acid in bekasam K were oleic acid, linoleic acid, a-linolenic acid and aracidonic acid.

Fatty acid content in K was the highest. It was caused by the the use of rice as the only carbon source. It would also result in lower total plate count and lower lipid hydrolysis. Bekasam K and B had higher total plate count and the lipid hydrolysis were higher. While those results have shown at **Table 5.**

3.5. Fatty acids content in milkfish bekasam

Table 5. The result of fatty acid content of fresh milkfish and milkfish bekasam with different carbohydrate source (%)

No.	Analysis	Result			
	Unsaturated Fatty Acid	Fresh Milkfish	K	A	B
1.	Oleic Acid (C18:1)	16,19	17,71	19,24	21,51
2.	Linoleic Acid (C18:2)	-	6,72	4,90	3,41
3.	A-Linolenic Acid (C18:3)	1,6	2,49	1,19	1,9
4.	Aracidonic Acid (C20:4)	3,86	2,24	3,68	3,28

According to Umaiya (2015), lactic acid bacteria had lipase. Su'i et al. (2010), lipase could hydrolyze lipid and the result was free fatty acid. Lipase hydrolyze lipid (triglycerides), diglycerides and monoglycerides into free fatty acid and glycerol.

A-lenolenic acid (Omega-3) and linoleic acid (Omega-6) in every sample increased after fermentation. Bekasam K was the highest in linoleic acid and a-linolenic acid content. Aracidonic acid (Omega-6) decreased in every sample. Bekasam K was the lowest. Oleic acid (Omega-9) increased after fermentation, bekasam B had the highest Oleic acid content. According to Umaiya (2015), lactic acid bacteria had lipase that can hydrolysis lipid into fatty acid and glycerol. In addition Winarno (2004), said that fatty acid could be synthesized from compounds that have carbon like asetic acid, acetaldehyde and ethanol. Fatty acid synthesis happened in anaerob condition with spesific strain of bacteria.

Increasing and decreasing fatty acid in each fatty acid is different. Ngadiarti et al. (2013), reported that fermentation with lactic acid bacteria could change the composition of fatty acid. Saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) increased and polyunsaturated fatty acid (PUFA) decreased. Fermentation using lactic acid bacteria could cause lipolysis and hydrogenation.

3.6. Hedonic value of milkfish bekasam

Table 6. Average value of lipid content (% db) in milkfish bekasam with different carbohydrate source

Spesification	Value		
	Bekasam K	Bekasam A	Bekasam B
Color	2,300 ± 0,952 [a]	3,300 ± 0,794 [b]	3,430 ± 0,817 [b]
Aroma	3,130 ± 0,507 [a]	3,430 ± 0,626 [b]	3,700 ± 0,595 [b]
Taste	1,900 ± 0,952 [a]	3,130 ± 0,681 [b]	3,600 ± 0,621 [c]
Texture	2,900 ± 0,803 [a]	3,430 ± 0,568 [b]	3,670 ± 0,546 [b]

Information :
• Data is the average of three times ± standard deviation
• Data followed by different superscript showed significant differences (P<0.05)

Color

From the hedonic value, it could be seen that A has no significant effect on B but has significant effect on K. Bekasam K had a pale white color, A and B had the same / yellow color, so that A and B were more attractive than K. This was caused by the more dominant influence of brown sugar so that the appearance of the bekasam was sharper and was more preferred by panelists. According to Nuraini et al. (2014), suspected that the influence of the addition of brown sugar t causes the brownish yellow color of the products. The color was more preferred by panelist than pale color (due to no addition of brown sugar) because it looked more appetizing.

Aroma

The hedonic value of the aroma indicated that the bekasam A with B does not have any real effect, but on the bekasam K gave a real effect. Bekasam K had a salty aroma, bekasam A had a sweet aroma while B had a sweet savory aroma, so the A and B were more preferred by the panelist. The result showed that due to the addition of a little garlic the smell of brown sugar was more dominant thus gave a distinctive aroma and was more preferred by the panelist. According Sutrisno (2014), brown sugar had a distinctive aroma due to the content of organic acids. In addition, brown sugar also had a distinctive aroma of caramel.

Taste

Bekasam K had a salty taste, bekasam A had a sweet and sour taste, while bekasam B had a sour taste, salty, and tasty. This suggested that each of the samples with different carbohydrate sources could give a sweet, sour, and salty taste to create a savory taste in the paste favored by the panelists. Nuraini et al. (2014), explained that the addition of brown sugar could unite the taste of salty sour and slightly sweet, then Muller et al. (1999), said that the addition of garlic as a flavoring agent could also stimulate the growth of lactic acid bacteria that affected the hedonic characteristics of Som-fak products, especially texture and taste.

Texture

The texture that belonged to bekasam K was more compact while the texture of bekasam A and B were softer. The addition of brown sugar and garlic added the carbon sources and stimulated the growth of LAB, which them more actively decomposing the protein in the fish body. According to Widayanti et al. (2015), the addition of garlic concentration affected the growth rate of lactic acid bacteria which increased the amount of lactic acid in the waste product, then lactic acid improved the protein decomposition into simpler form and affected the texture of the meat so easily detached from the thorn and the product is more preferred by the panelist.

4. Conclusion

1. Different carbohydrate source in the fermentation process affected the fatty acid composition. Carbohydrate used by microorganism as a energy to growth, hydrolisis and synthesis a fatty acid.
2. Bekasam K had the highest result in nutrition lipid content, fatty acid content, lowest TVBN as an putrefaction indicator. Although the sensory score was not high as other treatment.

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