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PREFACE

By the Grace and Blessings of Allah the Almighty, we would like to present, with great pleasure, the Volume 02 number 01 of *Food ScienTech Journal (FSJ)*. This journal is part of the Universitas Sultan Ageng Tirtaya series of journal.

This journal was envisioned and founded to represent the growing needs of food technology as an emerging and increasingly vital field, now widely recognized as an integral part of agriculture and human living. Its mission is to become a voice of the food technology and science community, addressing researchers and practitioners in areas ranging from chemistry to management, from microbiology to industry, presenting verifiable methods, findings, and solutions.

The journal is intended as a forum for practitioners and researchers to share their research, idea, and solutions in the area of food science and technology. We would like to request for the reader to participate on writing the articles in this journal.

Thank you for your kind attention and support, hopefully this journal will provide lots of benefits for you and society.

Serang, July 2020

Editorial Team

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A REVIEW OF STARCH DAMAGE ON PHYSICOCHEMICAL PROPERTIES OF FLOUR

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ABSTRACT

Starch damage is starch which is damaged by mechanical treatment, such as milling process in flour production. The purpose of this review is to determine the importance of the amount of starch damage affected by the milling process in the physicochemical properties of flour. Starch damage in flour product is influenced by some factors, such as the milling time, methods, and the roller on the milling machine. Beside milling process, the starch modification is also cause damaged to starch, such as fermentation. The presence of starch damage is an important parameter to determine the physicochemical properties of flour. Starch damage in flour will affect the physicochemical properties, such as particle size, amylose content, thermal and pasting properties, retrogradation, microstructure of starch granule, etc. It is very important to pay attention to produce the food product that have a good physicochemical characteristics. The starch damage in flour has an important role in food processing technology, for example: bread, cake, and cookies product. Furthermore, starch damage also affects the shelf life of product that has correlation with retrogradation process.

Keywords: Flour, milling, physicochemical properties, starch damage

INTRODUCTION

Starch is used on several industries, both food and non-food industries. Starch that needed on food industry is closely related to the formation of paste as an adhesive, and is stable to heating and cooling (Nadia *et al*, 2014). Paste as a thickener can form a smooth gel and remain flexible in cold conditions, thereby increasing viscosity, texture, and mouthfeel in food processing product. Functional properties of starch is influenced by it's physicochemical properties. The physicochemical properties of starch are influenced by several factors such as starch source, amylose and amylopectin ratio, and starch gelatinization.

Starches of different types show amylose content, gelatinization profile, starch granules morphology, and starch crystallization are different from the others. In addition, the milling process in the flour process production

will also affect the physicochemical properties of starch. According to Leon *et al*, (2006), during milling process some starch granules suffered mechanical damage, that the level of damage varied depending on the hardness of the seeds and conditions of milling process.

Hard seeds need more energy for milling than soft seeds milling for reduce endosperm to flour, and during this milling process the number of starch granules that suffer more damage. According Jovanovich *et al* (2003), the damage can be found in the milling process, which the endosperm is broken and crushed, some starch granules are physically damaged. Damage to starch granules due to mechanical treatments such as milling process is often called starch damage.

The amount of starch damage is an important parameter in evaluating flour quality (Allister *et al*, 2016). Previous studies shoen that there is correlation between the level of

damage to starch with water absorption, enthalpy gelatinization, and others (Jovanovich *et al*, 2003). The amount of starch damage on flour production can be changed by certain treatments, such as by increasing pressure on the miller. Another factor that influences the amount of starch damage is the type of wheat (hard or soft wheat), that a harder endosperm will produce the amount of starch damage more (Manley in Jovanovich *et al*, 2003).

In addition to milling process and the hardness of seeds, the milling method (dry and wet milling) will also affect the amount of starch damage. The amount of starch damage using wet milling method is lower than dry milling method.

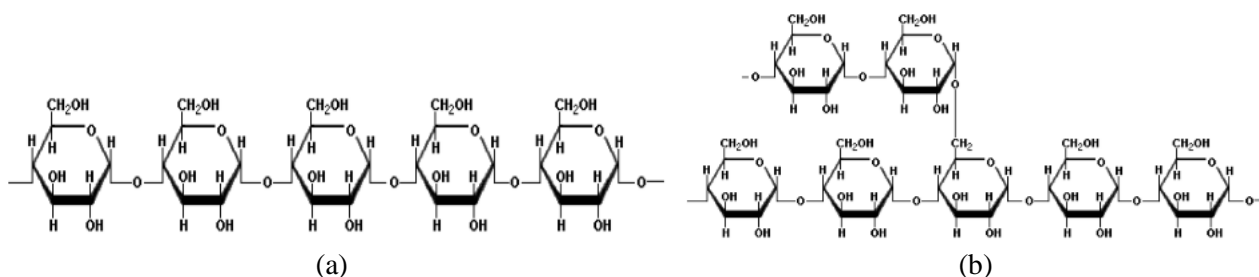


Figure 1. Starch structure chain (a) amylose structure chain, (b) amylopectin structure chain (Zulaidah, 2011)

Starch Damage

Starch damage mainly is caused by mechanical forces obtained during the milling process. During milling process, 5-12% of the starch is damaged. The difference between native starch and starch damage can be seen on Figure 2.

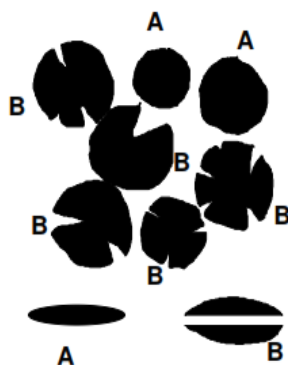


Figure 2. The difference of native starch and starch damage (Loubersac, 2007)

The larger starch granules usually suffer more damage. In Figure 2, it can be seen that native starch has a shape that is still round and flat on its surface, while the starch damage has irregular shape on its surface or is no longer

STARCH

Starch Granule

Starch is composed of particle that have different characteristics, such as size, shape, morphology, composition, and supramolecular structure which depend on botanical source. Starch granule diameters generally range from less than 1 μm to more than 100 μm , and they are generally round, ovale, angular, or can be quite regular. According to Bertolini (2010), starch is composed by two polysaccharides with α -D-glucose structures, namely amylose and amylopectin (Figure 1). Starch granules are composed by different amylose and amylopectin depend on botanical source. Amylose has a straight D-glucose chain, an average glucose unit between 500 and 6000 distributed between 1-20 chains.

circular. According to Dubois in Dubat (2004), there are two types of starch damage namely cracks and breaks (Figure 3).

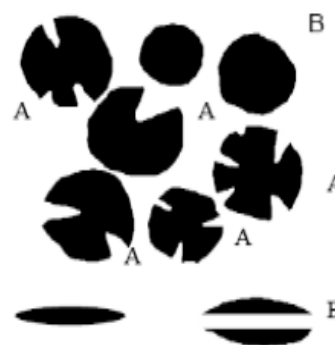


Figure 3. Types of starch damage, A is breaks, B is cracks (Dubois in Dubat, 2004)

According to Dubat (2004), starch damage can affect both the positive and negative effects. One of the positive effect is the presence starch damage causes the water absorbency of starch to be higher, which is 2-4 times its original weight. Pati is said to be 100% damage if it can absorb a number of water that is same with the number of starch at a temperature 30°C, while the native starch is

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only able to absorb water 0.4 times its original weight. This is economically important, because water is one of the inexpensive ingredients to increase the yields as bread and noodle.

On the other hand, too much water absorption can cause the dough to become sticky, it makes difficult to form. Starch damage also allows some specific enzymes to

grow such as β -amylase, and increase the digestibility of starch more. Starch damage shows several similar physical properties that can be seen in Table 1 such as digestibility by α -amylase and β -amylase, birefringence properties, X-ray type, crystallinity, paste viscosity, absorption capacity, solubility, and component that leach.

Table 1. Characteristics of native starch and starch damage

Characteristics	Native Starch	Starch Damage
Digestibility		
a. α -amylase	Slow	Fast
b. β -amylase	-	Fast
Birefringence Properties	Positive	-
X-ray type	A type	-
Crystallinity	Have	-
Paste Viscosity		
a. Cold	Low	Medium
b. Hot	High	Medium
Absorption Capacity	0,5	3-4
Solubility	Low	High
Component that Leach	-	Amylopectin

Source: Arora (2003)

The amount of starch damage can increase with increasing the grain hardness, roll speed of the milling machine, the level of milling, and milling time; also decreasing the rate of material intake (Arora, 2003). According to Morrison and Tester (1994), the differences milling time using a ball mill can changes physical and functional properties of

wheat flour that is produced such as colour, swelling volume, and gelatinization profile. Flour industry can set the amount of starch damage, both by increasing or decreasing it. The way to set the amount of starch damage can be seen on Table 2.

Table 2. The way to set the amount of starch damage

Increasing Starch Damage	Decreasing Starch Damage
Squeeze a firm roll of milling machine	Prevent excessive roll density
Increase a layer compactness	Decrease a layer compactness
Decrease flake disrupter	Choose an efficient flake disrupter
Cover the flour sieves in front of the milling machine	If possible, increase the ash content using grooved rolls

Source: Willm in Dubat (2004)

THE EFFECT STARCH ON FLOUR QUALITY

Different milling methods in producing flour are thought to cause damage to starch and different chemical compositions. During soaking process, the hydrolysis of the constituent polymer become to component that has more solubility in the soaking media, so it can cause the chemical compositions of flour is changed (Usansa *et al*, 2009). In addition,

mechanical process such as milling can also cause starch damage 5-14% (Dubat, 2004).

Particle Size

Milling methods will also affect the particle size of the flour. Using dry milling method, corn flour which is produced has the larger particle size is about 20-40 μm than on corn flour that using wet milling method, its particle size is about 10-25 μm . It is because particle of flour form the starch granule

aggregate and protein matrix, so it has a larger particle size. The smaller particle size will give a smoother and softer texture of product (Suarni and Firmansyah, 2008). The relationship between starch damage and starch particle size can be seen in Figure 4.

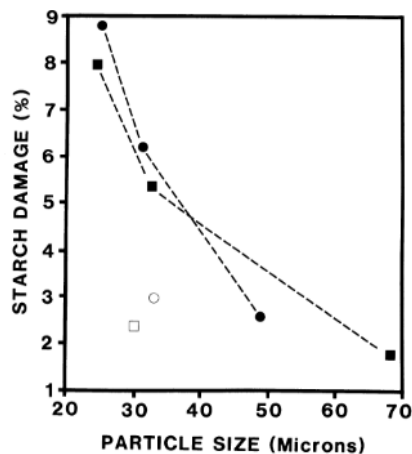


Figure 4. The relationship of starch damage and starch particle size (Gaines *et al*, 1988)

In Figure 4, it can be seen that the amount of starch damage has negative correlation with starch particle size, the larger percentage or the amount of starch damage has the smaller particle size. Flour that is produced using dry milling method has the larger the amount of starch damage and the smaller particle size.

The Amount of Starch Damage

The milling method also affects the amount of starch damage. Corn flour produced by wet milling method has lower an average of starch damage than corn flour produced by dry milling method. According to Suksomboon *et al* (2005), the absorbed water also functions as a lubricant thereby decrease mechanical power generated and give a cold effect during milling. Because of the decreasing mechanical force and milling temperature cause the amount of starch damage also decreases. Conversely, if the dry milling method the material is still intact and hard, thereby it can increase the resistency during milling that cause increasing the amount of starch damage (Bolade, 2009).

Amylose Content

The differences treatment of flour production method such as soaking and milling will affect the flour properties due to chemical compositions changes in flour and damage to starch. On corn flour which is produced using

wet and dry milling method have a different average of amylose content. The average amylose content of corn flour which is produced by wet milling method is lower (28% bk) than corn flour which is produced by dry milling method. This is caused by activated amylase enzyme on soaking process using wet milling method. It can hydrolyze amylose into soluble dextrin (Usansa *et al*, 2009).

Physicochemical Properties

According to Jovanovich *et al* (2003), enthalpy of starch gelatinization has positive correlation with the amount of starch damage when the differences level of starch damage result from differences in the roll pressure used during the milling process. In wheat, the level of starch damage depends on the hardness of the grain and its milling method. Hard wheat flour is more difficult to change into flour particle size, so the hard wheat flour has a larger the average particle size than soft wheat flour (Barrera *et al*, 2007).

Hard wheat flour produce more starch damage during milling process. In addition, Leon *et al* (2006) conducted a study to determine the effect of the amount of starch damage on the thermal properties of flour and bread staling. The results of this study indicate that the amount of starch damage has a large effect on falling number tests on flour but not on triticale flour.

Analysis using DSC showed a higher amount of starch damage significantly decreased the enthalpy of gelatinization of starch and flour formations such as amylose-lipid complex, but was not examined in pasting properties, which is an amylose activity might be able to hydrolyze starch damage. The effect disc mill time on the amount of starch damage, amylose content, falling number, and enthalpy gelatinization of starch samples can be seen in Table 3.

In addition, Leon *et al* (2006) also performed an analysis using Rapid Visco Analyzer (RVA) to determine the pasting properties of samples that are affected by the amount of starch damage. When the starch dispersion in water is heated and shear is applied, the starch granules absorb water and swell, thereby increasing viscosity. During this period, the integrity of the granules is disrupted and amylose molecule comes out. Paste viscosity increases to the point where the intact

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starch granules swell maximally. Peak viscosity indicates the ability of starch to absorb or bind the water (Thomas and Atwell in Leon *et al*, 2006). The broken of starch

during high temperature and shear cause decreasing viscosity into minimum (minimum viscosity).

Table 3. Effect of disc mill damaged starch and amylose contents, falling number, and gelatinization enthalpy of starch

Sample	Milling Time (minute)	Starch Damage (%)	Amylose Content (%)	Falling Number (s)	Gelatinization Enthalpy (J/g)
B0	0	9,3 b	21,5 a	694 f	3,92 d
B1	2	14,7 e	22,2 a	568 d	3,70 c
B2	5	17,2 f	21,6 a	519 c	2,76 a
DE0	0	8,4 b	21,3 a	630 e	4,45 e
DE1	2	12,8 d	20,1 a	505 c	3,57 c
DE2	5	17,7 f	20,1 a	419 b	2,85 a
T0	0	6,1 a	20,5 a	234 a	4,32 e
T1	3,5	10,4 c	20,3 a	229 a	3,64 c
T2	7	14,0 e	19,8 a	231 a	3,27 b

Note: B (Baguette), DE (Klein Don Enrique), T (Tatu); 0, 1, and 2 corresponded to the milling time. Source: Leon *et al* (2006)

When gelatinized starch is cold, amylose rearrangement occurs so that viscosity increases again, it form the gel in end the test (final viscosity). This increasing viscosity is

called setback and it shows retrograde amylose chain. The results of the analysing of the pasting profile can be seen on Table 4.

Table 4. Effect of the amount of starch damage on flour pasting properties

Sample	Peak Viscosity (cP)	Minimum Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)
B0	1880 ± 30	1133 ± 47	752 ± 52	2364 ± 33	1231 ± 23
B1	1834 ± 56	1075 ± 34	759 ± 42	2359 ± 40	1284 ± 46
B2	1742 ± 51	1007 ± 55	735 ± 47	2228 ± 24	1221 ± 27
DE0	1697 ± 36	1151 ± 16	546 ± 31	2405 ± 19	1254 ± 39
DE1	1628 ± 32	1056 ± 36	572 ± 48	2371 ± 37	1315 ± 41
DE2	1439 ± 38	922 ± 44	517 ± 26	2132 ± 38	1210 ± 32
T0	947 ± 18	269 ± 31	678 ± 39	830 ± 48	561 ± 29
T1	882 ± 31	283 ± 22	599 ± 28	1019 ± 51	736 ± 34
T2	836 ± 28	263 ± 51	573 ± 35	992 ± 53	729 ± 21

Note: B (Baguette), DE (Klein Don Enrique), T (Tatu); 0, 1, and 2 corresponded to the milling time. Source: Leon *et al* (2006)

Microstructure of Damaged Starch

Different milling methods in producing flour are thought to cause damage to starch and differences in the microstructure of starch granule. Furthermore, microstructure of native starch is different with microstructure of modified starch. Microstructure of starch granule can be analyzed using trinocular microscope and Scanning Electron Microscopy (SEM).

According to Putri *et al* (2018), MOCAF starch granules that produced using dry and wet milling method with the differences of

fermentation time is different. The particle size of MOCAF starch granule after fermentation is smaller than before fermentation process.

Furthermore, the particle size of MOCAF starch granule using wet milling method is also smaller than MOCAF starch granule using dry. It is caused in wet milling method the milling process is twice. The differences of MOCAF starch granule microstructure are presented in Figure 5.

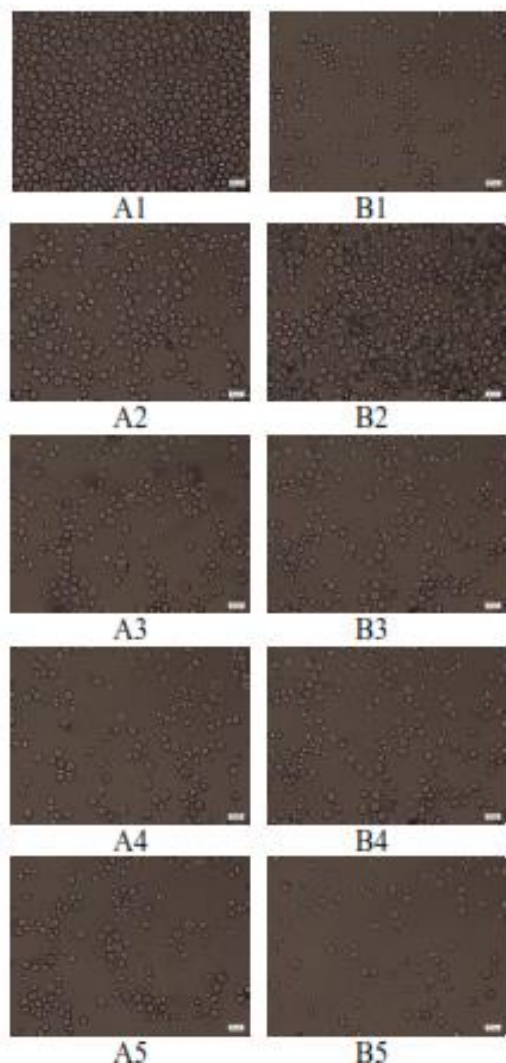


Figure 5. MOCAF starch granule microstructure with different milling method, (A= dry milling method, B= wet milling method), and fermentation time (0,6,12,18,24 hour) (Putri *et al*, 2018)

Scanning Electron Microscopy (SEM) of native and oxidized cassava starch granule shown in Figure 6. According to Sangseethong *et al* (2010), native cassava starch granules had round shape with a truncated end on one side. The surface of native starch granules was smooth with no evidence of any fissures.

In general, similar pattern of changes on external morphology of starch granule was observed for oxidized starches produced by either hypochlorite or peroxide oxidation (Figure 7). After 60 min, a slightly roughened surface was observed, with the oxidation time 120 and 300 min, the granule surface became rougher (Sangseethong *et al*, 2010).

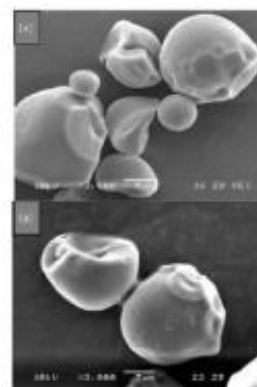


Figure 6. Microstructure of cassava starch, (a) native cassava starch, (b) modified cassava starch (Putri, 2018)

According to Putri (2018), all of starch granule MOCAF samples shown two type a damage of starch are cracks and breaks due to milling process (Figure 8).

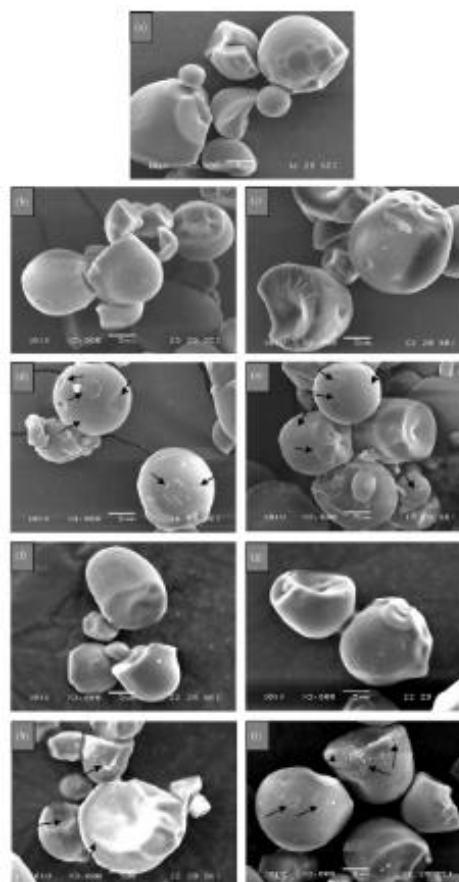


Figure 7. Microstructure of cassava starch, (a) native; (b-e) oxidized cassava starches prepared with hypochlorite, and (f-i) peroxide at various reaction times: 30 min (b and f), 60 min (c and g), 120 min (d and h), and 300 min (e and i) (Sangseethong *et al*, 2010)

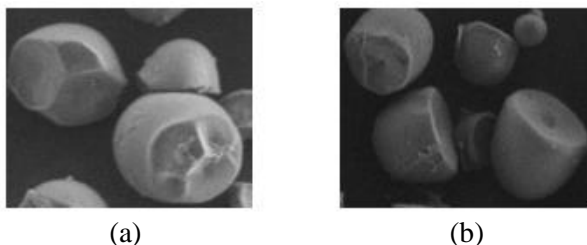


Figure 8. Starch damage type in MOCAF (a) breaks, (b) cracks (Putri, 2018)

CONCLUSION

The main factor causing damage to starch is mechanical treatment, such as milling process. Starch modification can also cause damage to starch. Starch damage can affect physicochemical properties such as particle size, amylose content, thermal and pasting properties, retrogradation, and microstructure of starch granule. It is important parameter to determine quality of flour.

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SENSORY EVALUATION OF YOGURT IN VARIOUS SUGAR CONCENTRATION

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ABSTRACT

The purpose of this study was to determine the hedonic test of various concentrations of sugar in UHT milk with the help of *Lactobacillus casei* in making yogurt on the color, aroma, taste, and thickness of the resulting yogurt. In this study, there are treatment factors, namely the concentration of granulated sugar. The results showed that the concentration of sugar affects the color, aroma, taste, and thickness of the resulting yogurt. In the hedonic test, the results were observed that in each provision of sugar found a difference in values obtained.

Keywords: Hedonic test, milk, *lactobacillus casei*, yogurt

INTRODUCTION

Milk is a food ingredient that is rich in nutrients that are beneficial to the organism. Milk contains calories, protein, fat, vitamins and minerals. Some people don't like to consume fresh milk because they don't like the taste, aroma, or appearance of the milk itself. Therefore, various attempts were made to change the taste, aroma, and appearance without reducing the nutritional value, so that more people would prefer to even attempt to increase the nutritional value of the milk. One of the dairy products is yogurt. In yogurt, there is an additional microbial population that is very beneficial for the human body.

The bacteria or microbes are *Lactobacillus casei*, the bacteria metabolize in the milk so that there is a change in the taste, aroma, and appearance of the milk. The aroma of milk feels fragrant and delicious with a fresh and slightly sour taste and the milk becomes rather thick. With the taste, aroma, and appearance of milk like this, some people who initially did not like milk came to like it so that their body's need for nutrients can be fulfilled (Asri *et al.*, 2017).

Yogurt is a product made from milk through the fermentation process of lactic acid bacteria, *Lactobacillus bulgaricus* and

Streptococcus thermophilus (Collins, et al., 1992). Yogurt is very good for health, especially to maintain stomach acidity and can suppress the growth of pathogenic bacteria in the intestines. Besides, yogurt also contains protein with high levels, even higher than milk protein. This is due to the addition of proteins from microbial synthesis and protein content from these microbes (Winarto, 2003).

The pattern of life of the people who realize the importance of health causes food needs are not limited to the fulfillment of conventional nutrition for the body and mouth gratification for a good taste but are expected to be able to function to maintain health and fitness. Such food products are commonly called functional food products. One example of conventional food products is fermented foods or drinks.

Fermented food and beverage products from various materials have long been made and known to humans. One of the fermentation products is yogurt. Yogurt is a fermented milk product made from full milk and skim milk that has been pasteurized or sterilized and then added the microbial culture *Streptococcus thermophilus* and *Lactobacillus bulgaricus* symbiotic products lactic acid from the characteristic flavor of yogurt (Oberman,

1985). Yogurt is consumed because of its freshness, aroma and distinctive texture. The distinctive taste in yogurt arises because of the fermentation process (Yusmarini, 2004). Yogurt is also a product that is more easily digested in the digestive tract than whole milk or whole milk (Prayitno, 2006).

Fresh cow's milk is a very high nutritional food, so it is not only beneficial for humans but also microorganisms. Therefore we need processing in terms of milk. One of the milk processing efforts that can overcome this is by the way of fermented milk. This fermentation of milk processing will make cow's milk turned into cow's milk yogurt.

Apart from fresh milk, various alternative ingredients can be used as the main ingredient of yogurt as well as skimmed milk powder. The quality of skimmed milk powder is very important in producing good quality yogurt.

UHT milk, UHT milk is milk that is made using a heating process that exceeds the pasteurization process, generally refers to a certain combination of time and temperature to obtain sterile commercial products, the selection of the right combination of time and temperature is also called the UHT sterilization technique (Eniza, 2004). The advantage of UHT milk is its very long shelf life at room temperature, which reaches 6-10 months without preservatives and does not need to be refrigerated (Ide, 2008).

MATERIALS AND METHODS

Tools and Materials

The main ingredients used in the research are UHT milk, culture *Lactobacillus casei* pure, and granulated sugar. The tools used in this research are scale pipettes, stoves, pans, stirrers, thermometers, measuring cups, plastic cups with cups lid, and incubators.

Methods

The study was conducted in the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University in November 2017. The process of making yogurt in this study by boiling 1 liter of UHT milk (for each treatment) until the temperature is 90 0C in an open container while stirring for 1 hour.

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Then 75 grams of sugar, 100 grams, 125 grams, and 150 grams are added and stirred until dissolved and than store it in a sterile bottle.

After the temperature becomes 40-50°C, the milk is inoculated with *Lactobacillus casei* bacteria and the bottle is tightly closed again. Then incubated at 40°C for 2-3 days, to form 3 layers, namely the first layer is yellowish white, the second layer is clear which contains *Lactobacillus casei* and the third layer is thick white which is protein.

RESULTS AND DISCUSSION

The hedonic yogurt test results can be seen in the Table 1.

Table 1. The result of yogurt hedonic test.

The Test	Treatment			
	A (75 g)	B (100 g)	C (125 g)	D (150 g)
Colour	60	60	68	43
Smell	43	49	56	57
Flavour	35	42	52	65
Aroma	48	57	62	66

The results of the analysis showed that there were differences in the values of each treatment to the color, aroma, taste, and thickness of yogurt. This is because increasing of sugar concentration, will on decreasing the bacterial activity so that the formation of lactic acid from lactose is reduced and affects the color, aroma, taste, and thickness of yogurt. This is following the statement of Winarno *et al* (1980) if bacteria, yeast, and mold are placed in a concentrated solution then the water in the cell will come out through the membrane and flow into the sugar solution.

The colors that appear on yogurt produce varying values in the hedonic test. Because the colors that appear in all four treatments produce the various color. For taste and aroma attributes, the highest value was found in the treatment 150 gram of sugar. Similarly, the resulting aroma. This is caused by the culture of *Lactobacillus casei* pure which is still actively producing lactic acid. Lactic acid causes an increase acidity (Kosikowski, 1982).

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Giving sugar can inhibit *Lactobacillus casei* to produce lactic acid, increasing of sugar

concentration, will on decreasing the sour taste in yogurt and affect to the taste and aroma.



Figure 1. The yogurt result

The highest level of consistency is found at the highest sugar concentration, which is at 150 gram sugar concentration. Increasing of sugar concentrations, will held the thicker of yogurt this is because dissolved solids are formed. Purwati (2006) said that high acidity can cause the protein to clot, and cause product thickness. So the level of viscosity produced in the treatment of 150 grams of sugar concentration is lower than the level of viscosity in other treatments.



Figure 2. Participant of the yogurt hedonic test

CONCLUSION

Based on the results of the study it can be concluded that the yogurt hedonic test there are various kinds of differences in color, aroma, taste, and thickness of yogurt in the treatment of various concentrations of sugar. The highest hedonic yogurt test results are at the highest sugar concentration of 150 grams.

It is recommended in making yogurt should use a sugar concentration of 150 grams/liter and use fresh UHT milk so that yogurt products can be obtained with better quality and much preferred.

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CHEMICAL COMPOSITION AND AMINO ACID PROFILE OF FRESH AND STEAMED COBIA (*Rachycentron canadum* L.)

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ABSTRACT

Cobia (*Rachycentron canadum*) is economically important fish with good prospects because of fast growing and easily cultured. Cobia is usually processed by many methods including steaming. The purpose of this research was to determine the effect of steaming process on proximate and amino acids composition. The chemical composition of fresh and steamed cobia's meat were determined by proximate analysis. Amino acid compositions of fresh and steamed cobia's meat were measured using HPLC (high performance liquid chromatography). Steaming process reduced 11.31% of water, 1.06% of ash, 26.04% of fat, and 9.11% of protein. Fresh and steamed cobia's meat contained 17 amino acids consisting of 9 essential amino acids and 8 nonessential amino acids. The highest essential amino acid in fresh cobia's meat was arginine (2,262 mg/100 g) and the highest nonessential amino acid was glutamic acid (3,894 mg/100 g). Steaming process reduced amino acids generally. The highest essential amino acid in steamed cobia's meat was leucine (1,379 mg/100 g) and the highest nonessential amino acid was glutamic acid (2,370 mg/100 g). Taurine content of fresh cobia's meat was 120.84 mg/100 g and changed into 94.33 mg/100 g after steaming process.

Keywords: Amino acid, cobia, essential, nonessential, steaming

INTRODUCTION

Cobia fish (*Rachycentron canadum* L) is a very important fish for the people of Indonesia. Cobia fish have an economic value of US \$ 0.5 per seed size of 10 cm, US \$ 6 per kg for consumption and US \$ 4-6 per kg in frozen form (Liao and Leano 2008). This fish attracts the attention of the public both in research and commercial fields to be cultivated. The advantages such as rapid growth, high conversion efficiency, easily changed in maintenance in cages and very resistant to disease.

Fish is a raw material that has nutrients that are beneficial to humans. Fish contain protein in sufficient quantities, which is 18-20%. Protein as one of the macro nutrients has a function in the body that is to create new tissue and maintain existing tissue. Protein can be used as a required fuel energy that not met

by fat and carbohydrate. Proteins are composed of twenty different amino acids. The human body cannot synthesize nine amino acids containing isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine. Amino acids known as essential amino acids are only obtained through food intake (Abdullah *et al.* 2013).

The excess protein of the fish is easily digested by the body and has complete amino acids. The excess released by this protein does not need to be supported by its nature which is easy to repair changes and damage. Physical or chemical treatment of fish food ingredients from the beginning, processing, storage and finally reaching consumers is often the cause of damage to nutritional value, especially protein.

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Steaming is one of the processing method that uses high temperature which has an impact on low nutrient loss, but this depends on the type of food and steaming method. The steaming process will affect the levels of amino acids contained in food so it needs to know about the content of amino acids lost during processing. This research is based on the research of Nurjanah *et al.* (2014) which defines the steaming factor for the chemical composition and mineral red wine, and Jacoeb *et al.* (2012) about steaming factors for crab amino acids. This study aimed to determine the content of yield, proximate, and amino acids of fresh and steamed cobia fish.

MATERIALS AND METHODS

Tools and Materials

The material used in this study was adult cobia fish taken from the Kalianda area, Lampung with a length of 80-100 cm totaling 5 individuals with a harvest age of 80 days. The ingredients used in proximate analysis, amino acids and taurine are selenium, concentrated H₂SO₄ (Merck), NaOH (Merck), H₃BO₃ (Merck), HCl 0.1 N, bromcresol green methyl red (Merck), hexane solvent, HCl 6 N (Merck, Germany), 0.01 N HCl (Merck, Germany), potassium borate buffer pH 10.4 (Merck, Germany), orthoflaaldehyde (OPA) reagents (Merck), methanol (Merck), mercaptoethanol (Merck), 30% Brij-30 solution (Merck), acetonitrile 60% (Merck), sodium carbonate buffer (Merck), Carrez reagents (Merck), danzil chloride solutions (Merck) and methylamine hydrochloride (Merck). The instrument used is a tool for proximate testing as well as amino acid and taurine testing using HPLC (Shimadzu, Japan).

Research Method

This research consists of several stages, namely: 1) Measurement of dimensions and weights of cobia fish; 2) Calculation of yield of cobia fish by separating meat, viscera, bones and fins, and heads; 3) Analysis of chemical composition consisting of analysis of water content, ash content, fat content, and protein content (AOAC 2007); 4) Analysis of the composition of amino acids and taurine.

Procedural Analysis

Amino Acids (Abdullah *et al.* 2013)

Amino acid analysis using HPLC (Shimadzu, Japan) consists of four stages, namely: the stage of making protein hydrolyzate, drying, derivatization and injection and analysis of amino acids.

a. Hydrolizate Protein

Samples were weighed as much as 0.1 g and crushed. The destroyed sample was added with HCl 6 N (Merck, Germany) of 10 mL which was then heated in an oven at 100°C for 24 hours. Heating is done to accelerate the hydrolysis reaction.

b. Sample drying

Filtering aims to make the resulting solution really clean, separate from the solid. The filter results are taken as much as 30 µL and added with 30 µL drying solution. Drying solutions are made from a mixture of sodium carbonate, carrez reagents (merck), danzil chloride solutions and methylamine hydrochloride (merck, Germany) in a ratio of 4: 4.

c. Derivatization

A 30 µL derivatization solution was added to the drying product, the derivatization solution was made from a mixture of methanol (Merck, Germany), sodium acetate (Merck, Germany) and triethylamine (Merck, Germany) in a ratio of 3: 3: 4. The derivatization process is carried out so that the detector is easy to detect compounds in the sample, then dilution is done by adding 20 mL of 60% acetonitrile (Sigma, UK) or 1M sodium acetate buffer (Merck, Gemrany), then left for 20 minutes.

d. Injection to HPLC

The filter results were taken as much as 40 µL to be injected into the HPLC (Shimadzu, Japan). Calculation of the amino acid concentration present in the material is done by making a standard chromatogram using ready-made amino acids that undergo the same treatment as the sample. Amino acid levels in ingredients can be calculated by the formula:

Amino acids (%)

$$= \frac{\text{sample area} \times C \times FP \times BM}{\text{standard area} \times \text{sample weight}} \times 100\%$$

Information:

C = standard amino acid concentration ($\mu\text{g} / \text{mL}$)

FP = dilution factor

BM = Molecular weight of each amino acid (g / mol)

Taurine Analysis (AOAC, 2007)

The content of taurine can be analyzed using a HPLC device (Shimadzu, Japan). Testing the level of taurine, the sample was weighed as much as 0.5 g and put into a 100 mL measuring tube, then added 80 mL of distilled water and 1 mL of carrez reagents (Merck, Germany) and then shaken until homogeneous. Then dilution is done by adding distilled water to the mark and shaking it until it is homogeneous. Then the solution is filtered using whatman filter paper. The filtrate is stored in an erlenmeyer and stored in a dark place. The derivatization step is then carried out by taking 1 mL of sample extract into 10 mL measuring flask, then adding 1 mL of sodium carbonate buffer and 1 mL of dansil chloride solution. After that the sample was allowed to stand for 2 hours then shaken and added 0.5 mL of methylamine hydrochloride latency (Merck, Germany) then shaken again until homogeneous. The result of derivatization is taken as much as 40 μL and then injected into the HPLC to determine the content of taurine in the sample.

Data Analysis

Data from the research of chemical composition and amino acids used descriptive statistics with 3 replications and standard deviations. Data results were compared with relevant literary studies.

RESULT AND DISCUSSION

Size and Weight of Cobia

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The cobia studied were 5 fish and were taken from the Center for Sea Water Cultivation Development, the Ministry of Maritime Affairs and Fisheries, Lampung with a harvest age of 80 days. Cobia fish samples are presented in Figure 1. The average size and weight of cobia fish are presented in Table 1. Chuang *et al.* (2010) stated that cobia fish can reach 8 kg in weight per year from the initial seed size weighing 50-100 g. This is different from the study sample fish which have an average weight of 1.83 kg with an age of 8 months. According to Nurjanah *et al.* (2014), differences in feed, habitat, and age of harvest lead to differences in weights.

Table 1. Morfometriks cobia fish, n=5

Parameter	Value
Long (cm)	64.3 \pm 3.77
Wide (cm)	7.42 \pm 1.20
Height (cm)	14.4 \pm 1.86
Weight (kg)	1.83 \pm 12.1

Rendement of Cobia

Cobia fish used in the study were prepared and the yield was calculated. The yield of cobia fish is presented in Table 2. The meat yield is the largest yield, so the cobia fish meat is suitable for processing. These results prove that the cobia fish can be utilized for processing. Amiza and Aishah (2011) stated that the cobia fish processing waste consisting of skin and bones, has high collagen levels. This skin can be used as a gelatin ingredient that provides benefits in the food industry.

Table 2. Average size and weight of cobia fish, n=5

Part	Value (%)
Meat	36.83 \pm 0.45
Head	28.67 \pm 0.03
Bone and fin	16.42 \pm 0.5
Offal	11.21 \pm 0.4
Skin	6.87 \pm 0.9

Chemical Composition of Cobia

Comparison of the chemical composition of cobia fish meat results of

water and decreases its solubility, so that the protein will be released from meat (Silva *et al*



Figure 1. Cobia Fish and its component
A= cobia ; B.= Gill cobia; C=Offal Cobia; D= meat cobia

research with the results of research by Chuang *et al.* (2010) are presented in Table 3. The difference in results is thought to be due to several internal and external factors. Internal factors include fish species, age, and genetics, while external factors are environmental conditions, both the availability of food, and its competitors, as well as the water quality of their habitat.

The 11.31% decrease in water content is influenced by several factors, namely surface area, concentration of solutes in hot water and stirring of water. The decrease in water content is thought to be caused by the steaming process which makes the water contained in the meat of the fish release and evaporate because of the heat transfer process in fish meat (Pepino *et al.* 2015). Heat transfer and movement of water flow causes the process of evaporation and drying of food ingredients (Selcuk *et al.* 2010). Warming up the protein can cause both expected and unexpected reactions. Provision of heat causes the protein to be denatured, which in turn loses the binding power of the

2017).

The decrease in ash content by 0.2% is caused by the breakdown of mineral particles that are bound to water due to heating so that the minerals in the meat of the cobia fish dissolve into the water when steaming and the water molecules are released from the fish meat tissue (protein). Steaming also causes a decrease in nutrition in an ingredient.

Decrease in fat content by 4.12% due to the giving of heat to cobia fish causes volatile compounds, for example aldehydes, ketones, alcohols, acids and hydrocarbons to evaporate when heating. Fat melts when given heat so that the fat contained in the meat of the cobia fish comes out of the tissue. High temperature heating in the presence of air, fatty acids, aldehydes and ketones which are volatile volatile with water, therefore a decrease in fat content after steaming (Nurjanah *et al.* 2014).

An increase in protein content of 2.16% is thought to be caused by applying heat during steaming of cobia fish meat. Meat protein is divided into 3 based on its solubility namely

water soluble protein (sarcolemma), salt soluble protein (myofibril) and connective tissue protein (stroma).

Table 3. Proximate result of cobia fresh meat and steaming without skin, n=5

Component	Wet basis		Dry basis		Fresh* (%)
	Fresh (%)	Steam (%)	Fresh (%)	Steam (%)	
Moisture content	77.64±0.04	66.33±0.02	0	0	77.14±1.9
Ash	1.1±0.13	0.9±0.768	4.89±0.43	31.39±0.11	1.39 ± 0.11
Protein	10.34±0.45	12.50±0.13	46.22±0.9	19.21±1.1	9.21 ± 1.10
Fat	9.19±0.15	5.07±0.98	41.1±2.7	52.64±1.46	2.64 ± 1.46
Carbohydrate	1.73±0.7	14.81±0.7	7.73±3.2	43.99±3.2	9.62 ± 0.2

*Source: Chuang *et al.* (2010), n=9

Table 4. Essential amino acids content of cobia fish

No.	Amino acids	Fresh (%)	Steam (%)	Fresh * (%)	<i>Scomber japonicus</i> steamed (g.a.a./16g N)**
1	Leucine	2.26±0.02	1.38±0.6	3.6±0.5	8.87
2	Arginine	2.26±0.05	1.29±1.0	7.1±7.2	5.58
3	Lysine	1.98±0.4	1.22±0.3	16.0±15.5	7.44
4	Threonine	1.49±0.5	0.96±0.2	2.1±0.3	4.47
5	Isoleucine	1.49±0.1	0.90±0.57	2.3±0.5	4.59
6	Valine	1.41±0.3	0.92±0.42	2.7±0.4	5.85
7	Phenylalanine	1.24±0.45	0.73±0.3	1.7±0.5	4.56
8	Methionine	0.94±0.34	0.58±0.8	2.3±0.7	2.33
9	Histidine	0.85±0.46	0.51±0.9	2.0±1.0	6.49

*Source: Chuang *et al.* (2010) **Sumber: Oduro *et al.* (2011)

Table 5. Non-essential Amino Acid Content of cobia fish

No.	Amino acids	Fresh (%)	Steam (%)	Fresh * (%)	<i>Scomber japonicus</i> Steam (g.a.a./16g N)**
1	Glutamic acid	3.90±0.23	2.38±0.3	3.3±0.1	15.99
2	Aspartic acid	2.34±0.34	1.56±0.5	0.3±0.2	10.53
3	Alanine	1.39±0.5	1.14±0.4	11.4±2.0	5.85
4	Glycine	1.38±0.7	1.07±0.7	12.7±2.5	6.19
5	Serine	1.16±0.54	1.29±0.8	1.7±0.7	4.74
6	Proline	1.03±0.34	0.64±0.2	5.2±4.6	5.28
7	Tyrosine	0.99±0.47	0.63±0.4	2.5±0.4	3.11
8	Cysteine	0.36±0.29	1.00±0.5	0.4±0.4	-
9	Taurine	0.12±0.12	0.94±0.9	37.3±12.0	-

*Source: Chuang *et al.* (2010) **Sumber: Oduro *et al.* (2011)

Water-soluble proteins have relatively low molecular weights, high isoelectric pH and spherical structures. These physical characteristics may be responsible for the high solubility of sarcolemmas in water, so that the water-soluble protein contained in the flesh of cobia fish dissolves into steamed water. Water-soluble proteins can inhibit the formation of gels, because these proteins have a low water-

binding capacity, so to remove the sarcoplasmic protein, washing can be done with water, for example washing with water, as in the processing of surimi and kamaboko products.

Salt-soluble protein is the largest part in the aquatic meat network of commodities that function for muscle contraction. This protein plays an important role in clumping and gel

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formation during processing. The effect of heating from the steaming process causes denatured proteins, so that the salt-soluble protein loses its functional properties. Some of the protein myofibrils dissolved during steaming takes place due to high temperatures, but the amount dissolved is not as much as the sarcoplasmic protein. This is because the general nature of this protein is salt soluble. Protein solubility depends on the temperature, the higher the temperature the more denatured proteins.

Amino acids composition

The content of essential amino acids in cobia meat is presented in Table 4. The highest essential amino acid content in fresh cobia meat is leucine and arginine with a value of 2.26%, whereas in steamed cobia meat that is leucine with a value of 1.38%. Cobia fish are fish whose habitat is at sea so they have high levels of ureum in their blood to adjust the osmotic pressure in the seawater environment (Nurhayati *et al* 2017). Arginine in fish is involved in the synthesis of ureum in the liver (Nurjanah *et al* 2014). This is what causes high arginine content. Leucine is the most common amino acid found in food sources of protein, especially fish and meat. The function of leucine in fish is that it plays a role in the body's energy production process and controls protein synthesis, as it is known that the amount of protein is the most in the meat of cobia fish, including amino acids leucine (Schweigert *et al.* 2010).

The non-essential amino acid content of cobia fish meat is presented in Table 5. The highest non-essential amino acid of fresh and steamed cobia meat is glutamic acid of 3.90% and 2.38%. Nonessential amino acids are mostly found in animal muscle tissue, namely alanine, glycine and glutamic acid (Abdullah *et al.* 2013). High levels of glutamic acid are caused by an analysis process that uses acid hydrolysis with a higher degree of analysis. Glutamine amino acids undergo deamination to form glutamic acid so that glutamic acid levels increase. Hidayat (2010) states that

glutamic acid is the most important component in the formation of flavor in seafood so that the food tastes delicious. Glutamic acid contains glutamic ion which can stimulate several types of nerves on the human tongue. Cobia fish meat can be said to have a delicious taste.

The results of the analysis of the composition of essential and nonessential amino acids in cobia meat differed from the results of a study conducted by Chuang *et al.* (2010). The difference in amino acid content is thought to be due to various factors namely; type of organ observed, age of harvest and physiological processes of the organism itself (Nurjanah *et al.* 2015). Three amino acids that are not detected are asparagine, glutamine and tryptophan, this is due to the hydrolysis process. The hydrolysis process destroys all tryptophan and cysteine, and if there are metal ions, there will be a loss of methionine and tyrosine (Abdullah *et al* 2017).

Each type of food has a limiting amino acid. This amino acid is an amino acid with the least amount, so it is called a limiting amino acid (Harris and Karmas 1989). The limiting essential amino acid in fresh and steamed cobia fish is histidine with values of 0.85% and 0.51%. Limiting nonessential amino acids in fresh and steamed cobia are cystine with values of 0.36% and 1.00%.

Amino acids of cobia fish meat both essential and nonessential have decreased due to processing. Decreased amino acid content in fish meat after processing is caused by the use of high temperatures. Meat processing using high temperatures causes protein denaturation, so the protein loses its functional properties. Some of the protein dissolved during steaming takes place due to high temperatures (Conrat *et al.* 2010).

Heat treatment aims to make it easier for the body to digest food because complex proteins and carbohydrates change their structure, but the levels of vitamins, minerals and amino acids are reduced. The decrease in amino acid levels is also caused by the formation of the Maillard reaction which is

different from the product during heating (Oduro *et al.* 2011).

Each type of amino acid has different characteristics from each other. Effect of processing in general using heat can cause a decrease in the amount of amino acids depending on the type of processing, temperature, and length of processing (Nurjanah *et al.* 2014). A decrease in amino acids that exceeds 10% has a significant effect on the quality of the foodstuff.

Taurine composition

The content of taurine of fresh cobia fish is 120.84 mg / 100 g while steamed cobia meat is 94.33 mg / 100 g. The decreasing taurine content is caused by food processing, namely steaming (Jiancheng *et al.* 2010). Water vapor produced by heat dissolves the taurine that is in food. Abdullah *et al.* (2017) states that taurine is a type of amino acid that is soluble in water. Cooking with high temperatures causes taurine to be released from food and then dissolved in water and come out carried away by water vapor so that its content is reduced. Washing cobia fish meat can also dissolve amino acids.

The levels of taurine in the cobia fish meat results of the study differed from those of Chuang *et al.* (2010). This is allegedly due to differences in age, catch season and stages in the organism's life cycle (Nurjanah *et al.* 2015). Taurine in marine animals has the function of regulating osmoregulation to remain balanced (Abdullah *et al.* 2013).

CONCLUSION

Cobia fish meat contains 9 types of essential amino acids and 8 types of nonessential amino acids and taurine. The highest essential amino acid content in fresh cobia meat is leucine and arginine. The highest nonessential amino acid content in fresh cobia meat is glutamic acid. The highest proximate composition is protein. Steaming affects the proximate content, amino acids, and taurine.

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THE STUDY OF MARSHMALLOW'S PREFERENCES LEVEL WITH THE ADDITION OF GREEN GRASS JELLY (*Cyclea Barbata* L. Miers)

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ABSTRACT

The purpose of this study is to determine the level of panelists' preferences for green grass jelly marshmallows. The stages of the study consisted of two stages, which are making green grass jelly leaves extract and making green grass jelly marshmallows. The percentage of addition green grass jelly leaves extract as much as 25%, 50% and 75% based on the weight of the liquid needed in making marshmallows. This study uses a hedonic test that aims to determine the response of the panelists' preferences level to the taste, flavor, texture and color of green grass jelly marshmallows. The hedonic test results for the taste of green grass jelly marshmallows showed that products with the addition of green grass jelly leaves extract by 25% got the most preference from Panelist with mean score of 3.50 ± 0.73 (neutral). While the hedonic test results for the flavor, texture and color of green grass jelly marshmallows, showed that products with the addition of green grass jelly leaves extract by 50% got the most preference from Panelist with mean scores of 3.50 ± 0.68 (neutral), 3.50 ± 0.51 (neutral) and 3.30 ± 1.06 (neutral). Based on the results of determining the best product, panelists preferred marshmallows with the addition of green grass jelly leaves extract by 50%.

Keywords: marshmallows, green grass jelly leaves, preferences level

INTRODUCTION

Green grass jelly (*Cyclea barbata* L. Miers) is commonly found in various regions in Indonesia. There are three types of grass jelly known to the public namely green grass jelly, black grass jelly, and shrubs grass jelly. However, Indonesian people are more fond of green grass jelly, because physically it has thin and limp leaves so it is easier to squeeze to produce grass jelly gel.

Green grass jelly leaves are widely used by the community as traditional food to help reduce body heat, heartburn, stomach aches (nausea) and diarrhea. Grass jelly leaves are known to contain chlorophyll, as well as bioactive compounds polyphenols, saponins, flavonoids and fats. These four components are generally known as antioxidants, anticancer, and anti-inflammatory. Seeing the benefits of grass jelly leaves on the human body, it is necessary to develop a food product based on green grass jelly leaves that are attractive and

acceptable and liked by all ages. One food product that can be added with green grass jelly is marshmallows.

Marshmallows is one type of soft candy which has a texture like foam, chewy, soft, and when eaten will melt in the mouth (Nakai & Modler, 1999). Marshmallows is not only liked by children, but also adults like it. The price is relatively cheap and easy to find on the market. Some researchers have developed marshmallow products that provide more benefits to marshmallows. Research from Jalasena RA *et al.* (2016), marshmallows are processed with the addition of broccoli as an alternative high-antioxidant product. Research from Kinadari (2013), showed that marshmallows are processed with the addition of spirulina as a natural coloring agent. Research from Rumadana (2015), showed that marshmallows are processed with the addition of seaweed as a stabilizer. Research from Chandra (2013), showed that marshmallow is

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processed by adding angkak extract as a natural coloring agent. Whereas in this study, the use of green grass jelly leaves aims as a thickening agent and natural coloring. This innovation is expected to be liked by the community, as well as provide references for new food products based on green grass jelly for the community. The purpose of this study was to determine the level of community preference for green grass jelly marshmallows.

MATERIALS AND METHODS

Materials

Ingredients used in making green grass jelly marshmallows are sugar, green grass jelly extract, gelatin, salt, cornstarch and refined sugar. The green grass jelly itself was collected from the yard self-owned by researcher. The equipment used to make green grass jelly marshmallows are hand mixer, dough compost, spatula and mold.

Methods

The experiment of making green grass jelly marshmallows took place in the Laboratory of Culinary Art Program, Akademi Kesejahteraan Sosial Ibu Kartini, located on Sultan Agung street Number 77, Gajah Mungkur, Semarang City, Central Java Province. Ingredients used in making green grass jelly marshmallows are sugar, green grass jelly extract, gelatin, salt, cornstarch and refined sugar. The equipment used to make green grass jelly marshmallows are hand mixer, dough compost, spatula and mold.

This study compared the level of preference of Panelists to marshmallows with addition of green grass jelly leaves extract by 25%, 50% and 75%. The percentage addition of green grass jelly leaves extract based on the weight of the liquid needed in making marshmallows. The stages of this study consisted of two stages, namely the stage of making green grass jelly leaves extract and the stage of making green grass jelly marshmallows.

The stage of making green grass jelly leaves extract requires green grass jelly leaves that are old and clean, then mashed and kneaded using warm water with a ratio of 1:3, then filtered to produce green grass jelly leaves which are ready for use in making marshmallows. Meanwhile, the stages of

making green grass jelly marshmallows can be seen in Figure 1.

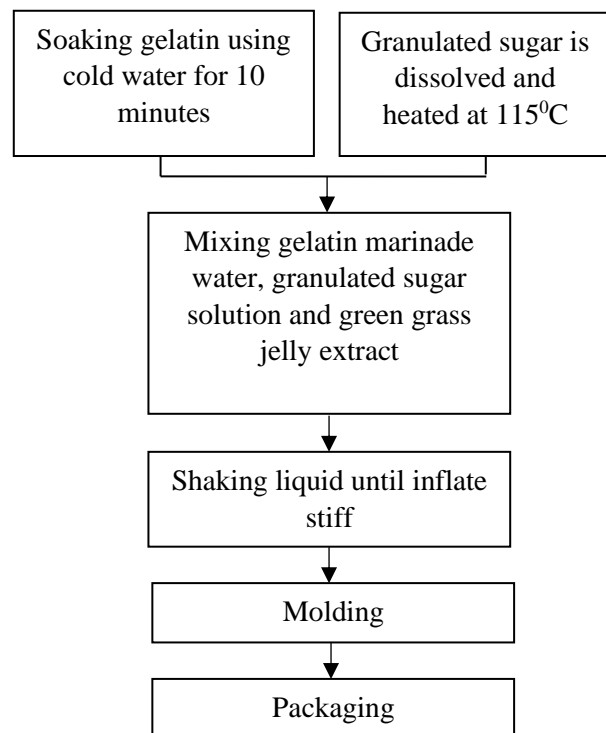


Figure 1. Flow chart of the process of making green grass jelly marshmallows

Sensory Evaluation

This study uses a hedonic test to determine the response level of the Panelists' preference for the taste, flavor, texture and color of green grass jelly marshmallows. The number of Panelists who took the hedonic test were 30 untrained Panelists.

The hedonic green grass jelly marshmallows test questionnaire uses a hedonic scale with very likes, likes, neutral, dislikes, and very dislikes criteria. These criteria are given a score of 5 for very likes, a score of 4 for likes, a score of 3 for neutral, a score of 2 for dislikes, and a score of 1 for very dislikes.

Calculation of the mean score and standard deviation using the Microsoft Excel program. The mean scores and standard deviations of each experimental product are then compared in the form of bar charts. Panelists also gave the most preferred product ratings. Analysis of the data used is descriptive analysis by evaluating and explaining experimental results.

RESULT AND DISCUSSION

Representation of the marshmallows with addition of green grass jelly leaves extract by 25%, 50% and 75% can be seen in Figure 2.



(a)



(b)



(c)

Figure 2. Product of marshmallows with (a) 25%, (b) 50%, and (c) 75% grass jelly leaves extract

Taste

The mean score of the Panelists' preference level for the taste of green grass jelly marshmallows can be seen in Figure 3. Based on Figure 3, the hedonic test results on the taste of green grass jelly marshmallows showed the highest mean score of 3.50 (± 0.73) in the neutral rating range or closed to like the choice of products with addition of 25% green grass jelly leaves extract. Panelists prefer the

The study of marshmallow's preferences ... taste of marshmallows with addition of 25% green grass jelly leaves extract because it has a sweet taste and the taste of green grass jelly is not so strong compared to products that are added green grass jelly leaves extract as much as 50% and 75%. The more the green grass jelly leaves extract on marshmallows added causes more bitter taste. Marshmallows with the addition of 75% green grass jelly leaves extract has the lowest mean score of 2.40 (± 0.50) which is not acceptable to Panelists.

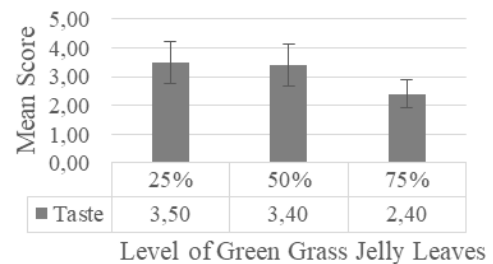


Figure 3. Mean score the Panelists' level of preference for the taste of green grass jelly marshmallows

Flavor

The mean score of the Panelists' preference level for the flavor of green grass jelly marshmallows can be seen in Figure 4.

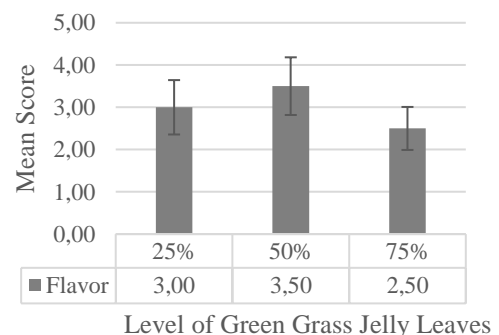


Figure 4. Mean score the Panelists' preference level for the flavor of green grass jelly marshmallows

Based on Figure 4, it is known that the results of the hedonic test on the flavor of green grass jelly marshmallows show that the highest mean score is 3.50 (± 0.68) in the neutral rating range or closed to like the product with the addition of green grass jelly leaves extract by 50%. Panelists prefer the flavor of marshmallows with the addition of green grass jelly leaves extract as much as 50% because it has a distinctive flavor of green grass jelly but not too sharp, so that it can still be accepted by Panelists compared to products that are given

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additional green grass jelly leaves extract as much as 75%. Whereas marshmallows with the addition of green grass jelly leaves extract as much as 25% do not have the distinctive flavor of grass jelly, so it cannot be accepted by Panelists with an mean score of 2.40 (dislike). Basically the smell of grass jelly is not strong. The smell of grass jelly comes from volatile components, such as linalool, styrolyl, this component is a group of flavortic compounds (Dalimartha, 2005).

Texture

The mean score of the Panelists' preference level for the texture of green grass jelly marshmallows can be seen in Figure 5.

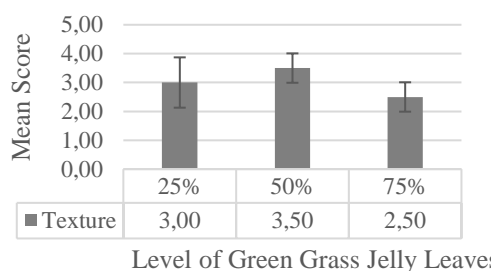


Figure 5. Mean score the Panelists' preference for the texture of green grass jelly marshmallows

Based on Figure 5, it is known that the hedonic test results on the texture of green grass jelly marshmallows shows that the highest mean score is 3.50 (± 0.51) in the neutral rating range or closed to like the product with the addition of green grass jelly leaves extract by 50%. Panelists prefer the texture of marshmallows with the addition of green grass jelly leaves extract as much as 50% because it has a chewy and soft texture.

For marshmallows with the addition of green grass jelly leaves extract as much as 25% produces a chewy texture but is too soft so it is easily broken. Whereas marshmallows with the addition of green grass jelly leaves extract as much as 75% produce a chewy texture that is too hard so that it cannot be accepted by Panelists.

Marshmallow's texture can be influenced by material formulation and manufacturing processes. Marshmallows composition ingredients such as granulated sugar, green grass jelly extract and gelatin affect the solidity of marshmallows. The function of gelatin as a stabilizer can form a gel layer that binds water molecules so that the formed marshmallows

become stiff and chewy. While the effect of the manufacturing process on the texture of marshmallows if the marshmallows dough shaking process is not right will result in low amounts of air trapped in marshmallows causing marshmallows to have a hard texture (Rohman, 2013)

Color

The mean score of Panelists' preference level for the color of marshmallows green grass jelly can be seen in Figure 6.

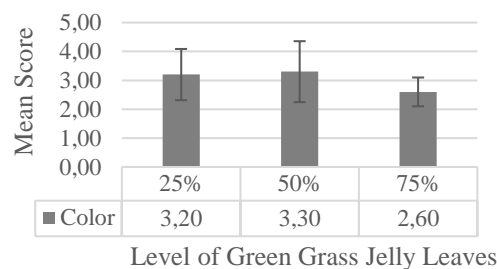


Figure 6. Mean score the Panelists' preference for the color of green grass jelly marshmallows

Based on Figure 6, the hedonic test results on the color of green grass jelly marshmallows showed the highest mean score of 3.30 (± 1.06) in the neutral rating range of products with 50% green grass jelly leaves extract.

Green grass jelly leaves contain chlorophyll pigments that produce a green color, producing marshmallows that are produced green (Palupi, 2015). The panel prefers the color of marshmallows by using green grass jelly leaves extract as much as 50% because it has a white color with green spots scattered from the grass jelly leaves. For marshmallows by using green grass jelly leaves extract as much as 25% produces a pale white color. While marshmallows using green grass jelly leaves extract by 75% produces a light green color but at this percentage it cannot be accepted by Panelists.

Based on the best product selection results, Panelists preferred marshmallows by agreeing to 50% green grass jelly leaves extract. Because, these products are more easily accepted by Panelists in terms of taste, flavor, texture and color. The higher the green grass jelly extract, the lower the Panelists' preference because of the dominant the taste and flavor of green grass jelly extract.

CONCLUSION

The hedonic test results for the taste of green grass jelly marshmallows showed that products with the addition of green grass jelly leaves extract by 25% had the highest mean score of 3.50 ± 0.73 (neutral or closed to like). While the hedonic test results for the flavor, texture and color of green grass jelly marshmallows, showed that products with the addition of green grass jelly leaves extract by 50% had the highest mean scores of 3.50 ± 0.68 (neutral or closed to like), 3.50 ± 0.51 (neutral or closed to like) and 3.30 ± 1.06 (neutral). Based on the results of determining the best product, Panelists preferred marshmallows with the addition of green grass jelly leaves extract by 50%. Understanding the limitation of this research, researcher suggest to improve this kind of research in the future with complimentary of proximate analysis.

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PREPARATION AND CHARACTERISTICS OF PACIFIC CODFISH (*Gadus macrocephalus*) MYOFIBRIL FOR SURIMI

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ABSTRACT

Myofibril is contributing to gel-forming. Every species of fish have different myofibril concentration. Pacific codfish has white flesh which is expected to make surimi. The objective of this research was to analyze the characteristics of surimi prepared from Pacific codfish myofibril (SPM). The method of this research was used ionic strength by using NaCl. The observe parameters of this research were protein solubility, color, microstructure, molecular weight, and texture. The results showed that SPM have 3-dimensional network with rigid and porous structure than other surimi gels. The major molecular weights were 150 kDa (zetalin) and 40 kDa (tropomyosin). The hardness, cohesiveness and adhesiveness of SPM were 0.071338 N/cm², 0.259 gf/sec and 116 gf.mm respectively. These results were shown that Pacific codfish was suitable to be used as surimi raw material because it can make a good gel to form surimi.

Keywords: Codfish myofibrillar protein, molecular weigh, mirostructure, texture

INTRODUCTION

Fishes are one of the food resources with high protein content. Fresh fish compositions per 100 gram are moisture (76%), protein (17%), fat (4.5%), mineral and vitamin (2.52–4,5%). Fish is excellent food material because of the high nutritive value of its muscle protein. Fish proteins are classified by sarcoplasmic (20–30%), myofibrillar (65–75%), and stroma (1–3%) (Suzuki, 1981).

Myofibril is a basic rod-like unit of a muscle cell. Myofibrils are composed of long proteins including actin, myosin, and titin, and other proteins that hold them together. Myofibril protein dissolves in the salt solution with medium ionic strength. Myofibril is contributing to gel forming. Every species of fish have different myofibril concentration.

The previous research, surimi made from Alaska pollock. This study compared the semi-gel system at a low temperature with a heat-induced gel system. The result showed that the formation process of heat-induced gel is intertwined while it is temporally separated in cold storage. Overall, myosin was selected as

the starting point for establishing a schema chart to characterize the gelation processes of the cold semi-gel and heat-induced gel (Liu, et.al., 2019). Black mouth croaker of surimi have the proximate composition contains protein (14.77±0.506%), lipid (0.94±0.081%), ash (0.58±0.007%) contents and yield rate (36.56±0.732%). It was known that the surimi from Black mouth croaker was an appropriate raw material for surimi production although it was a pellagic fish (Shekarabi, et.al., 2014). On the other hand, surimi from silver carps showed that the variations in chemical interactions were strongly correlated to gel properties across different washing processes, especially for hydrogen bonds. In terms of the enhancement of gel properties, washing with 0.2% CaCl₂ could bring high value when applied to the aquatic industry. (Zhang, et. al., 2018).

Pacific codfish (*Gadus macrocephalus*) is demersal fish found in huge schools confined to temperate waters in the northern hemisphere. The Pacific codfish is found in both eastern and western regions of

the Pacific. This fish can grow up to 49cm and weigh up to 15kg. Production of Pacific codfish in Japan was 6,610,000 tonnes (FAO, 2014).

Pacific codfish has been an important economic commodity in international markets and popular as a food fish with a mild flavor, low-fat content and a dense white flesh. The majority, Pacific codfish was sold in fillet flesh form. Whereas, Pacific codfish has white flesh and potential use of gelling product raw material.

White flesh is better using for product that have gelling ability product such as surimi. But every white flesh has different characteristics. So it's important to know the characteristic of surimi from Pacific codfish. The objective of the research was to know the characteristics of myofibrillar Pacific codfish myofibril (SPM) for surimi.

MATERIALS AND METHODS

Raw material

Fresh Pacific codfish (*Gadus macrocephalus*) with an average weight of 400–500 g were purchased from the local supermarket (Hiroshima, Japan). Upon the arrival, fish were immediately cut in small size and minced to uniformity by using a grinder.

Preparation of fish myofibrillar protein

Fish myofibrillar protein from Pacific cod mince was prepared according to the method of Subagio, Windrati, Fauzi, and Witono (2004) with some modification. Fish mince was added with 3 volumes of 0.5 % NaCl in 0.1 M phosphate buffer pH 7 and stirred at 4 oC for 3 min, followed by centrifugation at a speed of 3,000 g 4 oC for 10 min. The residue was added with 3 volumes of 0.5 % NaCl in 0.1 M phosphate buffer pH 7 and stirred at 4 oC for 3 min, followed by centrifugation at a speed of 3,000 rpm 4 oC for 10 min.

The residue was filtered by using filter cloth 4 layers. The filtrate was added with 3 volumes of 0.5 % NaCl in 0.1 M phosphate buffer pH 7 and followed by centrifugation at a speed of 3,000 g 4 oC for 10 min. The residue is myofibrillar protein. Myofibrillar protein was added with 5 % sucrose (w/w) and dried by using freeze-drying and kept in an airtight chamber.

Preparation of SPM

Myofibril powder was added in 4% NaCl solution with ratio 1:3 (myofibril powder: NaCl solution) and followed by blend it's all by using pounder. After all material was blended, the mixture was heated at 90 °C for 30 min. SPM was kept at room temperature for 1hr to decrease the temperature. After that kept SPM in 4 °C before until analyzed time.

Protein solubility

The soluble content of myofibrillar gel protein was determined using the Folin-Lowry method (Najafian and Babji, 2015) with some modification. Preparation of the sample was started by made sample concentration 2.5 mg/mL and stirred by using magnetic stirrer for 1 hr and 24 hr at 4 oC and 25 oC, followed by centrifugation at a speed of 3000 g for 5 min.

The supernatant of 0.5 mL of the sample was mixed with 2.5 mL of an alkaline-copper reagent and incubated for 10min at room temperature. The mixture was added to 0.25 mL of Folin-Ciocalteu's phenol reagent at 2 times dilution with deionized water and left for 30 min at room temperature. The absorbance at 750 nm was measured with a spectrophotometer (Model U-2001, Hitachi, Japan). The soluble protein content was quantified using bovine serum albumin as the standard with absorbance value were 0.128, 0.159, 0.292, 0.387, 0.500, 0.604. Equation of BSA standard curve was $Y = 0.0022x + 0.0655$.

Color

The color of myofibrillar gel protein was determined using a colorimeter (Konica Minolta CM-700d/600d). L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were measured, and whiteness was calculated as described by Lertwittayanon, et al. (2013) as follows:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

Microstructure of protein

The microstructure of myofibrillar gel protein was determined using a scanning electron microscope (Arfat and Benjakul, 2012) with some modification. Samples with a thickness of 0.5x0.5 mm were fixed with 2.5 % (v/v) glutaraldehyde in 0.2 M phosphate buffer

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(pH 7.2) for 2 hr. The samples were rinsed in distilled water before being dehydrated in graded ethanol with serial concentrations of 50%, 70%, 80%, 90%, 95%, and 100% (v/v). Dehydration was conducted for 5 min in each solution. Dried samples were mounted on a bronze stub and sputter-coated with gold. The specimens were visualized with a Scanning Electron Microscopy (Hitachi TM 3000).

Molecular weight (SDS-PAGE)

Myofibrillar gel protein was examined for protein patterns based on their molecular weight according to the method of Fowler and Park (2015) with some modification. To prepare the protein sample, 3.75 mL of 20 mM Tris-HCl buffer pH 8 containing 8 M urea, 2 % SDS, and 2 % 2- mercaptoethanol was added to the sample (0.2 g). The mixture was boiled at 99 oC for 2 min and shook more than 20 hr at 30 oC for dissolve the sample. The mixture was taken 200 µL and added 50 µL of 50 mM Tris-HCl buffer pH 8 containing 5 % SDS, 5 % 2-mercaptoethanol, and 50 % glycerol. The sample was boiled at 99 oC for 1 min.

Sample (2.5 µL, 5 µL, 7.5 µL, 10 µL) were loaded onto the polyacrylamide gels comprising a 12.5 % running gel and subjected to electrophoresis at a constant current of 20 mA by using an electrophoresis unit (AE 6530 serial number 5009405) for 75 min. Gels were fixed and stained in 0.125 g/100 mL Coomassie brilliant blue R-250, Followed by rinsed with distilled water 3 times. Molecular weights of bands were determined by comparison to a molecular weight standard (XL-Lader Broad Range SP-2110).

Texture

The texture of myofibrillar gel protein was determined according to Lertwittayanon, et. al. (2013) with some modification. Gels were equilibrated and evaluated at room temperature. One cylinder-shaped sample with a length of 3.0 cm was prepared and subjected to determination. Hardness, cohesiveness, and adhesiveness were measured using the Rheometer (Fudoh Rheometer) equipped with a spherical plunger with diameter 7.0 mm. The result was will be plotting in the equation.

Hardness (N/cm²) = force (g) X 0.0098 X
cross-section area of
probe

Cohesiveness (gf/sec) = A2 (peak area of
second time) / A1
(peak area of first
time)

Adhesiveness (gf.mm) = peak area drawn at Y-
axis negative
direction; A3

RESULT AND DISCUSSION

Protein solubility

Solubility is an important property in the utilization of proteins. Protein solubility of SPM in water was investigated. In all the conditions tested, protein in water phase was not detected. It was suggested that SPM was stabled in water even at room temperature for 24 hr. Another hand, the preparation of fish myofibrillar protein by using 0.5 % NaCl in 0.1 M phosphate buffer pH 7 made the SPM stabled.

According to Kim, Y. S. (2002), protein solubility in aqueous solution was dependent on pH. The isoelectric point was the pH at which a protein has zero net charge in solution. For most of proteins, minimum solubility occurs at the isoelectric pH. Solubility of Pacific whiting (*Merluccius productus*) protein was the lowest at pH 5.5. The pH more than 5.5 made the solubility was stabled. Gao, et. al. (2018) reported that myofibrillar protein of surimi gel was stabled in 0 day. But it would be decreased after 3 days.

Color

Lightness (L*) value of SPM was 71.097, a* value was -2.947 (small redness), and b* value was 4.733 (small yellowness). Whiteness value of SPM was 70.563. Pacific codfish has white flesh so whiteness value is higher. Whiteness value of SPM was lower than yellowtail barracuda surimi (78.24) (Lertwittayanon, et. al., 2013) although both of them have the same type of flesh. It caused by different comparison of white and red flesh every fish.

Another hand, whiteness affected by the type of cryoprotectant. Cryoprotectant from sugar reduction cause browning reaction. The combination of sucrose, sorbitol, and STPP can reduced the browning reaction (Susilo, 2010). SPM only use sucrose as cryoprotectant so whiteness value was not high.

Microstructure of protein

The 3-dimensional network structure of gel is an important determinant of texture and functional properties, such as water and fat holding capacity (Chen, et. al., 2007). The microstructure of SPM was shown in Figure 1.

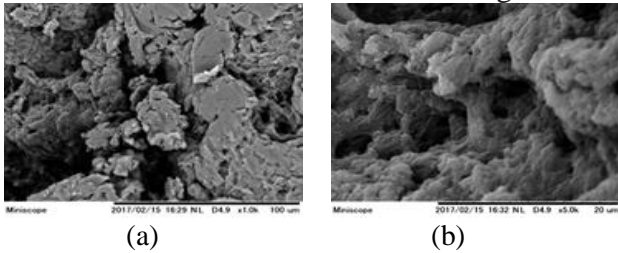


Figure 1. Scanning electron microscope image of SPM. (a) = magnification 1000X; (b) = magnification 5000x

Figure 1 (a) was shown that 3-dimensional network structure formed closely. Fig. 1 (b) was shown that 3-dimensional network structure of SPM more rigidly. Every protein attached mightly. This result was similar with the research of Gao, *et. al.* (2018). They reported that the microstructure of surimi was made 3-dimensional network structure rigidly. It is caused by ionic strength. Higher ionic strength will make aggregation gel structure rigidly. Hermansson, *et. al.* (1986) indicated that ionic strength also affects the microstructure of myofibrillar gels. They found that, at low ionic strength (0.25 M KCl), fine-stranded gel structures were formed, whereas, at high ionic strength (0.6 M KCl), coarsely aggregated gel structures were formed in the pH range 5.5 to 6. A higher rigidity was reached by the fine-stranded structure than that of the coarsely aggregated structure.

Molecular weight

The molecular weight of Pacific cod fish was different with the other fish. Protein pattern of SPM in different concentration was shown in Figure 2. According to the Figure 2, myofibrillar gel proteins of Pacific codfish have 2 main bands with molecular weight 150 kDa and 40 kDa respectively. Other bands were 30–40 kDa and 15–20 kDa.

According to Vigoreaux (2005), zetalin have molecular weight 107–210 kDa and tropomyosin has molecular weight 40 kDa. Molecular weight of protein myosin regulatory light chain, Glutathione-S transferase 2 and Troponin I were 24–30 kDa, 32–35 kDa, and 25–29–35 kDa respectively. Molecular weight ADP/ATP transcolase and flightin were 33

kDa and 20 kDa. Troponin C and myosin essential light chain have the same molecular weight 18 kDa.

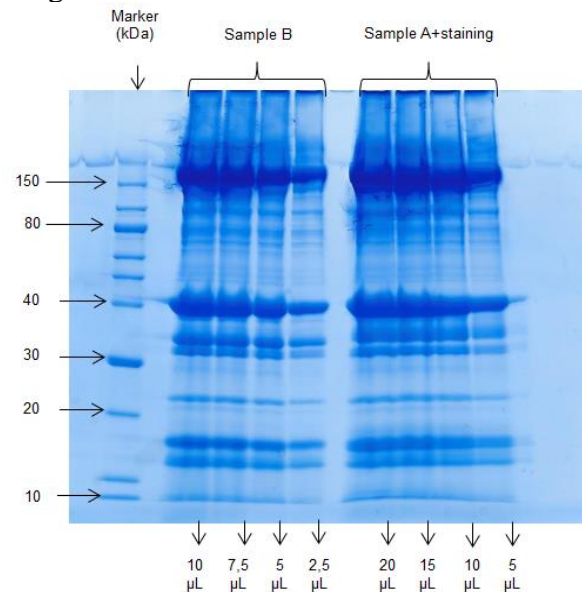


Figure 2. Protein pattern of SPM in different concentration. Sample B: sample after add 50 µL of 50 mM Tris-HCl buffer pH 8 (containing 5% SDS, 5% 2-mercaptoethanol, and 50% glycerol) without staining; Sample A+staining: sample after add 3.75 ml of 20 mM Tris-HCl buffer pH 8 (containing 8 M Urea, 2% 2-mercaptoethanol, 2% SDS), boiled and shake + staining

Texture

Rheological properties of myofibrillar gel protein were calculated as hardness, cohesiveness, and adhesiveness. The hardness, cohesiveness and adhesiveness of myofibrillar gel protein of Pacific codfish were 0.071338 N/cm², 0.259 and 116 respectively. The hardness affected by ionic strength. Higher ionic strength makes myofibrillar gel more rigidly.

Myosin in low ionic strength conditions (0.2 mol/L KCl) existed in the form of filaments, while in high ionic strength conditions (0.6 mol/L KCl), myosin usually existed in a monomeric or dimeric form (Boyer, et. al., 1996). In high ionic strength solutions, protein swelled, unfolded and became flexible upon absorbing solvent. The swelling and unfolding of actomyosin, in turn, increased its effective volume and shortened the distance between the protein molecules (Liu, et. al., 2008), which was beneficial for the crosslinking of protein during heat-induced gelation.

Pamujiati, et. al.

Samejima, et al. (1981) reported that the rigidity of rabbit myosin gel increased with the increase of ionic strength from 0.2 to 0.6. Laure, et. al. (2014) also found the increase of brine salt content had a positive impact on breaking stress of heat-induced pork gels.

CONCLUSION

According to the research, SPM have 3-dimensional network with rigid and porous structure. The major molecular weights were 150 kDa (zetalin) and 40 kDa (tropomyosin). The hardness, cohesiveness and adhesiveness of SPM were 0.071338 N/cm², 0.259 gf/sec and 116 gf.mm respectively. These results were shown that Pacific codfish was suitable to be used as surimi raw material because it can make a good gel to form surimi.

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IDENTIFICATION OF TOTAL PHENOLIC AND ANTIOXIDANT ACTIVITY OF FERMENTED RICE BRAN EXTRACTED BY ELECTROLYZED WATER

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ABSTRACT

Fermentation of rice bran is applied to facilitating the release of and may lead to higher yields of bioactive compound in its extraction product. The use of electrolyzed water as the solvent and the influence of this water on antioxidant activity of the fermented rice bran extract compounds is unclear. The bran of white (Jalawara) and red (Marahmay) rice from Banten Province are fermented using *R. oligosporus* and extracted using electrolyzed water at 2.5, 6.5, 7, 9.5, and 11.5 of pH value. This research presents that Marhmay contains more active antioxidant compounds with 422.11 ppm of IC50 by DPPH evaluation method and effectively extracted by electrolyzed water at pH 2 yielded of 3% extracted phenol more than water at pH 7. Furthermore, this treatment gives insights on the potency of electrolyzed water as solvent on extraction method of biological compound.

Keywords: Fermented Rice Bran, electrolyzed water, Antioxidant, Phenol

INTRODUCTION

The availability of rice bran in Indonesia is high since rice is the staple food of the citizen and paddy is the agricultural main crop. This is the favorable condition regarding to the fact that rice bran extracts may have the potential to be further exploitation as antioxidant rich products (Razak *et al.* 2017; Razak *et al.* 2014).

Rice bran is solid substrate by product of rice milling. Even though it consists of nutritive and functional component, the existences of the components are trapped on organic matrix of the bran. Fungal fermentation on enhanced the nutritional qualities and antioxidant activities on agricultural-by product solid substrates followed by both antioxidant activity and phenolic acid content of rice bran is also enhanced by solid-state fermentation using fungi (Razak *et al.*, 2014). Enzymatic reaction on fermentation process also could derive the derivative components that could have the highest biological functionality (Abubakr, 2012; Korhonen and Pirlanto, 2006).

The changes of physicochemical properties of magnetized water (Mosin and Ignatov, 2014; Hasaani. *et al.*, 2015) could be effective on extracting the bioactive compounds as well as done on partially remove hemicelluloses in the cell wall of *Miscanthus* using alkaline and acid electrolyzed water (Wang *et al.*, 2009).

This research elaborates the effect of electrolyzed water in different pH value on fermented rice bran (FRB). Bioactive substance of red rice (Marahmay) and white rice (Jalawara) especially phenolic compounds are evaluated both the quantity and the quality of its antioxidative activity using DPPH assay.

MATERIALS AND METHODS

Tools and Materials

Rice bran were taken from two local varieties of paddy those are cultivated in Banten. Jalawara paddy was collected from Lebak Distric and Marahmay was collected from Pandeglang Distric, Banten Province, Indonesia. *Rhizopus oligosporus* obtained from Collection of Laboratory of Microbiology, PAU, Bogor Agricultural

University. The fungi were maintained on PDA (Potatoes Dextrose Agar) until sporulating stage (7 days incubation) before it inoculated on the rice bran. Electrolyzed water obtained using LeveLuk JR II which could produce the water with pH 2.5, 6.5, 7, 9.5, and 11.5.

Fermentation and Extraction

Rice bran (RB) was weighed and added by water until 30% from its dry basis mass. The RB is autoclaved and then inoculated by *R. oligosporus*. The mixing of RB and inoculants is fermented in incubator at 37°C for three days to be the Fermented Rice Bran (FRB). The FRB then soaked by electrolyzed water using the ratio of 1:3 for dry basis FRB : electrolyzed water. The soaking is done in incubator at 60°C overnight. Next, the liquor is extracted using vacuum pump. The extract was then lyophilized and both of the dry extract and precipitate were stored at -180°C for further analyzes.

Proximate and total phenol

The Folin–Ciocalteu methodology was used to determine the total phenolic content in each sample. A 1 ml aliquot of the samples was allowed to react with 5 ml of Folin–Ciocalteu reagent and 4 ml of 7.5% sodium carbonate solution for 2 h at room temperature and in dark condition. Absorbance was measured at 765 nm using a spectrophotometer and the results were expressed as lg gallic acid equivalent (GAE)/gram sample.

Antioxidative test by DPPH

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (50, 100, 150, 200 and 250 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

Identification of bioactive compounds and its ...

$$\% \text{inhibition} = \frac{(As - Ac)}{Ac} \times 100\%$$

Where Ac is the absorbance of the control and As is the absorbance of the sample

RESULT AND DISCUSSION

Proximate analysis was done before the bran is inoculated and fermented by *R. oligosporus*. Table 1. present the result of proximate analysis of Marahmay and jalawara rice bran before it is fermented.

Table 1. Composition of Pre-Fermentation Rice Bran

Rice bran Type	Dry Basis Composition (%)				
	Ash	Fat	Protein	Carbohydrate	Total Phenol
Jalawara	4.65	8.14	13.95	74.42	0.35
Marahmay	13.19	12.09	9.89	63.74	0.26

The macromolecule components of these two varieties are different in all items and become the basis on the yield prediction of the extract. Rice bran could be extracted for many technical methods, but in general it is divided into two basic, polar and non-polar. Non-polar basic method is done to extract the oil component such as tocopherols, tocotrienols, and γ -oryzanol (Zhang, 2010). Polar basic method is done to extract components such as phenolic compounds, anthocyanins, and also flavonoid (Kapcum, 2016; Muntana and Prasong, 2010).

The use of electrolyzed water in this research was to evaluate its effectiveness on extracting not only polar compounds but also non-polar compounds because of its electromagnetic power changes which could interact with. Table 2. presents the composition of precipitate and extract after fermentation.

The total phenol content of precipitate or waste of extracted FRB (Fermented Rice Bran) is higher than non-fermented/pre-fermented rice bran and it indicates that fermentation is improve the quality of biological compounds composition (Razak *et al.*, 2014; Oliveira, 2010). Moreover, this result also informs that more pH values of electrolyzed water shift

toward neutral the extracted phenol becomes more seditious.

Table 2. Comparison of Phenol composition between Precipitate, Extract and its antioxidative activity

No	Variety	pH	Precipitate	Extract		
			Total phenol (%)	Solid content (g/ml)	Extracted Phenol (%)	IC50 (ppm)
1	Marahmay	2,5	0.16	0.0467	0.30	422.11
2	Marahmay	6.5	0.22	0.0608	0.24	500.03
3	Marahmay	7.0	0.33	0.1097	0.13	>800.00
4	Marahmay	9.5	0.46	0.0470	0	>800.00
5	Marahmay	11,5	0.16	0.0404	0.30	689.61
6	Jalawara	2,5	0.22	0.0980	0.29	>800.00
7	Jalawara	6.5	0.51	0.1095	0	653.71
8	Jalawara	7.0	0.51	0.1593	0	>800.00
9	Jalawara	9.5	0.26	0.1095	0.25	>800.00
10	Jalawara	11,5	0.25	0.1589	0.26	>800.00

Marahmay FRB that was extracted using electrolyzed water at pH value 2.5 and 11.5 give the least mass of solid content, 0.0467 g/ml and 0.0404 g/ml. Otherwise, the extract from electrolyzed water at pH value 2.5 and 11.5 give the highest yield of extracted phenol, 0.3% of each. The same pattern also presented in yield of extracted phenol of Jalawara at the pH 2.5 and 11.5. In these pH values, the yield of phenol is also the highest on number of 0.29% and 0.26% respectively.

Mosin and Ignatov (2014) hypnotized that water magnetization influences three condition of water: purifying water because of coagulation and precipitation of metallic ion, decreasing of ionic solubility because of water-ionic polarization, and deformation of water molecule because of dissolution of hydrogen bonding between molecule and polarization of OH⁻ and H⁺. Deformation of water causes the change in density, surface extension, viscosity, pH value, and other physicochemical properties (Mosin and Ignatov, 2014; Hasaani, et al, 2015).

This change of molecule formation and properties facilitate the water to interact with the polar component such as phenolic compounds so that in pH 2.5 and 11.5 of electrolyzed water yielded the highest amount of extracted phenol. This pH value also renders the breakage of hemicelluloses in rice bran which lead the effectiveness of the extraction (Wang et al, 2010). This easiness of extraction

also is facilitated by the enzymatic breakage of carbohydrate chain in cell wall because of enzymes excretion by the inoculants (Jones, et al. 1992).

IC50 was used to describe the strength of antioxidant activity of the FRB water extract. The strength antioxidant activity of the Marahmay extract obtained by extraction with water at pH 2.5 gives the best result at 422.11 ppm. However, almost all of water extract of Jalawara give the weak antioxidant activity. Only the extract from pH 6.5 which showed the concentration of IC50 under 800 ppm. This condition is expected that the biological compounds between Marahmay and Jalawara are different. Marahmay as red rice variety is estimated contain more rich on antioxidative compounds (Zhang, et al, 2010).

CONCLUSION

Marahmay and Jalawara rice bran have different amount of macromolecule and total phenol composition. Fermentation by *R. oligosporus* improves the extracted phenol in fermented rice bran as describe by the result on yield of precipitate fermented rice bran extract. Electrolyzed water in both alkaline and acidic pH could extract more phenol at least 3% more higher than water at pH 7. Further work should be focused on analysis of the component in the extract beside phenolic compounds.

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APPLICATION OF BUSINESS MODEL CANVAS IN PRODUCTION AND MARKETING OF SOLOG (ANALOGUE SAUSAGE)

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ABSTRACT

Business Model Canvas (BMC) is an alternative option to create new business models. BMC includes customer segments, value propositions, channels, customer relationships, revenue streams, key resources, key activities, key partners, and cost structure. They are able to support companies aligning their business activities. By applying BMC, companies have several alternatives of business models. A research was conducted in order to apply BMC for Small Medium Enterprises (SMEs) in Jember Regency. The descriptive method was employed to arrange BMC, while the qualitative analysis was applied for data analysis. Primary data collection was obtained by customers' interview. This paper describes the business plan of SOLOG (Analogue Sausage) product and application of BMC in the SMEs. The BMC creation for SOLOG was done in three stages. The first stage was a problem test, the second stage was a solution test and the last stage was verification model business. The last stage was chosen as the business model recommendation that is suitable as a reference in the business activities of SOLOG product.

Keywords: Business Model Canvas, Analogue Sauges , Small Medium Enterprises

INTRODUCTION

Comprehensive information about chicken sausage with analogue meat substitution (SOLOG) has not been available. To support product development, a suitable business model is required to be implemented by small and medium enterprises (SMEs). One method that can be used is creating a business model canvas (Osterwalder & Pigneur, 2012).

A business model plays an essential role, because it presents the competitive advantage of the business (Amit *et al.*, 2010). A business model also helps a business to determine an effectiveness and an efficiency of business activities (Amit *et al.*, 2010). Business model canvas is also a method for projecting financial analysis of a business (Osterwalder & Pigneur, 2012). The analysis of financial projection is a crucial stage of determining the feasibility

of a business (Ferreirra, 2012). By performing business model, the new product is expected to be successful accepted by the market.

RESEARCH METHODOLOGY

Preparation of business model for new product by using business model canvas was necessary, especially for SOLOG. The compilation of a business model canvas is created to facilitate small and medium enterprises (SMEs) to adopt the stages in establishing a SOLOG business. The first step was making an initial hypothesis for nine business components in the business model canvas. The next step was to direct confirm to the prospective customers. The confirmation was conducted by testing both the problem and the solution (Blank & Dorf, 2012). Furthermore, it was verified by selling

products to the market. Confirmation and verification were carried out as improvements to the initial hypothesis of business components. Data analysis was carried out regarding to the Moleong (2012) theory, as follow:

- a. Data collection from various sources through questionnaires.
- b. Data reduction by making abstractions in the form of summaries of the essence of research, processes, and statements following the research objectives.
- c. Data categorization by arranging data into categories based on views, opinions, or certain criteria.

The obtained data were then mapped to the business model canvas, so that the changes that occur can be recorded on the business model canvas (Dewabroto, 2011).

RESULT AND DISCUSSION

3.1 Business Model Hypothesis

A. Customer Segments

The customer segments element describes the target market of the product or the service offered. Customers are the core of all business models. The survival of a company depends on the customers. To satisfy customers, companies grouped customers into different segments based on similar needs, behavior, or other attributes.

The development of SOLOG business model is classified as an open market (existing market). It is because this product has not been well known by consumers. However, product advantages are required to compete in the market (Blank & Dorf, 2012). So, the selected customer segments for the initial canvas business model include all levels of society (unsegmented).

B. Value Propositions

The value proposition element is the benefit or value offered by the company to the market segments. Osterwalder and Pigneur (2012) asserted that "value propositions are the added value of a product or service that becomes the reason

for customers to use it". The value proposition for initial business model canvas may be similar to an existing offered market, with the addition of features and attributes. Several attributes contribute to customer value creation, namely novelty, performance, customization, design, brand, price, cost reduction, risk reduction, and convenience.

The business development of SOLOG can be based on adding value to the product. Adding value to the product can be a new attraction to consumers while answering consumer problems. The value propositions in the initial business model canvas of the SOLOG are as follows:

1) Health

SOLOG is a healthy product. It has good quality because it does not contain preservatives. Besides, this product healthier because it uses analogue meat instead of chicken meat, without reduce the nutrition value in the sausage itself

2) Brand Image

Attractive brand image is one of the initial value propositions. This can attract consumer because, attractive appearance increases the consumer's interest. By naming the product by "SOLOG" or analogue sausage, it makes potential consumers curious to try it.

3) Cleanliness (hygienic)

The "SOLOG" hygiene is an advantage of this product because the cleanliness of the home industry is somewhat questionable. However, the SOLOG production process is made by producing good food (CPMB) or good manufacturing practices (GMP) according to existing standards.

C. Channels

Channels are a means for companies to deliver value propositions to the customer segments served. The types of channels are divided into two, namely direct and indirect (Osterwalder & Pigneur, 2012). In the initial "SOLOG" business model, the channels required include direct selling by producers and indirect sales through retailers.

D. Customer Relationships

Customers relationship requires a special approach because the business venture SOLOG is still relatively new. Producers and sellers need suggestions and even criticism from consumers for product improvement. In this case, several ways to build a good relationship between producers and consumers is essential. One form of that relationship is personal assistance. According to Osterwalder and Pigneur (2012), The personal assistance relationships is form of relationships based on human interaction. Customer service personnel provided communication services to the customer and assist the sales process or services after complete sales. This communication can be done at the point of sale, through a call center, e-mail, or other channels. So that in the SOLOG business venture, it is necessary to build customer service, which accommodate criticism and suggestions form customers.

E. Revenue Streams

Revenue received by the company is from each market segment. Revenue streams are usually measured in terms of money the company receives from its customers. In the initial business model canvas, revenue streams of chicken sausage with analogue meat substitutes were obtained from direct selling and retail products to consumers.

F. Key Resources

Key resources (main resources) can be categorized into four, namely physical, intellectual, human, and financial (Osterwalder & Pigneur, 2012). In the SOLOG business venture, the implementation of key resources is in the form of raw material, production resources, technological resources, human resources and capital resources. Raw material resources include chicken meat, gluten, soy protein isolates, *anjasmoro* soy flour, *gembili* tuber flour, tapioca, and others food additives. Technology resources are the

science of process production and the equipment. Human resources needed include the production department, the research and development section and the marketing section. Capital resources are sources of funding that make it possible to carry out industrial activities. SOLOG business capital resources are obtained from personal capital and debt from banks.

G. Key Activities

The key activities is the main activity that must be mastered by the company to run a business. Like key resources, key activities are needed to create and offer value propositions, reach markets, maintain relationships with customer segments, and earn revenue. Key activities can be categorized by the production process, problem-solving, and network or platform (Osterwalder & Pigneur, 2012). The main activities of the business venture SOLOG are sausage production processes, product research and development, and promotional activities. Beside, the activity also include education activity about the benefits of analogue meat sausage chicken meat substitutes to the potential consumers to attract consumers.

H. Key Partners

Element key partners (partnerships) are the resources needed by the company to realize the value proposition, but not owned by the company. The intended partners in the business model are intermediaries' partners that have a role in turning the products or value into money.

Generally, there are four types of partnerships in a business to meet a certain conditions, namely strategic alliances between non-competitors, strategic partnerships between competitors, joint ventures to build new businesses and buyer-supplier relationships to guarantee reliable supplies (Osterwalder & Pigneur, 2012). In addition, company must also have three motivations in the building partnerships, namely optimization and economies of scale, reduction of risk and uncertainty, and

acquisition of particular resources and activities (Osterwalder & Pigneur, 2012). In the "SOLOG" business venture, business partners include distributors of raw materials and production support, that are:

- 1) Distributor of chicken meat
The chicken meat was obtained from suppliers located in Tanjung Market, Jember.
- 2) Distributor of gluten
Gluten was obtained from suppliers from various chemical firms in Jember
- 3) Distributor of soy protein isolates
Supplier of soy protein isolates obtained from PT Markaindo_Selaras located in Bogor.
- 4) Distributors of Anjasmoro Soybean Flour
Anjasmoro soybean flour suppliers are obtained from bean and tuber plant centres (Balitkabi) located in Banyuwangi Regency.
- 5) Distributor of *gembili* tuber flour
Gembili tuber flour obtained from the producer "Kusuka Ubiku" located in Bantul, Yogyakarta.
- 6) Distributor of packaging.

The Packaging was obtained from suppliers located in Jember Regency.

The other partner are:

- 7) Bank Rakyat Indonesia
Cooperation with Bank Rakyat Indonesia in the form of granting loans to SOLOG business.
- 8) Government

I. Cost Structure

The cost structure element is a part of the business model canvas element that explains the costs that arise when operating a "SOLOG" business model. In this study, cost analysis employs the full-costing method to calculate all cost components that exist in business operations. In this method, there are two main components, namely production costs and non-production costs. The production cost consists of variable costs and fixed costs. Non-production costs consist of investment costs and taxes.

In the initial stages of building a business model, a canvas business model was developed. The initial canvas business model can be seen in the **Figure 1**.

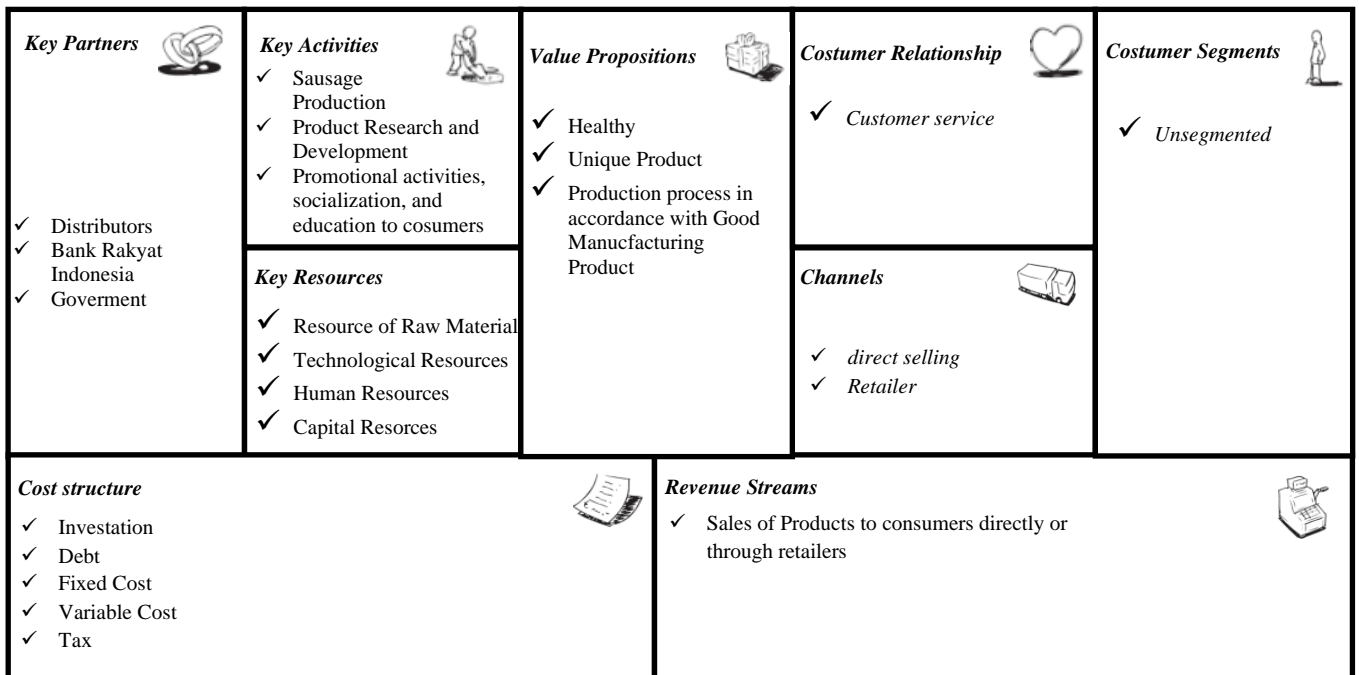


Figure 1. The initial stages of a business model

3.2 The Problem Test

The problem test was conducted by interviewing of 50 respondents. From the direct test through the interview process to the respondents, some information can be identified. This stage aimed to identify the issues that were being faced by the community related to healthy food/snack, and sausage products in the market. The results of the problem identification were solution that offered from the “SOLOG” product. There are three main elements of the business model to be tested in this test, namely:

- 1) Value Propositions (Natural)
- 2) Customer Segments (Unsegmented)
- 3) Channels (direct selling, retailer selling, online selling).

The results of the problem test by using questionnaire toward respondents can be seen in the **Figure 2**. Survey results showed that 100% of respondents had consumed sausages. Only 6% of respondents had ever consumed SOLOG. Furthermore, 76% of respondents were interested in trying to consume SOLOG and 64% were interested in buying the product. Nearly half of the respondents consume this product as a snack and all respondents reasoned they were interested in buying this

product because it was healthy and innovative.

Regarding market place to buy the products, 70% of respondents were willing to buy SOLOG at school and 84% of respondents were also willing to buy SOLOG in sausage agents and street vendors fried or grilled sausages. As many as 72% of respondents also wish to buy SOLOG at an online shop.

The result obtained from the test problems leads researchers to update the initial business model (hypothesis), especially, in the component value proposition that needs to be added with healthy and innovative attribute, in the channels components that needs to be replaced with schools, frozen sausage agents, fried or grilled sausage street vendors. In addition, the channel element need to accommodate the wishes of respondents about online purchasing. The producers can utilize social media such as Facebook, Instagram, and WhatsApp to promote and sell the product. However, the customer segment components were still unsegmented because business verification had not been done directly. Changing in the business model canvas can be seen in the **Figure 3**.

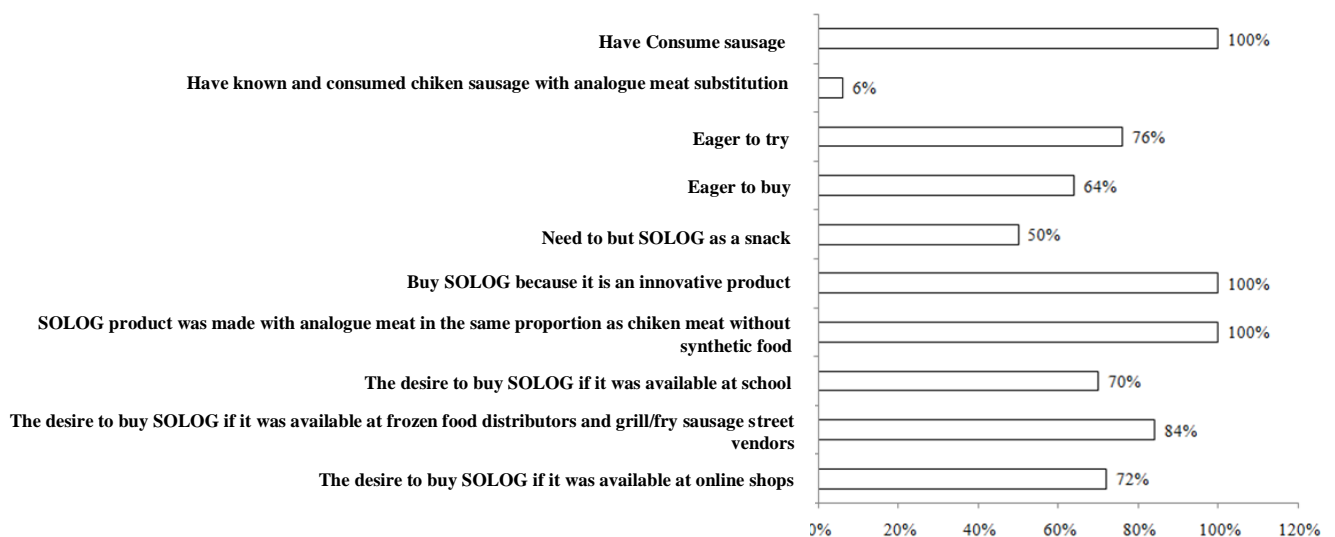


Figure 2. The results of the problem questionnaire test

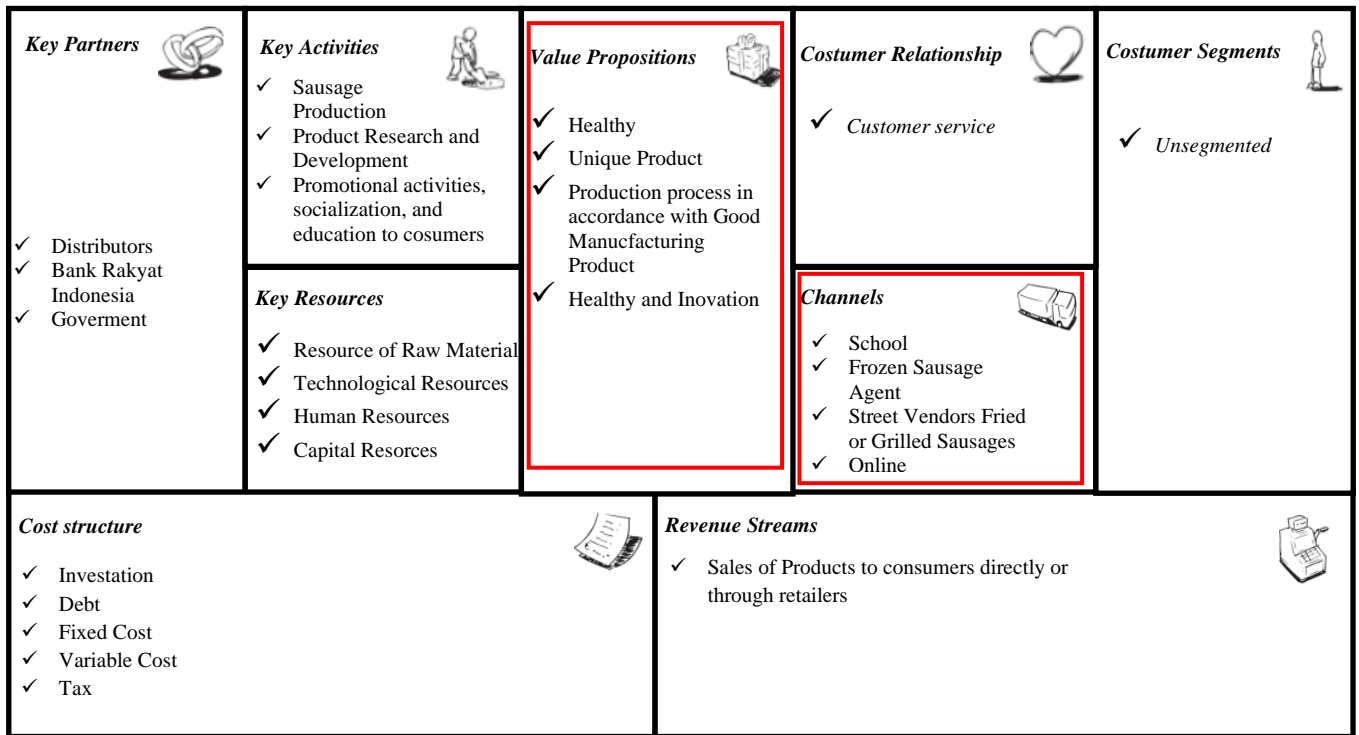


Figure 3 First stage of the business model canvas

3.3 Test the Solution

Solution testing was carried out by interviewing the same respondents in the problem testing. The test solution was intended to answer the problem and to identify the consumers' desire based on the results of the problem test. The results of

the solution test by using questionnaire can be seen in **Figure 4**.

The targeted market segment was indeed interested if there were an healthy and an innovative product. Furthermore, SOLOG was tested on respondents so that it could be re-verified on the interview's results.

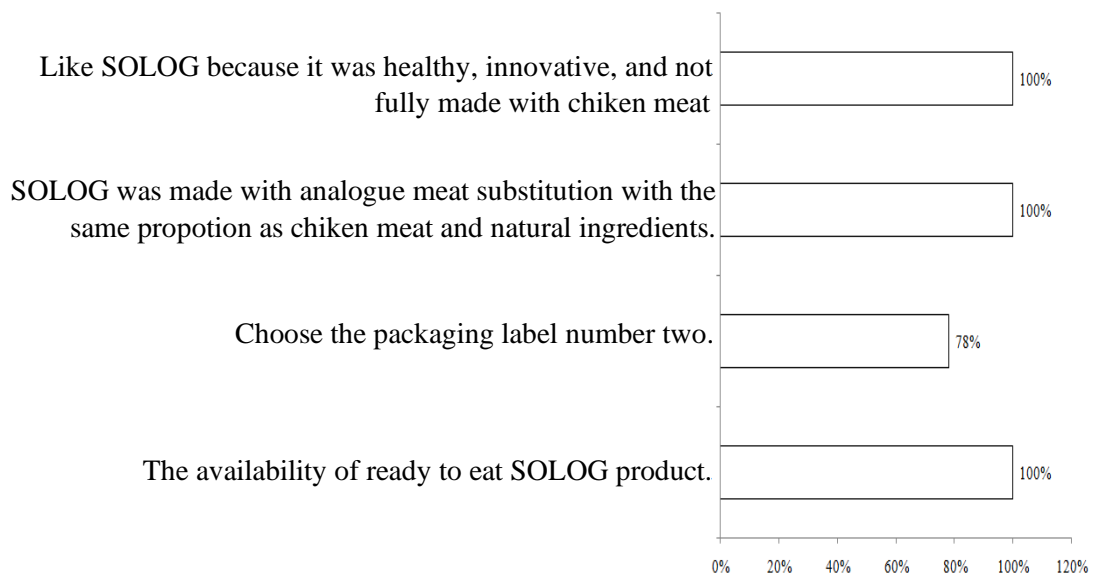


Figure 4. The results of the solution test questionnaire

The results of the interview are as follows:

- (a) All of respondents liked SOLOG because it was healthy and innovative. Beside it wasn't use 100% chicken meat as raw material.
- (b) All of respondents liked the value of SOLOG because it was made from analogue meat substitutes that came from natural ingredients and has free artificial food additives.
- (c) Respondents also like SOLOG because the analogue meat replace chicken meat in the same amount as raw material.
- (d) Some respondents want the existence of chicken sausage products with analogue meat substitutes that can be ready to eat without cooked.

As many as 78% of respondents preferred the second choice of the three alternatives provided by researchers. The majority of respondents liked the second design choice because it was attractive, it matched the product's color and it was bright.

Based on the results of the solution test, the business model canvas component of value proposition was improved, that are "SOLOG" must be ready to eat. Meanwhile other components of the problem test on the initial business model canvas were still

maintained. The complete changes of the second business model canvas can be seen in the **Figure 5**.

3.4 Verification of Business Model

Business model verification was done based on the results of the problem test and solution test. Verification was continued with preparing the final business model canvas or the third one. At this stage, the business model was verified by trying to sell SOLOG products to the market directly and through retailers. The direct selling included physical selling directly form the seller to consumers, online selling, and cash on delivery selling. The kinds of retailers/sellers included schools' canteens, distributors of frozen food, and street vendors of fried or grilled sausages. Selling product was conducted for approximately one month at the end of March to the end of April. For one-month, chicken sausages with analogue meat substitution were sold of 200-250gr packages. This activity was carried out in the neighborhoods of Summersari, Kaliwates, and Patrang. Based on sales data from one-month, it was known that as much as 15% of sales were sold directly, while as

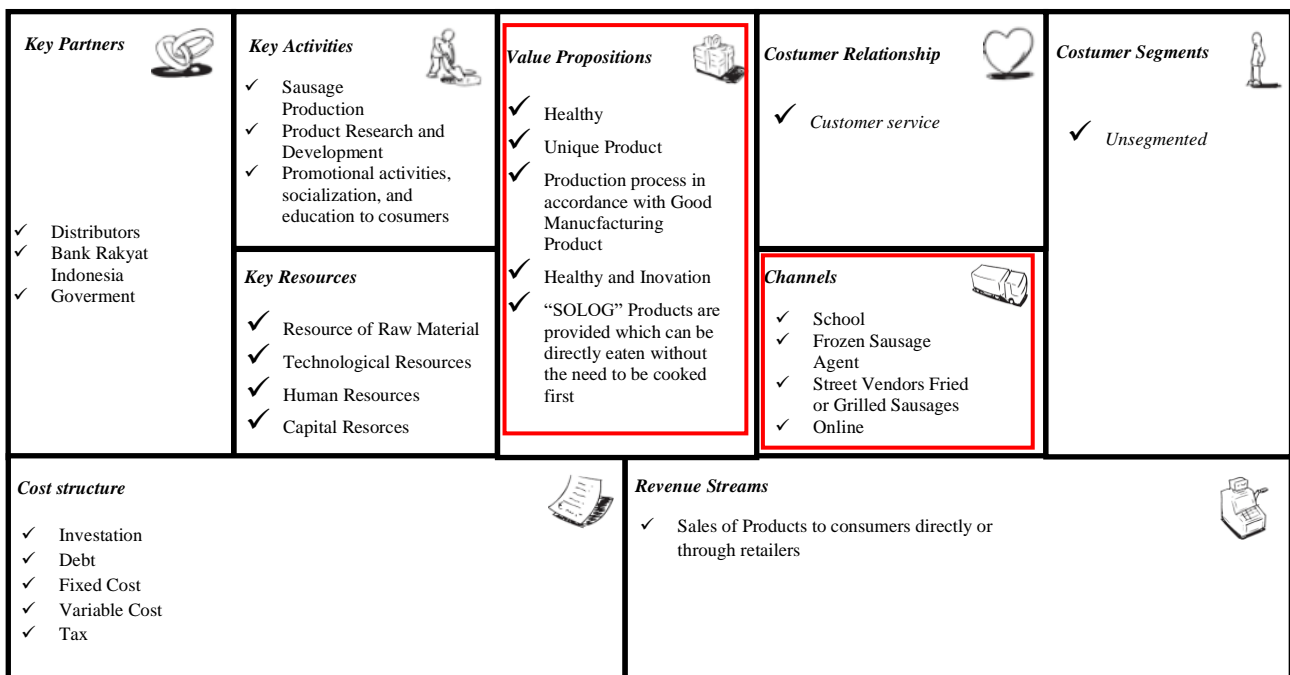


Figure 5. The second business model

much as 35% of sales were sold retail. Retail sales were further divided into two, namely distributors of frozen sausages and street vendors of grilled or fried sausages. Half the total sales of SOLOG product was from school's canteens.

Based on data from business verification results, the business model needs to be improved. In the revenue stream component, the business activity were obtained from direct sales, school canteens, street vendors fried sausages and grilled sausages and frozen sausage agents. The sales results in the school's canteens was the best sales result because snacks are an inseparable part of school lives (Suhardjo, 1989). Next, the customer segment components, which were originally unsegmented, were changed to the Jember area of the sub-districts of Summersari, Kaliwates and Patrang. Consumers were adults, men, women, and school-age children. At this stage, there was a change in the business model canvas with more detailed explanation was presented in **Figure 6**.

CONCLUSION

The compilation of a business model canvas for SOLOG was done by carrying out a series of stages, which were the problem test phase, the solution test and the business model verification. A series of test stages made changes the business model canvas for 3 (three) times. The change in the third business model was used as a reference of SOLOG business activities. In the last business model, it was found in the value proposition component that it was necessary to reduce the use of chicken meat. Consumers preferred a healthy and innovative ready-to-eat products. The school's canteens are the most suitable place to sell the product, along with other places such as frozen sausage agents, fried or grilled sausage street vendors, and online shops. Based on the data verification, the business model needs to be improved. In the revenue stream component, the improvements was made by adding direct sales, such as schools' canteens, street vendors fried sausages and

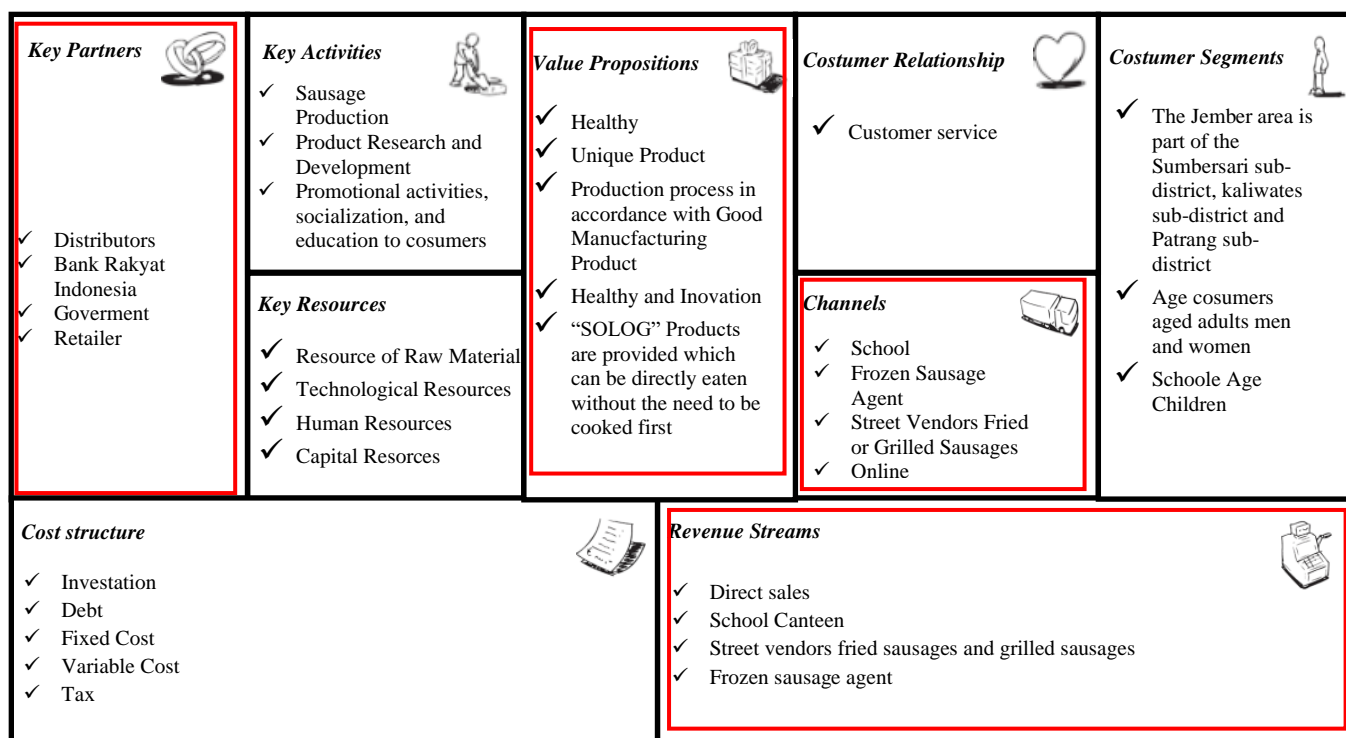


Figure 6. The final business model canvas

grilled sausages and frozen sausage agents. Furthermore, the customer segment component which was originally unsegmented was changed to the Jember area of the sub-districts of Sumbersari, Kaliwates, and Patrang. Age of consumers were adults of men, women, and school-aged children.

RECOMMENDATION

Further research can be done by developing business model using SWOT analysis when this business is no longer a start-up. So that, the product arise based on broader market demand and came at competition in the similar product businesses.

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Utilization of Milkfish (*Chanos chanos*) Bone Powder in Making of Rengginang, Local Food of Baduy Tribe

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ABSTRACT

Fishbone have a proportion of 10% of the total weight of fish and usually become fish processing waste that contains nutrients. One fish that has high nutritional value is milkfish. The utilization of milkfish bones can be an alternative way to provide a nutrient-rich food source because it contains a high number of mineral especially Ca and P. In Banten Province, milkfish bone is mainly waste from local food processing of Sate Bandeng (milkfish satay). This paper will discuss the results of research on the utilization of milkfish bone powder in making Rengginang, the local food of Baduy tribe. Baduy rengginang products are added milkfish bone powder with a concentration of 0%, 1%, 2%, and 3%. The products produced were characterized by parameters of linear expansion, crispness, hardness, organoleptic test, Total Plate Count (TPC), and proximate analysis. Rengginang product chosen was the treatment of adding 1% fish bone powder. Rengginang produced has the characteristics of volume expansion, crispness, and hardness in the range of 33.08-47.69%, 104.62-164.67 mm, and 958.20-2600.62 g. Water content, ash content, protein content and the amount of fat in the rengginang produced were 87%, 1.10-3.10%; 8.73-11.20%; and 0.48%. The water and protein content is very important because it is closely related to the physical characteristics of the rengginang produced.

Keywords: Rengginang, Milkfish Bone, Baduy tribe

INTRODUCTION

Baduy tribe is one of the dominant local ethnicity in Banten Province, Indonesia. This tribe has diverse food choice in terms of food stuff or food crops to provide food security in their areas. Baduy tribe acquired food produces its own food in agricultural land or buy in market or at a stall in their neighborhood (Khomsan 1993).

The Baduy main commodities are rice, fish and vegetables that are found in their surroundings. These commodities are processed to be a food named wajik, uli, rengginang, tapai ketan, gipang, getuk

dangdeur, getuk cau panggalek, wedang jahe, sayur hiris, and dodol (Eris *et al.* 2017). The exploration of Baduy local food is important as its cultural heritage for the ethnicity. This exploration also includes the nutritional value of the local processed food. Less nutrition value in food may possess negative impact to human, hence adding food additives is needed to enhance the nutrition value of Baduy local food.

Milkfish is one of the alternative commodity that can be utilized as food additives. Milkfish is a typical fish in Banten Province which is rich in nutritional value and beneficial for human health,

especially as a source of protein. Research related to diversification and processing of milkfish are: marks from milkfish (Candra et al. 2007), milkfish without thorns (Nusantari et al. 2016), making chips milkfish skin and floss by UKM (Sugito et al. 2019) This study aims to carry out fortification of the local food of Baduy, Rengginang, to increase nutritional value by adding milkfish as an effort of fishery products diversification.

MATERIAL AND METHODS

Materials and tools

The materials used in this study were the basic ingredients of Rengginang. Rengginang is a processed food of Baduy that are made by glutinous rice, garlic and salt. Other materials needed for proximate, microbiological, physical and sensory analysis were distilled water, 96% alcohol, sterile 0.85% NaCl solution, Plate Count Agar (PCA) media, Acidified Potato Dextrose Agar (APDA), Brilliant media. Green Lactose Bile Broth (BGLBB), Eosin Methylene Blue Agar (EMBA), pH 7 buffer, saturated NaCl, H₂SO₄, NaOH, Boric acid, indicator phenolphthalein.

Tools used in the production of rengginang were scales, basins, glassware, stoves and knives. The tools for analysis are

glassware, aluminum cup, desiccator, oven, furnace, stomacher, closed test tube, Durham tube, micropipette, incubator, bunsen, autoclave, hot plate, refrigerator, sealer, aluminum foil, spatula, pH meter, texture analyzer, chromameter, and reflux.

Stage of research

The experiment was conducted by direct field observation of the rengginang processing and by laboratory analysis. Sample of rengginang (control and fortified rengginang) were taken duplicates. The research began with conducting samples of rengginang. Milkfish bone was mixed with all ingredients of rengginang. The procedure for making rengginang is presented in Figure 1

The characterization of the rengginang product was carried out with experimental methods design with using a completely randomized design. The treatment given is concentration milkfish bone meal with four levels, namely 0%, 1%, 2%, and 3%. The treatment is carried out with three repetitions. Obtained data processed and tested ANOVA to see differences between treatments. If there was a significantly difference among treatments at $\alpha \leq 5\%$ then a further test was carried out using the real difference test smallest (LSD).

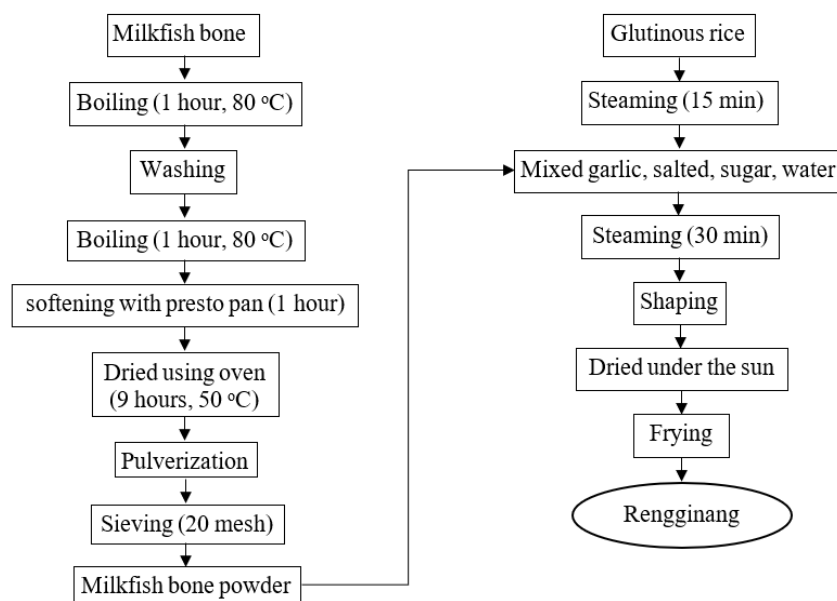


Figure 1. The procedure for making rengginang

The products were characterized by parameters of linear expansion, crispness, hardness, organoleptic test, Total Plate Count (TPC), proximate analysis, calcium and phosphorus content. Proximate analysis was then carried out to determine the content of protein, fat, ash, water, and carbohydrates. Protein analysis was done by using Kjeldahl semi micro method, fat analysis by using Soxhlet method (SII 2453-90), carbohydrate by using total carbohydrate by difference method and TPC (AOAC, 2005; Faridah *et al.*, 2008).

RESULTS AND DISCUSSION

Rengginang is one of the Baduy local foods. The results of proximate analysis of rengginang showed that rengginang had a water content of 0.47%, ash content of 1.33%, fat content of 31.10%, protein content of 7.19% and crude fiber of 2.49%. To complement the nutritional needs of rengginang, milkfish bone fortification was carried out to add calcium and increase protein. Salitus *et al.* (2017) described milkfish bone meal contains 35.22% protein; 9.68% calcium, 30.47% ash, and 23.06% fat and Bakhtiar *et al.* (2019) added that at 2.9 grams of milkfish bone meal

containing 5.24% calcium and 2.36% phosphorus.

a. Linear expansion

The linear expansion is an important physical characteristic of the development of cracker products, including rengginang with the addition of milkfish bone. Linear expansion is a percentage of the difference between the diameter of the cooked and raw rengginang with the volume of the raw rengginang. Linear expansion volume ranged from 33.08% to 47.69%. Based on the analysis of variance, the treatment of milkfish bone concentration and storage duration significantly ($P < 0.05$) affect on the linear expansion of rengginang, but there was no interaction between the two treatments. Rengginang with 0% concentration was significantly different from 2% and 3% concentration, but not significantly different from 1% concentration. In the storage time, the storage capacity stored for 0 weeks was significantly different from storage for 3 and 4 weeks, but not significantly different for 1 and 2 weeks. Rengginang linear expansion with variations in milkfish bone concentration is presented in the Figure 2.

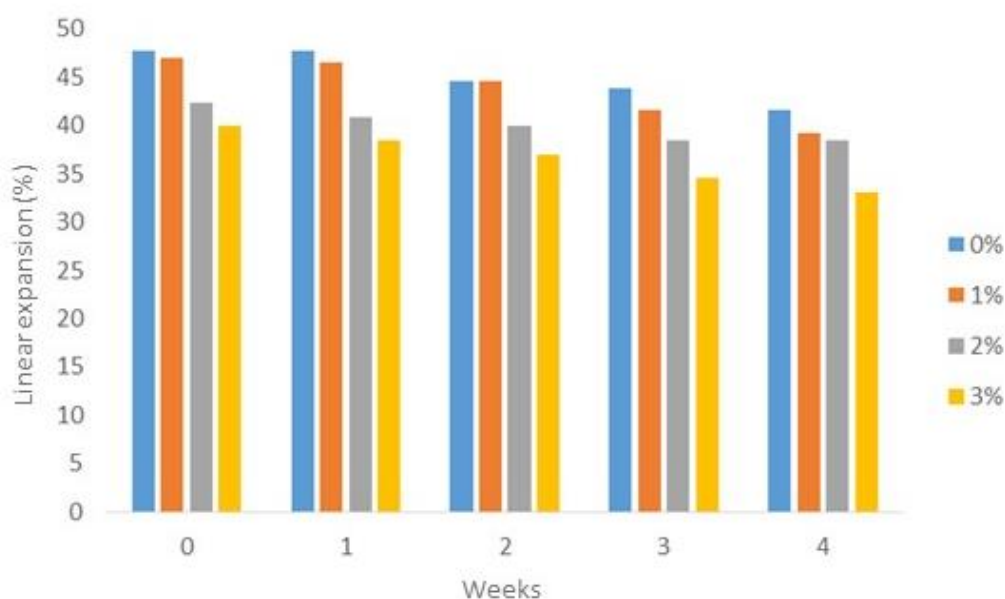


Figure 2. Linear expansion of rengginang with various concentration of milkfish bone

The results showed that the linear expansion decreased with increasing concentration of milkfish bone. According to Imaningsih (2012), when the mixture containing protein and tapioca is heated, the water will be used first to denature the protein before the gelatinization temperature is reached (75-76°C) with the result that the water is not enough to make the tapioca completely gelatinized. Increased concentration of milkfish bone caused the ability of starch granules to bind water decreases. When rengginang was fried, the air cavity decreases because it was filled with other materials and the linear expansion decreases. The best linear expansion was in the treatment with a concentration of 1% because it could expand better than other treatments.

The results also showed that the linear expansion of rengginang with the addition of milkfish bone decreased during storage for 4 weeks. This happened because of the high water content in rengginang with the addition of milkfish bone. This process rendered it to expand when it was fried during the frying process. At a concentration of 3%, the resulting rengginang did not swell well

but was more easily destroyed in texture.

The expansion mechanism of rengginang is the result of a number sparks of water that evaporate rapidly during the frying process so that air cavities are formed in the product. According to Fajriah (2014), the expansion of cassava rengginang is also influenced by the amylopectin content. Food with higher amylopectin content will have a tendency to expand more when it was fried. A good linear expansion is produced from the gelatinized starch so that the pores and surface area of the crackers become larger. Pores play an important role in the crispness and texture of the product. Crispy food will be difficult to chew if it does not have pores (Tsukakoshi *et al.*, 2008).

b. Crispness

Crispness is one of the important quality parameters in rengginang. Product crispness, also known as brittleness, describes how strong a material is in resisting the compressive force that causes it to break (Faridah *et al.* 2014). Rengginang crispness value with the addition of milkfish bone can be seen in Figure 3.

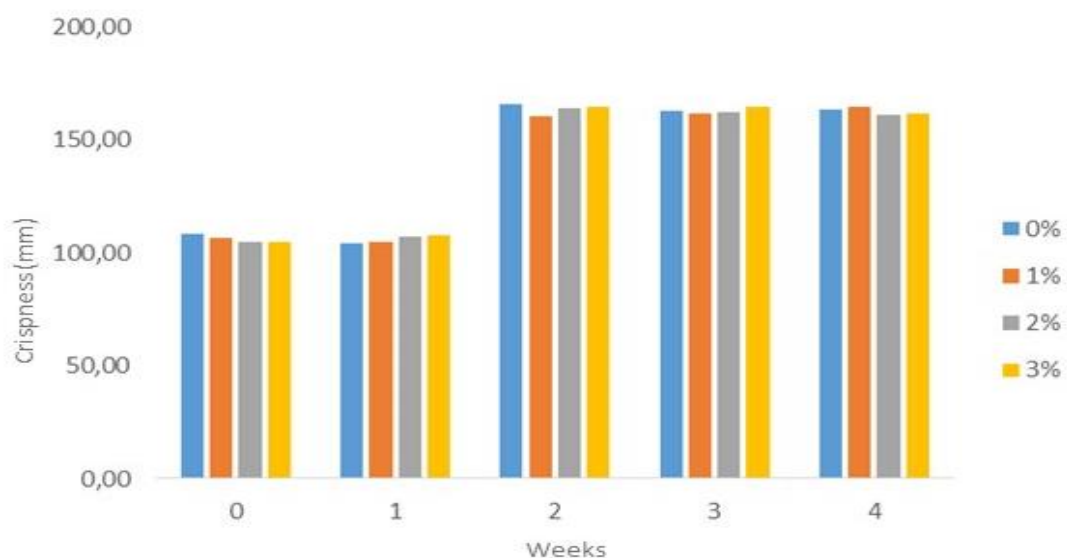


Figure 3. Crispness of rengginang with various concentration of milkfish bone

Based on the analysis of variance, treatment of fish bone and storage duration significantly ($P < 0.05$) affection crispness of product, but there was no interaction between treatments. Rengginang crispness in each treatment was not significantly different. Rengginang with 0week storage was not significantly different from 1 week, but significantly different from 2, 3, and, 4 weeks storage.

Rengginang crispness increased with the increasing of storage time. Significant improvement occurred from week 1 with a range of 104.50-108 mm to 164.06-165.81 mm in week 2. The duration of storage affected the water content in rengginang which was related to the level of crispness (Wijaya and Nocianitri 2008). The water content during storage is relatively stable, but water evaporation occurs during drying before frying. The diminished water content causes increased product pores to increase its crispness (Rosiani *et al.*, 2015).

Increasing the concentration of milkfish bone powder produced the same level of crispness of rengginang. The reason was the protein content that was relatively the same so it did not thicken the

amylopectin granules (Zulfahmi *et al.*, 2015). The process of heating the product before frying played a role in easing the tissue through the mechanism of starch gelatinization, decreasing cell adhesiveness, as well as the release of tissue-forming substances to the medium (Andersson *et al.*, 1994; Grizotto and Menezes 2002). In this study, the rengginang product was preheated in the sun for ± 3 hours so that it could increase crispness even though it had been stored for 4 weeks.

c. Hardness

Hardness is the durability of the material to break due to the compressive force applied. The value of hardness is shown by the deformation force, the force needed to break down food products. This measurement is intended to look objectively at the value of hardness in rengginang with the addition of milkfish bone. Hardness is closely related to crispness, the lower the hardness, the higher the crispness, because the force needed to crack the product is smaller. Rengginang hardness value with the addition of milkfish bone can be seen in the Figure 4.

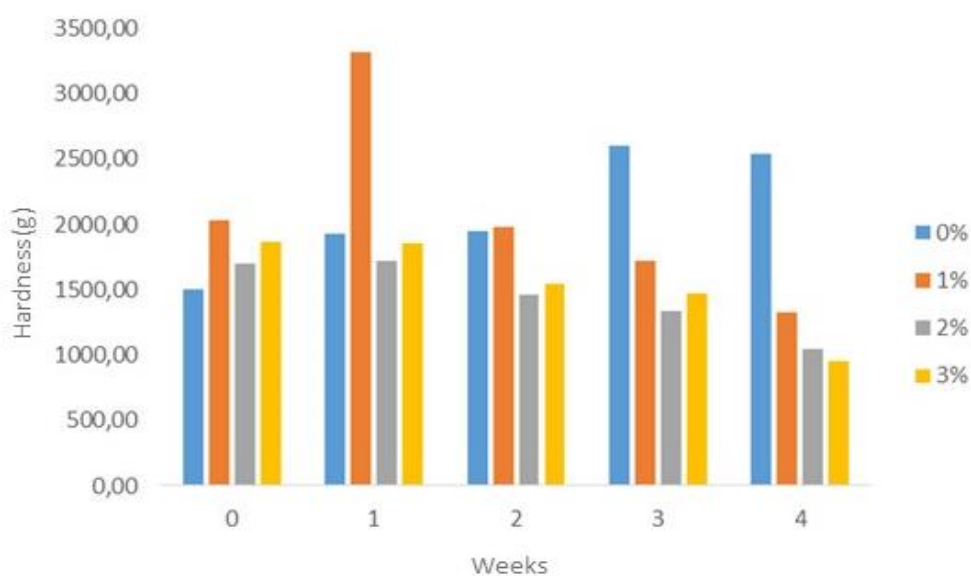


Figure 4. Hardness of rengginang with various concentration of milkfish bone

Based on the analysis of variance, the addition of milkfish bone had no significant effect ($P > 0.05$) on the hardness of rengginang. However, storage time had a significant effect ($P < 0.05$) on the hardness of the product. Rengginang at 0-4 weeks storage had a hardness that was not significantly different. At chilling temperature storage ($0-5^{\circ}\text{C}$), hardness of rengginang was influenced by the binding capacity of water and protein. The binding power of water was due to the protein repelling each other, as a result, the space between myofilaments becomes larger and water enters to the meat which causes the hardness to be smaller (Laiya *et al.*, 2014).

Rengginang produced in week 0 have a hardness with values in the range 1499.38 - 2034.91 gf. The product had a decreased hardness value along with the increase in the concentration of milkfish bone. This was due the amylose content was lower by increasing milkfish bone. Therefore, the product texture was not hard and tend to be crispy. Glutinous rice doesn't has amylose content (0-2%) so the texture was crispy and not hard. However, if the water content in rengginang increases, it could cause the product to have poor quality with low crispness and hard texture and cause the growth of microorganisms (Lertworasirikul and Tipsuwan 2008). Therefore, it is recommended that rengginang must have good and proper packaging so that the water content is maintained.

Figure 4 shows that the hardness value of rengginang tended to decrease with the increasing storage time. This happened because the water content of the product was maintained during storage so that physical and microbiological damage occurred slowly. According to Wijaya and Nocianitri (2008), rengginang has A_w of 0.4-0.6 so that bacterial growth is inhibited because it requires environmental conditions with a higher water activity. This was supported by the

statement of the lower the amount of free water in food, the lower the level of hardness and the higher crispness.

The hardness value of rengginang was inversely proportional to the value of crispness. This could be seen in the value of hardness that decreased every week, but the value of crispness was increasing. In week 2, the crispness value of rengginang increased by about 50% from the previous week with an average value of 106.09 mm. These results were in line with the decrease of hardness value of rengginang products from an average value of 2372.75 gf in week 1 to 1727, 92 gf in the following week.

d. Total Plate Count (TPC)

The result of analysis of variance showed that milkfish bone powder and duration of storage were not significantly effect ($P > 0.05$) to the total microbial number in rengginang. Total microbes in rengginang products ranged at 7.0×10^2 - 5.3×10^2 cfu / g for 4 weeks storage at chilling temperature ($0-5^{\circ}\text{C}$). Based on SNI 7388: 2009, the number of microbes for all calculations in rengginang is lower than the maximum limit of 1×10^5 cfu / g so that the resulting product still met the requirements. The total microbial count in rengginang with milkfish bone are presented in Figure 5.

The total microbial count of rengginang with a concentration of 3% increased until week 2, whereas at a concentration of 2% the increase of the total microbial occurred until the third week. Moreover, in the control, the total microbial count decreased until week 2, and increased until the end of storage. Microbial growth and activities are regulated by water content, protein, and temperature storage. Rengginang is a dry product that has slight water The water content in all rengginang produced was relatively the same so the effect of microbe was relatively small

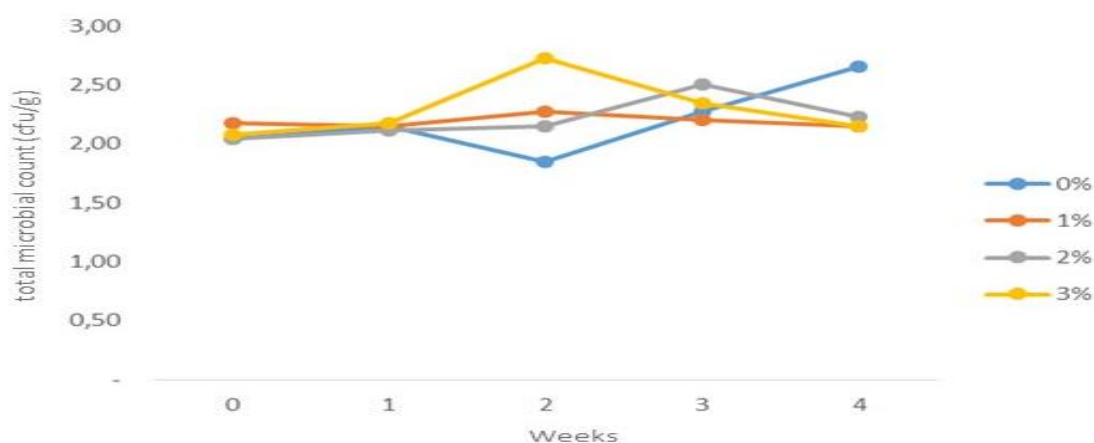


Figure 5. Total count of microbes in rengginang with various concentration of milkfish bone

Increased addition of milkfish bone causes the number of microbes in the rengginang to increase until week 2. This is because the nitrogen compounds in proteins can be used as microbial growth media. The presence of microorganisms in food degrades macromolecules (carbohydrates, proteins and lipids) into organic compounds. However, microbial growth also becomes stagnated due to storage at 0-5°C. Therefore, the number of microbes in the entire range was relatively small and in accordance with the standards.

e. Proximate Analysis

Moisture content

The result of analysis of variance showed that the addition of milkfish bone had no significant effect ($P > 0.05$) on the moisture content of the rengginang. However, the duration of storage had a significant effect ($P < 0.05$) on the moisture content of rengginang. Storage

for 0 week was not significantly different from storage for 1, 2, 3, 4 weeks. Rengginang moisture content with the addition of milkfish is presented in Table 1. Rengginang moisture content with the addition of milkfish bone tended to decrease during storage. This was due to the heating process for ± 3 hours before frying. The process caused the water contained in rengginang to evaporate so that the water content decreases during storage for 4 weeks.

According to Salamah *et al.* (2008), the moisture content of Opak crackers is influenced by humidity, thickness level and texture of the material. Water will easily evaporate on thin products so that the moisture content gets smaller and vice versa. Rengginang moisture content in this study was influenced by the drying factor. Rengginang drying process was done by using solar heat which was strongly influenced by weather conditions.

Table 1. Moisture content of rengginang with various milkfish bone concentration

Parameter	Week				
	0	1	2	3	4
0%	87.74	87.72	88.00	87.88	87.52
1%	87.02	87.19	87.01	87.06	87.00
2%	87.50	87.17	87.33	87.09	86.93
3%	87.17	87.41	87.16	87.25	87.21

Table 2. Ash content of rengginang with various milkfish bone concentration

Parameter	Week				
	0	1	2	3	4
0%	1.45	1.26	1.10	1.36	1.50
1%	1.75	1.81	1.89	1.33	1.91
2%	2.27	1.96	2.70	2.07	2.73
3%	3.02	2.69	3.30	2.06	3.10

Ash Content

The result of analysis of variance showed that the addition of milkfish bone and storage duration did not significantly effect ($P > 0.05$) on the ash content of Rengginang. Rengginang ash levels with the addition of milkfish are presented in Table 2. The results of the study showed that the ash content increased with increasing milkfish bone concentration, although it was not statistically significant. This value indicates the high levels of minerals that are important in terms of nutrition.

Ash content in rengginang products was relatively the same during 4 weeks storage. Minerals were stable to the high temperatures processing so that the ash content of rengginang did not changes significantly. Based on SNI 01-4307-1996, the content of rengginang ash with the addition of milkfish bone was still

above the maximum limit of 1%. Rengginang ash content produced ranged from 1.10 to 3.10%.

Protein content

The result of analysis of variance showed that the addition of milkfish bone powder had no significant effect ($P > 0.05$) on the protein content of Rengginang. However, the duration of storage had a significant effect ($P < 0.05$) on rengginang protein levels. Rengginang protein levels with the addition of milkfish bone can be seen in Figure 4. In t milkfish bone powder processing, protein content was eliminated as maximum as possible by protein hydrolysis. Protein removal aims to increase the mineral content in powder (Putranto *et al.*, 2015). Therefore, protein content in rengginang with the addition of milkfish bone powder was relatively the same.

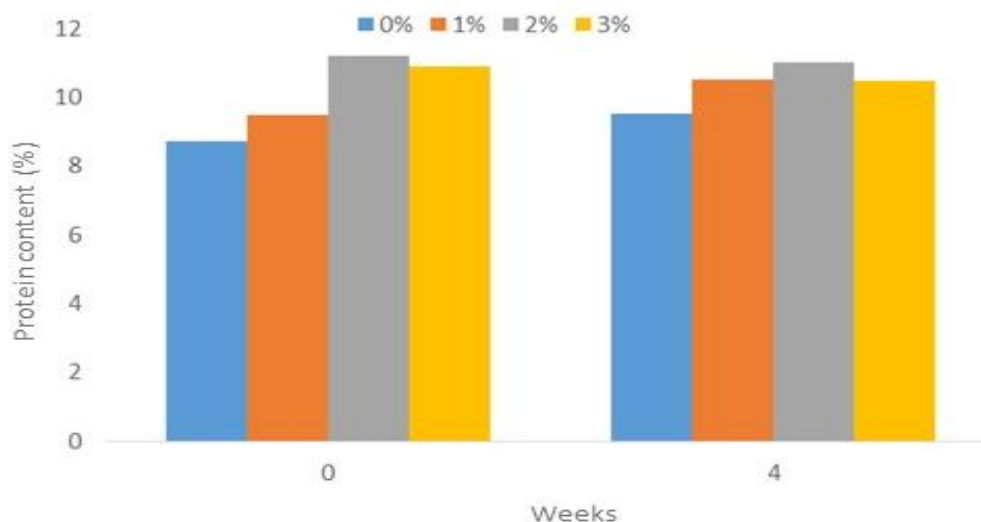


Figure 6. Protein content of rengginang with various milkfish bone concentration

Protein content in rengginang with the addition of milkfish bone powder tended to decrease with increasing of storage time. This could be caused by the process of protein denaturation in rengginang. Protein denaturation can occur due to heat, pH, chemicals, mechanics, and so on. Each of these methods has different effects on protein denaturation. Based on SNI 01-2713-1999, the protein content of rengginang met the standard of a minimum of 6%, the protein content of rengginang was in the range of 8.73% - 11.20%.

Fat content

The analysis of variance result showed that the addition of milkfish bone and storage time did not significantly affect ($P > 0.05$) on the fat content of rengginang. Rengginang fat levels with the addition of milkfish bone can be seen in Figure 6. According to Putranto *et al.* (2015), in milkfish bone powder, lower fat content is expected. Low fat content makes quality of rengginang more stable quality and uneasily to damage. High fat content causes powder to have fish taste and causes oxydative rancidity due to fat oxidation. Rengginang fat content with a concentration of 1% and 2% met the

maximum fat content requirements in the SNI 01-2713 (1999), 0.8%. Rengginang fat content with a concentration of 1% and 2% were 0.48% and 0.36%.

f. Selection of Best Product

Bayes Method is one of the techniques that can be used to conduct analysis in decision making of a number of alternatives with the aim of producing optimal results. Optimal decision making will be achieved when considering various criteria. The treatment is a criterion that needs to be considered in selecting the best rengginang. The selection of the best rengginang with the performance index analysis is based on the highest total value of each treatment.

The parameters assessed of rengginang were objective parameters (linear expansion, crispness, nutrient content, total plate count, and hardness) and subjective (taste, texture, color, and aroma). The results of the Bayes method calculation on rengginang products are presented in the Table 3. The results of subjective assessment, taste and aroma of rengginang were relatively the same so that it had a lower importance than the other parameters.

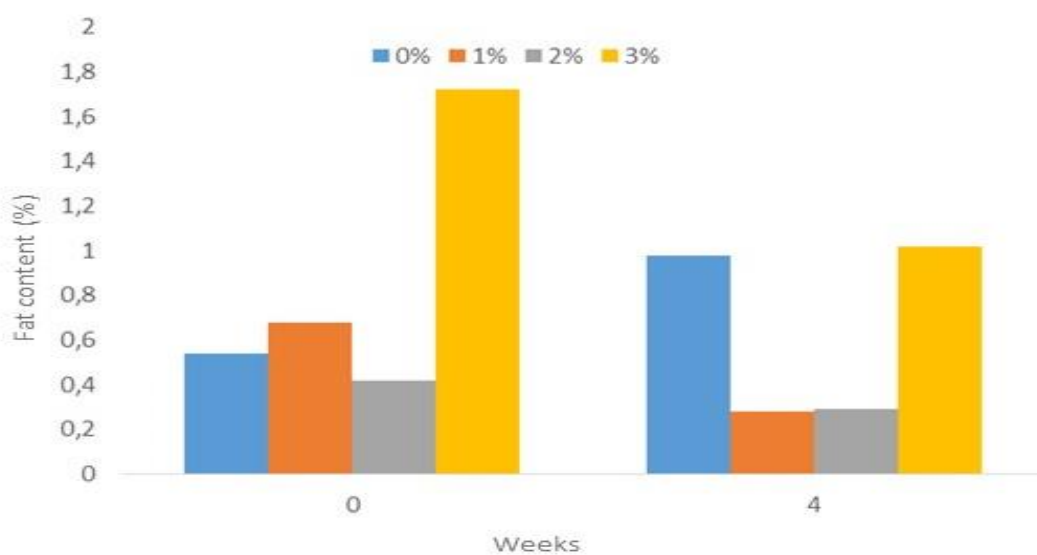


Figure 7. Fat content of rengginang in various milkfish bone concentration

Tabel 3. Bayes method result of rengginang

Parameter	Milkfish Bone Concentration			
	0%	1%	2%	3%
Linear expansion	4	3	2	1
Crispness	3	4	2	1
Protein content	1	2	3	4
Moisture content	1	4	3	2
Fat content	2	4	3	1
Ash content	4	3	2	1
Hardness	1	2	3	4
TPC	2	4	1	3
Texture	4	3	2	1
Aroma	4	3	2	1
Taste	4	3	2	1
Color	4	3	2	1
	2.83	3.17	2.25	1.75
Rank	2	1	3	4

Rengginang texture was different because the increasing milkfish bone content causing reduced crispness. Rengginang texture also decreased with increasing storage time. In addition, the aroma of rengginang produced was fishier with increasing fish bone concentration. The importance value of each parameter was based on a scale of 1 to 3, which is 1 representing ordinary, 2 representing important and 3 representing very important. The objective parameters that have the highest importance were linear expansion and crispness, followed by nutritional content were given a value of 2. Subjective parameters were given an assessment based on panelist acceptance. Based on the results of calculations with Bayes method, rengginang products with the addition of 1% milkfish bone powder was the best product. This was indicated by the highest average value compared to other concentrations.

CONCLUSION

The selected rengginang product was the treatment of adding 1% milkfish bone powder. Rengginang had characteristics of linear expansion,

crispness, and hardness with a range of 33.08-47.69%, 104.62-164.67 mm, and 958.20-2600.62 g. The moisture and protein content are very important because it is closely related to the physical characteristics of the rengginang.

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