

## CONTENTS

1. ANTHOCYANIN AS NATURAL COLORANT: A REVIEW
2. THE EFFECT OF IMPROVER ADDITION ON SWEET BREAD MADE FROM WHEAT FLOUR
3. SOURSOP FRUIT POTENCIAL AS A SUBSTRATE IN NATA DE ANNONA PRODUCTION
4. ANALOGUE SAUSAGE FORMULATION OF TEMPEH-WHITE OYSTER MUSHROOMS (*PLEUROTUS OSTREATUS*) WITH THE ADDITION OF CARRAGEENAN
5. PHENOLIC COMPOUND OF FRESH AND BOILED SEA GRAPES (*CAULERPA SP.*) FROM TUAL, MALUKU
6. BIODEGRABILITY OF NANOCOMPOSITE MADE FROM PVA, ZnO NANOPARTICLES AND STEARIC ACID
7. THE EVALUATION OF GOOD MANUFACTURING PRACTICES (GMP) IN FISH PROCESSING SME CENTER CASE STUDY OF BALIKPAPAN CITY
8. HAZARD ANALYSIS AND CRITICAL CONTROL POINTS IN AMPLANG PROCESSING

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## PREFACE

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By the Grace and Blessings of Allah the Almighty, we would like to present, with great pleasure, the volume 01 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 2019

Editorial Team

## TABLE OF CONTENTS

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ANTHOCYANIN AS NATURAL COLORANT: A REVIEW	1-7
THE EFFECT OF IMPROVER ADDITION ON SWEET BREAD MADE FROM WHEAT FLOUR	8-19
SOURSOP FRUIT POTENCIAL AS A SUBSTRATE IN NATA DE ANNONA PRODUCTION	20-23
ANALOGUE SAUSAGE FORMULATION OF TEMPEH-WHITE OYSTER MUSHROOMS ( <i>PLEUROTUS OSTREATUS</i> ) WITH THE ADDITION OF CARRAGEENAN	24-30
PHENOLIC COMPOUND OF FRESH AND BOILED SEA GRAPES ( <i>CAULERPA SP.</i> ) FROM TUAL, MALUKU	31-39
BIODEGRABILITY OF NANOCOMPOSITE MADE FROM PVA, ZnO NANOPARTICLES AND STEARIC ACID	40-44
THE EVALUATION OF GOOD MANUFACTURING PRACTICES (GMP) IN FISH PROCESSING SME CENTER CASE STUDY OF BALIKPAPAN CITY	45-53
HAZARD ANALYSIS AND CRITICAL CONTROL POINTS IN AMPLANG PROCESSING	54-64

## ANTHOCYANIN AS NATURAL COLORANT: A REVIEW

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### ABSTRACT

On this era, people very concern to their food. The first sensory quality which is seen to choose the food are the color. However, during food processing is often occurred the color degradation, so the colorant is added to the food. Today, natural colorant is consumer's selection because it has functional function. One of natural colorant is anthocyanin. Anthocyanin gives red, blue, and purple color. Anthocyanin has different types, it is depended to sugar and hydroxyl which bounded into structure. The types are pelargonidin, malvidin, cyanidin, delphinidin, petunidin, and peonidin. This pigment is stable on acid condition, away from light and oxygen, cold temperature, and away from polyphenol oxidase enzyme. Beside as colorant, anthocyanin also act as antioxidant because the structure is very reactive. The consequences of having antioxidant activity, anthocyanin can prevent cardiovascular disease, cholesterol, atherosclerosis, or colon cancer by blocking fat oxidation and DNA mutation. Anthocyanin source is very broad like from flowers, fruits, tubers, or fruit peels.

**Keywords:** Antioxidant, anthocyanin, colorant

### INTRODUCTION

On this era, people are very concerning with their food that they eat. They are not only interested in its taste, but also have noticed to sensory quality of the food that they eat, like color. Color is often used as first assessment of food quality determining. The example, if the color of apple peeled is brown, people will not accept it because of enzymatic browning, or the color of whole bread is greyish green, also people will not accept it because the mold is grow. On the other hand, also color can make food more attractive and increase food acceptability. One example, people will be more interested to bright color in food like rainbow cake than brown cake (Nurhadi & Nurhasanah, 2008).

Food processing often make the decreasing of sensory quality, including the color. Therefore, manufacturers often add colorant to their products. Food synthetic colorant are often used because of their high stability, but nowadays people are more attention to the functional properties of their

food that they eat, including natural food colorant. One of the natural food colorant is anthocyanin.

Anthocyanin is a pigment found in flowers, fruits, vegetables, and tubers. This pigment gives red, blue, and purple colors and there is a lot of availability in nature. This pigment can replace amaranth which are banned in the United States and Europe. Another advantage of anthocyanin is that it has a high antioxidant content that is good for health (Cahyadi, 2006; Sampebarra, 2018).

### STRUCTURE AND CHARACTERISTIC OF ANTHOCYANIN

Anthocyanin word comes from the Greek word *anthos* which means flower and *kyanos* which means dark blue. It is categorized as flavonoid pigment which is soluble in water and one of the main pigment groups in higher-order plants (Fennema, 1996). Anthocyanin pigment is found in plant cell vacuoles and it is flavonoid which is naturally in the form of glycosides from



flavylium or 2-phenyl benzopyrylium. This pigment belongs to the benzopyran derivative. The main structure of benzopyran is characterized by the presence of two benzene aromatic rings which are linked to three carbon atoms forming a ring. Anthocyanin is a glycoside group formed by aglycone and glycon (Hendry & Houghton, 1996). The basic structure of anthocyanin (Figure 1) consists of 2-phenyl-benzopyrylium or flavylium with a number of hydroxy and methoxy.

Most anthocyanin come from 3,5,7-trihydroxyflavylium chloride and the sugar part is usually bound to the hydroxyl group on number 3 carbon (Hendry & Houghton, 1996). If the sugar part of the anthocyanin is removed through the hydrolysis process, it remain aglycone and sugar-free pigment called anthocyanidin (Hendry & Houghton, 1996). The following structure of the anthocyanidin molecule can be seen in Figure 2.

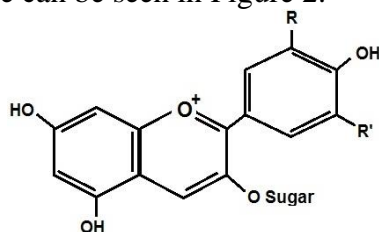


Figure 1. Anthocyanin structure (Winarno, 1998)

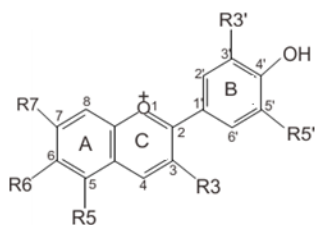


Figure 2. Anthocyanidin structure (Hendry and Houghton, 1996)

- A,B : Substituted Benzene (C6)
- C : Three carbon atom aliphatic chain
- R3' : H,OH/OCH<sub>3</sub>
- R5' : H,OH/OCH<sub>3</sub>
- R3 : O-Glycosyl
- R5 : O-H/Glycosyl
- R6 : H
- R7 : OH

In Figure 2, it show that the position of R3 is always filled with sugar. The sugar which can be bound is glucose, rhamnose, arabinose, xylose, and sometimes di- or trisaccharide. The position of R5 consists of glucose or rhamnose,

while the position of R7, R3', R5', and sometimes R4 'can be substituted by glucose. Each anthocyanidin is distinguished by each R substitute group whereas most anthocyanidin is a derivative of cation 3,5,7-trihydroxyflavylium (Hendry & Houghton, 1996).

The type of anthocyanidin are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. The differences of all the type are on R3' and R5'. The R3' and R5' of pelargonidin consists of atom H and the color expression are orange and reddish orange to dark red. R3' and R5' in cyanidin are OH and H, the expression are reddish orange, dark orange, and purplish red to bluish red. OH is on R3' and R5' of delphinidin and red, purplish red, and purple to blue are the color expression. The R3' and R5' of peonidin consists of OCH<sub>3</sub> and H, the color expression are purple, red, to reddish orange. R3' and R5' in petunidin are OH and OCH<sub>3</sub>, the expression are blue and purple to red. OCH<sub>3</sub> is on R3' and R5' of malvidin and blue and light purple to red are the color expression (Hendry and Houghton, 1996).

The concentration of the pigment is very important on determining of the colour expressed by anthocyanin. In lesser concentration, the colour is blue. Meanwhile in higher concentration, the colour is red. In medium concentration, the colour is purple. The increase of the hydroxyl group tends to strengthen the color to be more bluish (Fennema, 1996).

The double bond conjugated to the chromophore group in the anthocyanin structure makes it to be able to absorb the light with maximum absorbance in the visible light region. The more and the longer conjugated the double bond to anthocyanin, the stronger the colour. It will cause the light absorption to the longer wavelength. It is because the energy needed to the transition to the conjugated double bond is getting smaller, so that the light absorption will shift more to the larger wavelength (Mahmudatussaadah et al., 2014).

Anthocyanin can absorb light radiation in the ultraviolet area (UV) absorption until the visible light, but it absorbs stronger in the visible light area. It can absorb light at the wavelength of 250 - 700 nm, with two peaks as sugar groups (glycon) at the wavelength of 278

nm, and the main peaks as anthocyanin (aglycones) at the wavelength 490-535 nm (Mahmudatasaadah et al., 2014). The following is the maximum wavelength of each aglycone which can be seen in Table 1.

Table 1. Maximum Absorption Visible Light Wavelength of Aglycons

Aglycon	$\lambda_{max}$ (nm)	Color
Pelargonidin	494 nm	Orange
Cyanidin	506 nm	Reddish Orange
Peonidin	506 nm	Reddish Orange
Delphinidin	508 nm	Red
Petunidin	508 nm	Red
Malvidin	510 nm	Bluish Red

Sumber: Farahmandazad (2015)

### ANTHOCYANIN STABILITY

Anthocyanin is a reactive compound. This reactivity is because the flavylium cation nucleus experiences a lack of electrons, so that its colour easily degraded. It occurs due to changes in the red flavylium cation to an alkali colourless carbinol and eventually it becomes a colorless chalcone (Markakis, 1982). The changes in anthocyanin structure can be seen in Figure 3.

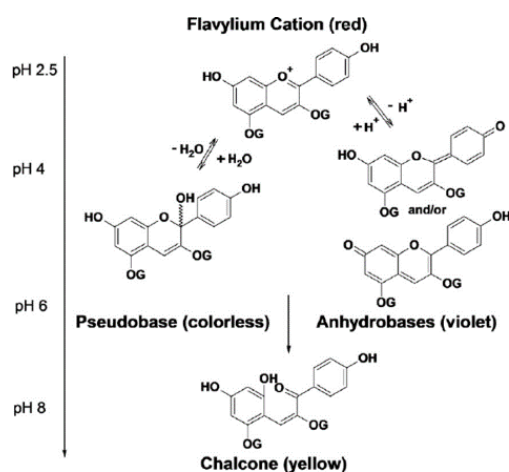


Figure 3. The changes of anthocyanin structure (Barnes, et al., 2009)

Anthocyanin is hydrophilic which is soluble in water easily (Husna et al., 2013). However, it can also be dissolved in polar organic solvents such as ethanol, methanol, acetone, and chloroform (Kristiana et al., 2012). Anthocyanin is very stable in acidic conditions, so that when extraction can be added organic acids such as acetic acid, citric acid, or hydrochloric acid (Sipahli et al., 2017).

A mixture of polar solvents and organic acids that will produce very acidic conditions can stabilize the anthocyanin which gives a red color. However, if the solvent is mixed with a weak acid, the anthocyanin will change into faded red at pH 3; purplish red at pH 4; purple at pH 5-6; and purple blue at pH 7. The form of anthocyanin at pH 1 is a flavylium cation, at pH 2-4 in the form of a mixture of flavylium and quinoidal cations, and at pH 5-6 form two colorless compounds namely pseudo-alkali carbinol and chalcone (Pedro et al., 2016; Sitepu et al., 2016).

Beside the acid, many other factors can influence the stability of anthocyanin, which is the presence of the polyphenol oxidation enzyme, temperature, light, and oxygen (Fennema, 1996; Hendry and Houghton, 1996). The enzyme reacts in the form of o-diphenol and oxygen which will oxidize anthocyanin. Polyphenol will initially oxidize o-diphenol to benzoquinone which will later react with anthocyanin in non-enzymatic reactions to form oxidized anthocyanin and other degradation products. High temperature can change the structure of anthocyanin in equilibrium reactions from flavylium cations to chalcones. The forming of chalcone because of high temperature occur in two stages, there are hydrolysis of glycosidic bonds and producing labile aglycone, and the ring of aglycone is opened and forms a colorless carbinol and chalcone group (Markakis, 1982; Hendry & Houghton, 1996; Fennema, 1996).

Chalcone compounds are able to degrade to form simpler colorless compounds, carboxylic acids such as substituted benzoic acid and carboxyl aldehyde compounds namely 2,4,6- trihydroxy benzaldehyde (Hendry and Houghton, 1996). Oxygen can affect the stability of anthocyanin directly and indirectly. The direct effect of oxygen is that it can oxidize anthocyanin into a colorless compound, whereas for indirectly effect, some hydroxyradical compounds can oxidize anthocyanins to form colorless compounds such as chalcone (Rein, 2005).

Light can degrade anthocyanin because it has energy that stimulates photochemical reactions (photooxidation). This reaction can cause the opening of the aglycone ring which begins with the opening of the carbon ring number two so as to form colorless compounds such as chalcone (Hendry and Houghton,

1996). The anthocyanin stability can be maintained by a copigmentation reaction. It is the interaction between the structure of anthocyanin with other molecules such as metals ( $Al^{3+}$ ,  $Fe^{3+}$ ,  $Sn^{2+}$ ,  $Cu^{2+}$ ) and organic molecules such as flavonoids (flavones, flavonones, and flavonols), alkaloid compounds (caffeine), and so on. The presence of copigmentation with metals and other organic molecules tends to increase the color stability of anthocyanins (Hendry and Houghton, 1996).

### THE ROLE OF ANTHOCYANIN AS ANTIOXIDANT

The existence of the conjugated double bond in the anthocyanin structure can make it very reactive and can function as an antidote to radical compounds or antioxidants (Barrowclough et al., 2015). The more phenolic hydroxyl groups that are bound to anthocyanin, the stronger the antioxidant activity (Han et al., 2017). Anthocyanin can react with various types of free radicals derived from reactive oxygen, such as peroxy ( $ROO\cdot$ ), hydroxyl ( $\cdot OH$ ), and singlet oxygen ( $O_2^{\cdot}$ ). The free radicals are compounds that can be formed in the structure itself by prooxidative enzymes as well as those from the environment such as cigarette smoke, pollution, fat oxidation, exhaust fumes, and exposure to other chemicals (Muttalib et al., 2014).

Because anthocyanin has antioxidant activity, it can prevent various degenerative diseases such as cardiovascular disease, cholesterol, colon cancer, and atherosclerosis. The mechanism of anthocyanin in reducing cholesterol levels in the blood is to oxidize LDL (low density lipid). Forbes-Hernandez et al., (2017) who tested strawberry methanol extract on HepG2 cells showed a decrease in cholesterol levels by 13.6% compared to before being given strawberry methanol extract. The mechanism that occurs is the termination of the chain of propagation of free radicals and all hydroxyl groups on ring B can contribute electrons so that free radical inhibition occurs. (Forbes-Hernandez et al., 2017).

The mechanism of antioxidants in preventing colon cancer is by blocking the cancer initiation stage by inhibiting DNA damage caused by carcinogens (Manson,

2003), in addition antioxidants can prevent the mutation of DNA by stabilizing free radicals and inhibit the occurrence of chain reactions from the formation of free radicals that can cause stress oxidative. Antioxidants can also act as free radical scavenger, decomposer peroxide, and reduce singlet oxygen (Glasauer et al., 2014).

### SOURCES AND EXTRACTION OF ANTHOCYANIN

Anthocyanin sources are very abundant in nature such as flowers, leaves, fruits, tubers, and fruit peels. The following anthocyanin sources and levels can be seen in Table 2.

Table 2. Anthocyanin Contents in Various Sources

Sources	Content
Rose	0,0925%/g
Hibiscus	0,0739%/g
Rosella	0,0795%/g
Four o'clock flower	0,0977%/g
Red spinach	6350 ppm
Purple sweet potato	0,24-0,44 mg/g
Red cabbage	11,11-17,80 mg/g
Strawberry	20,8 mg/g
Wine	0,267 – 1,9 mg/g
Mulberry	19,93 mg/g
Red dragon fruit	0,088 mg/g
Jamblang	1,61 mg/g
Red dragon fruit skin	22,593 ppm
Rambutan skin	4,1.10-3 mg/ml
Mangosteen skin	593 ppm
Jamblang skin	0,19 mg/g
Eggplant skin	750 mg/g
Jenitri's skin	0,2387 mg/g

Source: Djaeni *et al.* (2017); Sangadji *et al.* (2017); Ahmadiani *et al.* (2014); Pebrianti *et al.* (2015); Winata *et al.* (2015); Anggraini *et al.* (2018); Winarti *et al.* (2018); Zulfajri *et al.* (2018); Lestario *et al.* (2011); Handayani *et al.* (2012); Farida *et al.* (2015).

The table shows that anthocyanin sources are found in nature abundantly. Vegetable sources such as radish, purple sweet potato or red cabbage have been shown to provide higher percentage of acylated anthocyanins than fruits. Beside that, radish



**Winda Nurtiana**

and red potato have the potential to be used as an alternative for Federal Food Drug and Cosmetic Red No. 40 (Allura red). Acylated pelargonidin extracted from red radish given a red color close to Allura red at pH 3.5 (Shipp and Abdel-Aal, 2010).

Black carrot is a good anthocyanin source not only for its color, but also it has high ratio of monoacylated structures increasing color retention at low pH like small amounts of non-anthocyanic phenolics which is oxidize easily. Red wine is very good anthocyanin source because it can be reduced to form stable complexes during fermentation and generated intermediate products further react to yield color stable anthocyanin derivatives.

Red rice is commonly used as a food colorant in China and was approved by the Chinese Ministry of Health to improve the color of meat, fish, and soybean products. Red cabbage and radish extracts not only improve the color of food but also reduce unnecessary aroma and flavour compounds. Another applications of anthocyanin is to acid fruit preparations, jams and preserves. The viability of acylated anthocyanins from red radish, red cabbage, black carrot and grape skin extract to color dairy products such as yogurt and sour cream having pH levels around 4.2-4.5 (Shipp and Abdel-Aal, 2010; Giusti and Wrolstad, 2003).

Anthocyanin can be obtained by extraction process with acidic condition. Because cation flavylium form is very stable and give strong color on acid environment. The best organic solvent to extract is aquadest and tartrate acid as acidulant. This acidation is done to decrease pH so flavylium cation still stable and increase the extraction efficiency. Anthocyanin extraction process consist of destruction of anthocyanin source, maceration extraction about 6-10 hours, decantation, centrifugation to remove slurry, vacuum filtering, and vaporization to have concentrate of anthocyanin (Tensiska, 2007).

Beside water, anthocyanin can be extracted by methanol or ethanol with small amount of acid. Ethanol is more preferable than methanol because of less toxic in food. If the extract contains lipid part, organic solvent like hexane must be added to the extract for eliminate lipid-containing substances. The pH level has a significant influence on the color of the anthocyanin extracts. At lower pH (pH < 2), the color extract is a red to dark and at a higher pH (pH > 4) extracts exhibited a yellow color, and anthocyanin is decreased 94% in pH 5 than lower pH (pH 1) (Shipp and Abdel-Aal, 2010).

2), the color extract is a red to dark and at a higher pH (pH > 4) extracts exhibited a yellow color, and anthocyanin is decreased 94% in pH 5 than lower pH (pH 1) (Shipp and Abdel-Aal, 2010).

## CONCLUSION

Anthocyanin is one of pigment commonly applied as natural colorant in food. This pigment gives red, blue, and purple colors. Anthocyanin is a reactive compound. This reactivity is because the flavillium cation nucleus experiences a lack of electron, so that its color easily degraded. Anthocyanin is hydrophilic and very stable in acidic conditions. In addition to acidic conditions, it is stable when it is stored in cold temperatures, no oxygen, and no light exposed, and no polyphenol oxidase enzyme.

Besides acting as a coloring agent, anthocyanin can play as an antioxidant role. It is because the conjugated double bond in the anthocyanin structure makes it very reactive and can act as an antidote to radical compounds. Anthocyanin sources in nature are very abundant which come from fruits, flowers, and tubers.

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## THE EFFECT OF IMPROVER ADDITION ON SWEET BREAD MADE FROM WHEAT FLOUR

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### ABSTRACT

Modified starch produced in the MOCAF manufacturing process is still not widely used, so further studies are needed regarding the utilization of modified MOCAF-based starch. Starch can be used to improve the quality of bread. The raw materials used in the research are ADIRA cassava and cakra flour for sweet bread. The method used RAL with 3 factors, A type of control treatment improver (without addition of improver), addition of improver (native) and improver (modification), treatment B with variations in the amount of treatment added water (480, 510, 540 ml), and treatment C with variations in the concentration of the number of improvers added (0.125, 5 and 1%). In the application of sweet bread the data obtained from the results of the study were analyzed using the ANOVA test, Duncan's Multiple Range Test and continued the Effectiveness test to get the best treatment of bread produced. The lowest density and texture are the modification improver (1%+540 ml). The density value is  $0.17 \pm 0.00$  (g/cm<sup>3</sup>) and the texture value is  $36 \pm 6$  g/10 mm was obtained after storage on day 1. Based on the sensory test, the overall bread appearance score, the color of the inside of the bread, and the aroma, ranged from likes to the value of 4. The modification improver treatment (1%+540 ml) has a texture and taste which is preferred with values 4.25 and 4.25 and ease of swallowing with a value of 4.17. The effectiveness value of 0.8 in treatment modification (1%+540 ml). The conclusion is that the improver in modification treatment is best used as an improver on sweet bread.

**Keywords:** Improver, MOCAF, modified starch, bread, cassava

### INTRODUCTION

During the process of MOCAF making, many of unused modified starch is produced, so further studies are needed on the utilization of modified MOCAF side-processing starch. Selomulyo and Zhou (2007), reported that improver including hydrocolloid, emulsifier or other improver can be used to improve the quality of fresh and stored bread.

Cauvin (2000) states that an improver is an ingredient added to 'increase' the potential of flour-fed bread processing. Different bread processing uses different flour and different formulations. According to Wassermann (2009) improver is used specifically to improve production methods and quality of bread products. Improver can be derived from natural materials or with the addition of

additives, intended to facilitate or simplify the making of bakery product to exchange compensation for processing characteristics due to fluctuations in raw materials and to affect the quality of bakery product.

Improver applications are generally used not more than 10% out of the flour weight, improver is multifunctional product. Improver materials interact each other and are arranged in such a way as to meet the requirements of each type of flour and bakery, applied technology and quality of bread needed in bakery.

Starch contributes to bakery products in which it has important roles such as gelatinization, ability to absorb water and retrogradation (Taggart, 2004; Cui 2005). Starch gelatinization is important for building

the structure and texture of the starch products. The ability of starch to bind water can reduce the stickiness of the dough, improve the handling and softening the texture of bakery product (Radley, 1976; Taggart, 2004). The ability of starch to bind water can reduce the stickiness of the dough, improve handling, and increase the volume of bread. These characteristics can increase moisture and soften the texture of bakery products (Radley, 1976; Taggart, 2004).

Improver is a material that can be added to a product in order to make better product (product quality, process tolerance, and shelf life stability). The addition of improver into bread products is expected to increase expand power, decreasing staleness and density. The purpose of this research is to study the application of modified starch as an improver material on sweet bread.

The aim of the research are: to know the effect of adding starch (native / modified) to sweet bread product made from wheat flour, and the best improver treatment used in sweet bread made from wheat flour.

## **MATERIALS AND METHODS**

### **Tools and Materials**

The raw material used in this research is ADIRA cassava from Rowo Indah village, Jember Regency, aged of 10-12 months. Other research materials used are flour brand of Cakra Kembar Premium Bogasari, milk powder brand of Dancow, salt brand of Ship Cap, local sugar brand of Gulaku, instant yeast brand of Fermipan, blue band brand of Master, and HD plastic obtained in the city of Jember.

The tools used in this study include knives, plastic tubs, slicer, baking sheet, blender brand of Philips, 100 mesh sieve, Minolta color reader, Rheotex brand of Ogawa Saiki, oven brand of J Labtech. Rheotex Ogawa Seiki brand type SD-700, analytic balance brand of Matler Toledo AL204 type ( $\pm 0.01g$ ), oven brand of J Lab Tech, digital camera brand of Samsung with 10 pixel magnification. Scanner brand of Canon. Bread maker consists of proofing tools brand of Sinmag, Oven brand of Sinmag, bread mixer brand of Sinmag type SM-201.

### **Method**

In the first stage of research, starch production was used as an improver with fermentation time (hours) of 0, 24, 48 and 72

(F0, F1, F2, F3) with three replications and FB as a control with 10 hours fermentation time. Furthermore, physical, chemical, SEM and RVA analysis were measured. Followed by a non-factorial random test, which showed the F count was significantly different in all treatments at the 5% level.

Duncan test was further applied, the DMRT of 5% showed a real difference in the FB (native) and F3 (modified). Then 2 treatments were selected, namely FB and F3 to be applied as an improver on sweet bread products, to see how it affected the bread products made from flour such as swelling power, texture, density, H:D, water content, staleness and organoleptic properties. Furthermore, native starch and modification are used as improver with improver concentration of 0.125%; 0.5%; 1% and the variable addition of water 480, 510 and 540 ml, with three replications.

### **Data Analysis**

The processing of research data uses descriptive method. Observation data is displayed in table form, and to facilitate data interpretation, a histogram is created. In the application of sweet bread, the data obtained from the results of the study are analyzed by Variant analysis method (Analysis of Variant or ANOVA) with 5% confidence interval and continued with the Effectiveness test to obtain the best treatment of modified starch used.

### **MOCAF and Starch Production**

Cassava is removed from the skin, washed thoroughly to remove dirt and mucus. Then reduced the size in the form of chips with a thickness of 1-3 mm, weighed as much as 5 kg and added water with a ratio (1:2), and the starter is added until the concentration of marinade solution contains 100 ppm BAL. Stater is made by weighing as much as 0.5 g, 50 g of sugar, 50 g of dry MOCAF chips and as much as 1000 ml of water put into a 1000 ml of beaker glass, left for 24 hours until ready to use.

Furthermore, 5 kg chips are put into the tub and 9000 ml of water and 1000 ml of water are added, then fermented for 0, 24, 48 and 72 hours ( F0, F1, F2, and F3). After reaching the required fermentation time, the MOCAF chips are harvested and separated from the modified starch by filtering the liquid using a 120 mesh sieve and continued precipitation for 2 hours,



latter the fermented water is removed and the modified starch is dried underneath the sun.

As the FB made by crushing cassava with a blender then the juice is taken with a ratio of 5 kg of chips dissolved in 9000 ml of water and taking the starch then added with LAB as above as much as 1000 ml, precipitated for 10 hours, then set aside the fermented water and dried the starch under the sun. The flowchart starch production in the (FB).

Table 1. Fermentation time treatment

Fermentation Time (hour)	1	2	3
F0	F0.1	F0.2	F0.3
F1	F1.1	F1.2	F1.3
F2	F2.1	F2.2	F2.3
F3	F3.1	F3.2	F3.3

### **Application of Improver to Sweet Bread**

The second phase of the research is an improver application into sweet bread product. In this application, two types of improver that are still/original are selected, namely FB/native and those that have undergone modified, namely F3 improver. Based on physico-chemical characteristics, RVA and SEM. Native and modification improvers are selected in sweet bread applications since native and improvers had physicochemical characteristics, different chemistry in which native had granules, high break down (BD)  $2,620 \pm 30.45$  while modification has granules that have been modified by fermentation, which are more hollow and larger in size based on SEM, and break down (BD) is low  $1.494 \pm 148.28$  based on RVA.

The native improver has a higher peak viscosity (PV) with a value of  $4,389.00 \pm 58.39$  compared to modification with a value of  $4,207.00 \pm 143.04$ . The native improver has a higher set back value (SB) with a value of  $955.67 \pm 23.07$  compared to modification with a value of  $889.67 \pm 76.85$ . Application of native and modification improver on sweet bread is done to see the effect on the quality of the sweet bread produced, so that the control is used as a comparison with which improver is not added. Then formulation is carried out to see the effect of treatment, which is the addition of improver on sweet bread. Control is done by increasing the amount of water in

stages (480 ml, 510 ml and 540 ml). In native and modification, an increase in the number of improvers and water is carried out in stages (0.125%+480 ml; 0.50%+510 ml; 1%+540 ml). Furthermore, testing of the quality of sweet bread on controls, native and modification are added to the improver.

### **a. The Design Application of Starch on Sweet Bread**

The design of starch application in sweet bread products of wheat flour as the raw materials can be seen in Table 2. The composition of sweet bread and control composition of sweet bread can be seen in Table 3.

### **b. The Procedure of Making Bread**

The method used in making sweet bread is a straight dough method. The ingredients are weighed appropriately then stir the flour, sugar, vegetables, milk and modified starch until it is mixed well in a stirring machine for 2.5 minutes. Furthermore add the salt, eggs, water and stir for 5 minutes. Add blue band and stir evenly for 2.5 minutes. After all the dough is mixed well, increase the speed by 3 (three) and stir for 15 minutes until the dough is smooth. After it is smooth and a stirring film is formed, then stop the stirring. The dough is transferred to the table to be rounded and left for 10 minutes and covered with aluminum foil.

The dough is weighed as much as @ 80 g and shaped round, then let stand for 10 minutes on aluminum film cover. The dough is twisted 2-3x to remove gas on the surface then flipped and roll again 2-3 times, furthermore form the dough by rolling and put into mold that has been smeared in butter. Molds that have been filled with dough are arranged in a baking sheet to be included in the proofing room for 1 hour at 40°C. The proofing process has done as well as the increasing volume of the dough, and then put it into the oven at 180°C for 20 minutes. After the bread is baked, the bread is removed from the oven and cooled. The bread is cooled 6-8 hours then weighed and put into plastic and arranged in a platter for observation and testing.

### **c. Testing of Sweet Bread Products**

At this stage the quality of sweet bread products produced includes: expand power (%), density (g/cm<sup>3</sup>), texture (g/10 mm), moisture content (%), crust color (L, a\*,

b\*)with Color Reader, and sensory testing (color, taste, aroma, texture, ease of

swallowing, color of inside the bread and overall appearance).

Table 2. Design application of starch on sweet bread

Treatment	Control (C)			Native (N)			Modification (M)		
Water (ml)	480	510	540	480	510	540	480	510	540
Improver (%)	-	-	-	0,125	0,5	1	0,125	0,5	1

Table 3. Design application of starch on sweet bread

Treatment	Control (C)			Native (N)			Modification (M)		
Starch (%)	-	-	-	0,125	0,5	1	0,125	0,5	1
Water (mL)	480	510	540	480	510	540	480	510	540
Flour (kg)	1	1	1	1	1	1	1	1	1
Instant yeast (g)	22	22	22	22	22	22	22	22	22
Sugar (g)	220	220	220	220	220	220	220	220	220
Salt (g)	15	15	15	15	15	15	15	15	15
Powdered milk (g)	60	60	60	60	60	60	60	60	60
Butter (g)	150	150	150	150	150	150	150	150	150
Egg (g)	100	100	100	100	100	100	100	100	100

#### d. Measuring the Quality of Sweet Bread

**Expand Power** (Yuwono and Susanto, 2001)

The procedure for testing the expand power of the bakery is done by measuring and sticking it in the middle of the dough using a stick, so the measurement of the height before and after bakery can be known:

$$\% \text{ Expand Power} = \frac{B-A}{A} \times 100\%$$

Description:

A = the height before bakery

B = the height after bakery

**Density** (Eduardo et al., 2014)

Bread as many as (7) seven pieces are weighed and measured 60 minutes after the roasting process. Bread weight and bread volume measurement expressed in (g/cm<sup>3</sup>).

#### **Texture**

Bread is cut into pieces with a thickness of 3cm and measured the texture of 6-7 spots during storage of 0, 1, 2, 3, 4 and 5 days.

**Water Content** (Eduardo et al., 2014)

Bread samples were weighed 2 g, heated at 130°C for 2 days, then cooled in a desiccator. Measured fresh bread of (3) three pieces. Water content is measured by the formula:

$$\text{Water content (\%)} = \frac{\text{weight of water (g)}}{\text{weight of material (g)}} \times 100\%$$

#### **Lightness (L) Bread Crust**

The bread surface is measured in color after 180 minutes or (3) three hours out of roasting with total (4) four breads to measure. Determination of white degree is based on the Color Reader method. Previously, Color Reader is calibrated with standard porcelain. A number of samples are placed in a cup, then target the sample at seven points to find out the values of dE, dL, da, and db. The value of L \* (Lightness) is related to the degree of brightness, which ranges from 0 (zero) to 100 (one hundred). Brightness is stated to increase with increasing L value \*.

The L value is obtained based on the formula:

$$L = \frac{L \text{ porcelin standart (spesifik factory)}}{L \text{ in porcelin used}}$$

Description:

L porcelain standard (specific factory) = 94.35

L in porcelain used = 63.50

#### **Sensory Test** (Rampengan et al., 1985)

Sensory testing is done to determine the level of preference or feasibility of a product to

be accepted by the researcher (consumer). The test method used is the hedonic method (test of preference). Bread sensory testing carries out among overall (appearance, interior color of bread, aroma, texture, taste and ease of swallowing) through the scoring method by 35 panelists. The sensory test is carried out in a cross and random manner. Done step by step. Bread samples are placed on white paper which is given a 3 digit random number code. Panelists were asked to smell, hold, and observe the bread using their eyes, mouth and nose. The scores used are:

- 1 = extremely dislike
- 2 = dislike
- 3 = rather like
- 4 = like
- 5 = really like

**e. Effectiveness Test (Degarmo, et al.,1984)**

Determination of the best treatment is determined based on the index effectiveness method (DeGarmo et al., 1984). This method is based on the procedure as follows: variables are sorted by priority and contribute to results. Give value weight to each variable (BV) according to its contribution with relative numbers 0-1. This weight differs depending on the importance of each variable whose results are obtained as a result of treatment. Normal weight (BN) is determined from each variable by dividing the variable weight (BV) by the sum of all value weights.

Divide the variables analyzed into two groups, namely:

1. Group A, consists of variables in which the greater the average the better the value (desired for the treated product).
2. Group B consists of variables which are the greater the worse (not desired).

Determined the effectiveness value (Ne) of each variable, using the formula: the treatment value - the worst value and the best value - the worst value, for the variable with the greater average the better, so the lowest value as the worst and the highest value as the best. Conversely for the variable with the smaller value the better, the highest value as the worst value and the lowest value as the best. Calculate the result value (Nh) of each variable obtained from the normal weight multiplication (BN) with the value of effectiveness (Ne).

Add the results value of all variables and the best combination is chosen from the treatment combination which has the highest result value (Nh).

$$N \text{ Effectiveness} = \frac{\text{treatment value} - \text{the worst value}}{\text{the best value} - \text{the worst value}}$$

$$\text{Result value} = NE \times \text{weight}$$

**RESULTS AND DISCUSSION**

Application of native and modification improver on sweet bread is done to see the effect on the quality of the sweet bread produced, so that the control is used as a comparison of which the improver is not added.

**Expand Power**

Bread expand power is the ability of bread to experience increased size before and after cooking process. The lowest expand power is 114.1±4.2% in the native improver with the formulation (0.125%+480 ml), the highest expand power in the modification improver with a value of 304.1±34.6% in the formulation (1%+450 ml).

In the modification improver the old fermentation causes the improver to have a shorter chain that has been converted into simple sugars so that during the proofing process it is used by the yeast as an energy source to increase the expand power. Bread expand power can be seen in Figure 1.

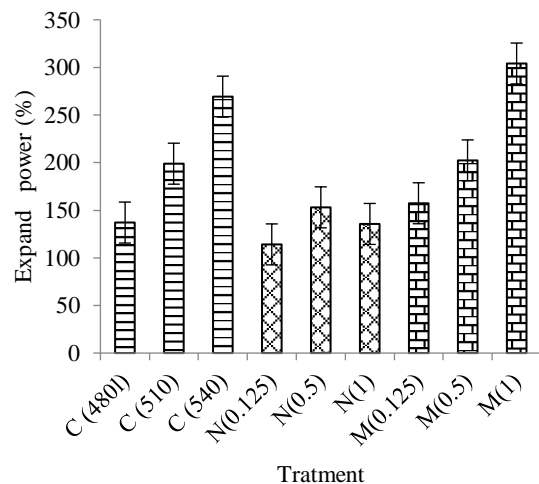


Figure 1. Expand power of sweet bread with the addition of improver: 0.125%; 0.5%; and 1%



The capacity of swelling is often related to protein and starch (Woolfe, 1992). Higher protein content in flour can cause starch granules to be embedded in the protein matrix, which then limits the access of starch to water and limits swelling strength (Aprianita *et al.*, 2009). Amilopectin is primarily responsible for granular swelling (Tester and Morrison, 1990). Moorthy and Ramanuhour (1986) also report that the swelling strength of the granules is an indication of the extent of associative strength in the granule.

### Density

Density or solidity is the amount of substance in a unit of volume, in bread it is used to measure the density of bread at a certain volume. The low density of the bread indicates that the bread has the greater volume, as well as the smaller the density. The highest density obtained in this study is  $0.20 \pm 0.01$  ( $\text{g}/\text{cm}^3$ ) in the native improver (0.125%+480 ml).

While the lowest density is equal to  $0.17 \pm 0.00$  ( $\text{g}/\text{cm}^3$ ) in the modification improver (1%+540 ml). Bread that is given an improver with an modification formulation (1%+540 ml) produced a lower density than an native improver (1%+540 ml). Density here relates to the size of the cavity in the bread, if the density is small then the cavity in the bread is getting bigger or more porous and the volume of bread that is increasingly expanding can be seen in Figure 2.

The low density shows that the bread is tenderer. The modification improver has a lower density value than the native improver, indicating that bread given an modification

improver is tenderer than native. Controls have lower values based on tenderness level, controls are softer than modification or native.

The density value can be seen on Figure 3.

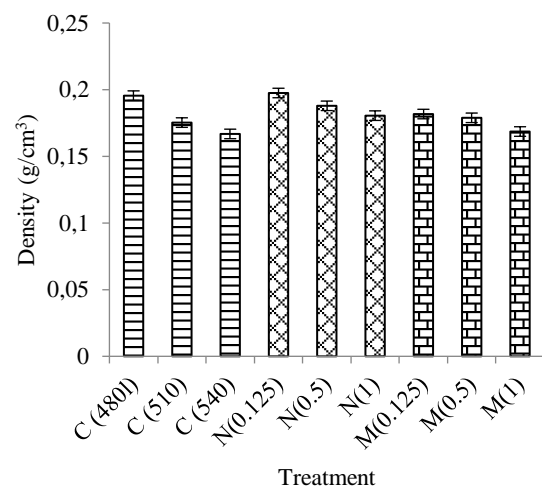


Figure 3. Density of sweet bread with the addition of improver: 0.125%; 0.5%; and 1%

### Texture

Texture is one of the most important factors for measuring bread quality, since the bread hardness increases during staling due to changes in amylopectin structure during gelatinization, and swelling of granules (Schoch, 1965). Texture ( $\text{g}/10$  mm) is count to measure the level of hardness on bread. The quality of bread, especially texture, is specifically related to the level of hardness and is associated with density, which means the smallest density, the tenderer the bread will be (Basman *et al.*, 2002).

The lowest texture is obtained after storage on day 1 of the modification improver (1%+540 ml) with a value of  $36 \pm 6$  g/10 mm and the highest value in the native improver (1%+540 ml) with a value of  $46 \pm 2$  g/10 mm. After the 5th day being stored, the lowest value is obtained in the modification improver (1%+540 ml) with a value of  $86 \pm 28$  g/10 mm and the highest value in the native improver (1%+540ml) with a value of  $92 \pm 26$  g/10 mm .

The addition of water decreases the texture of the control due to the addition of

excess water, therefore the control texture has a lower value than modification and native. This is presumably because the native improver added to the bread is still so the dough cannot absorb the water in the starch perfectly when it is mixed. Most of the granules are still close after the cooking process and when cooling retrogradation occurs by re-crystalline the starch which is characterized by an increasingly hard texture. In Figure 4.

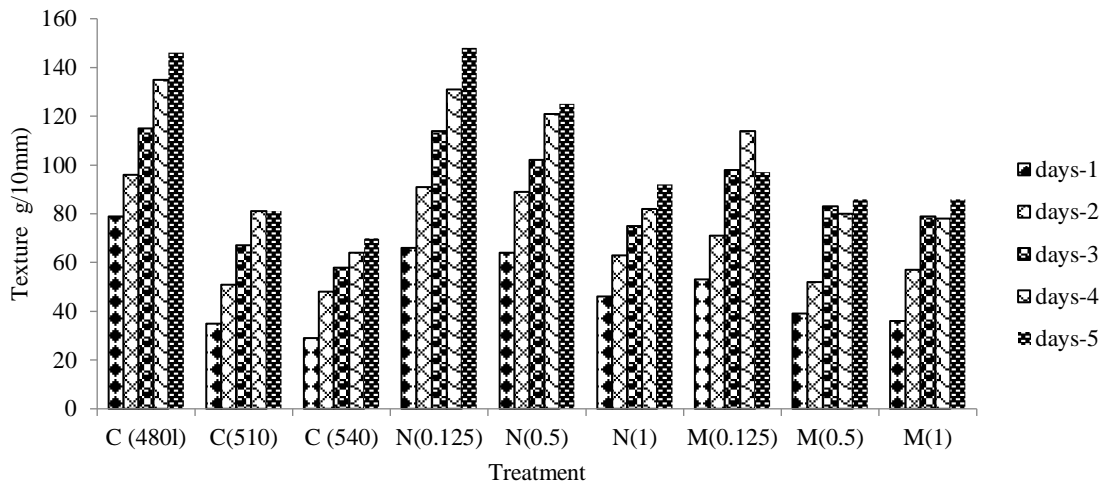


Figure 4. Texture of sweet bread with the addition of improver: 0.125%; 0.5%; and 1% (1st day until 5th day) from left to right

Hibi *et al.*, (1993) stated that starch granules have a semi-crystalline structure and do not dissolve at room temperature. Retrogradation in starch is unnecessary for food products such as bread, because it causes staling and shortens product shelf life (Karim *et al.*, 2000; Jane 2004). Starch in bakery products has important roles such as gelatinization, ability to absorb water and retrogradation. Starch gelatinization is important for building the structure and texture of the starch products. The ability of starch to bind water can reduce the stickiness of the dough, increase handling, and increase volume. This characteristic can increase moisture and soften the texture of bekeri products (Taggart, 2004). According to Be Miller (2007) the changes in bread will result in staling by the increase of bread texture, and the transfer of water from the bread to the skin/crust of bread.

According to (Zallie *et al.*, 1984) low amylose content can delay staling bread. Amylose proposition and amylopectin

structure have an important role in the speed and degree of starch retrogradation (Goodfellow and Wilson, 1990). Staling involves several physical and chemical phenomena, crystallization of amylose and amylopectin (Zobel and Kulp, 1996).

### Water Content

The water content in food ingredients also determines the level of acceptance, freshness and durability of the product. Most of the chemical and biochemical changes in food ingredients occur in the medium of water that comes from that material (Winarno, 2004).

The lowest water content obtained in this study is  $23.48 \pm 0.90\%$  in modification improver (0.125%+480 ml). While the highest water content found is  $30.66 \pm 2.27\%$  in native improver (0.125%+480 ml). During storage until the 5th day the water content of the modification improver is lower than the native improver. The results of the measurement of water content can be seen in Figure 5. The addition of improver does not have an effect on



the weight loss of bread. This shows the level of freshness of bread with native and modification improver has the same level of freshness as the control.

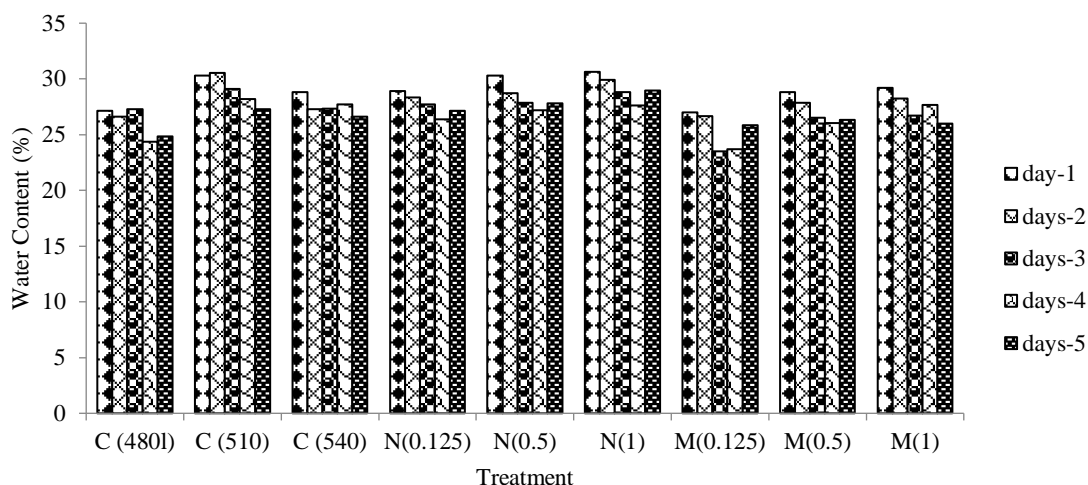


Figure 5. Water content of sweet bread with the addition of improver: 0.125%; 0.5%; and 1% (1st day until 5th day) from left to right

According to Stauffer (2000) water content is an important parameter for staling since the occurrence of retrogradation of starch slowly when the water content is high. The use of starch can increase the binding capacity of water (Zallie, 1988). Starch also has multifunctional properties in ingredients, including gelling and moisture storage (Pietrasik, 1999). Increasing water absorption capacity in food systems makes it possible to manipulate the functional properties of dough in bread products (Achinewhu and Orafun, 2000; Iwe and Onadipe, 2001). Water content is an important parameter in staling, when starch undergoes retrogradation in a state when the water content is high. The water content that rises 2% will increase the shelf life of bread one day (Stauffer, 2000).

Bread is produced using additional ingredients such as fat, milk, and additives, together with optimized parameters of the technological process found to improve its sensory and nutritional characteristics (Fik, 2004). Addition of other ingredients in the bakery process such as fat will also affect staling and water loss, as well as the processing of bread (temperature, processing). Bread shelf life can be increased by such methods involving freezing, packaging, heating processes, bioconversion and addition of chemicals and others (Karolak et al., 2014).

### Lightness (L)

Color is the most determining factor for whether or not a food product is attractive (Winarno, 1991). According to Fennema (1985), color is the most important quality attribute together with texture and taste. Color plays a role in determining the level of acceptance of a food, even Kartika et al., (1988) states that color is one of the visual profiles that become consumers first impression in assessing food ingredients. The result of the lightness (L) measurement on the control shows a darker color. This is presumed by the addition of water to the control, the dough is more fully mixed. The modification improver has a\* higher intensity value than the native improver. The values of L, a\*, and b\* on sweet bread can be seen in Table 4.

The bread color is directly related to the raw material, the formulation used and the condition of the bread (Silva et al., 2009). In general, the L value indicates the high value of brightness/light with brighter color results. The high values of a\* and b\* illustrate that the sample has strong red and yellow or golden/golden intensity (Esteller et al., 2004). Crumb lightness colour (L\*), red (a\*) and yellow (b\*). The bread crust color as a whole at the top is deep golden brown and light golden brown at the bottom (Kamman, 1970).

The intensity value of the bread surface/crust increases when it gets stronger red/gold color. In the control by adding water, the red color on the crust is getting stronger, this is presumably because of the sugar content, amino acids in flour are completely dissolved so that when working the non-enzymatic browning process or the maillard takes place more perfectly. According to Bamforth (2005) the reaction of red or brown color on the crust due to the maillard reaction, which is a heated sugar compound and free amino groups, namely amino acids, proteins and amines, will have an impact on flavor.

It can also occur in amino components and substrates other than sugar (carbonyl free group), which are ascorbic acid and molecules produced during lipid oxidation. Color is an important criterion for the initial acceptance of a bakery product to be accepted by consumers. Color development occurs at the stage of work

and it is a very complex stage in the production process (Zanoni *et al.*, 1995). Zanoni *et al.*, (1995) state the color of the bread surface depends on the physical and chemical characteristics of the raw material (such as water content, pH, reducing sugars, amino acids and operating conditions during the cooking process) such as temperature, air velocity, RH, and transfer heat.

According to Fayle & Gerrard (2002) during production there is a maillard reaction between wheat protein and added sugar and caramelization which is influenced by the distribution of water and added sugars and amino acids (Kent and Evers, 1994). The maillard reaction is related to temperature, time and the presence of  $a_w$  / water activity, and the crust color on the bread will be optimal as the occurrence of browning reactions (Eduardo *et al.*, 2013).

Table 4. Lightness of sweet bread

Treatment	L	a *	b *	
Control/C	480 ml	57,26	7,15	1,72
	510 ml	55,9	7,98	1,74
	540 ml	55,99	7,7	1,66
Native/N	0,125%+480 ml	55,11	6,84	1,11
	0,5%+510 ml	55,75	6,36	1,05
	1%+540 ml	56,98	6,79	1,55
Modification/M	0,125%+480 ml	55,3	7,09	1,22
	0,5%+510 ml	54,49	7,29	1,19
	1%+540 ml	56,69	7,29	1,28

### Sensory Test

Bread sensory tests with modification and native improver on various concentrations were carried out for overall bread appearance attributes, the inside color of the bread, aroma, texture, taste and melting were swallowed using the scoring method. Based on the sensory test results obtained the overall bread appearance score, the color of the inside of the bread, and the aroma, ranged from likes to a value of 4. The modification improver treatment (1%+540 ml) had a texture and taste that was favored with grades 4.25 and 4, 25 and ease of swallowing with a value of 4.17. Native improver treatment (1%+540 ml) has the preferred texture and taste with values of 3.75 and 3.73 and ease of swallowing with a value of 3.90. Based on the test, Modification

and native sensory improvers are received by consumers. The panelists' assessment of the overall appearance of the bread, the color of the inside of the bread, aroma, texture, taste and ease of swallowing can be seen in Figure 6.

Flavor (aroma and taste) is one of the most preferred sensory characteristics of bread (Caul, 1972, Martinez-Anaya, 1996). Flavor consists of aroma sensation, mouth feeling (Martinez-Anaya, 1996, Caul, 1972, El-D ash, 1967). Improvers, especially starch, protein and water contribute to structural architecture and mechanical strength of bread, assumed to play an important role in changing the nature of cell walls during bread storage (Gray and BeMiller, 2003). Elasticity is mainly influenced by interactions between starch

gelatinization and gluten dough, and can form a continuous spongy structure in bread after

feeding, which results in more elasticity of bread dough (Hoseney and Roger., 1994).

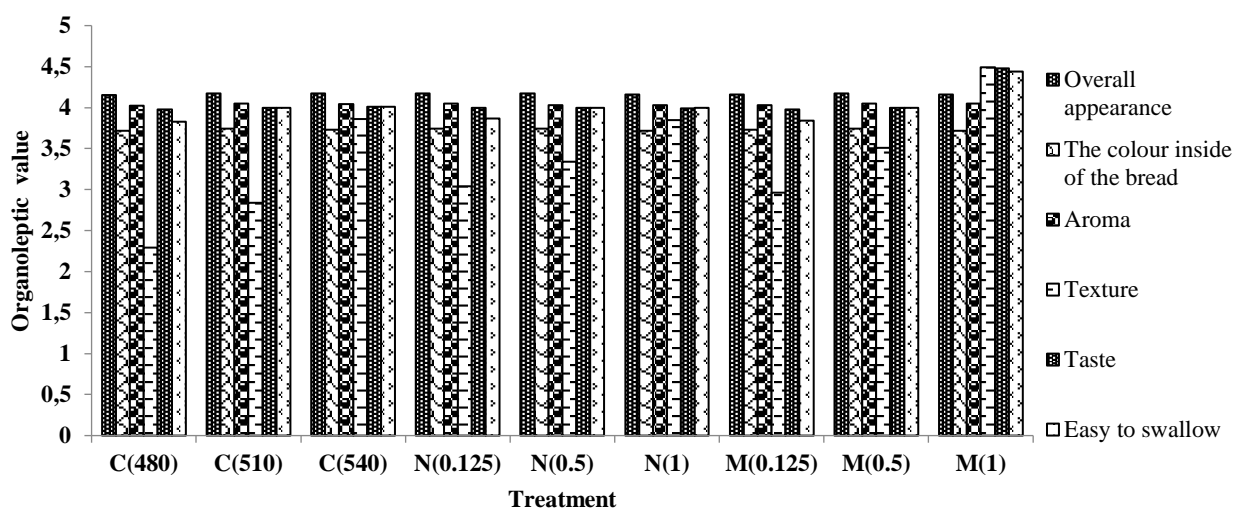


Figure 6. Sensory test of sweet bread with the addition of improver: 0.125%; 0.5%; and 1%

### Effectiveness Test

The effectiveness test is carried out to determine the best improver treatment used in sweet bread products. Effectiveness testing is carried out on all parameters, involving expand power, density, H:D, texture, water content, skin color/crust (L, a\*, b\*) and sensory test (overall appearance, inner color of bread, aroma, texture, taste and ease of swallowing, the color of the inside) on the bread.

The portion given for each parameter is different. The weight for expand power is 1.0; texture of day 5 is 1.0; density of 0.9; water content of 0.8. Weight for sensory test (texture of 1.0; taste of 1.0; overall appearance of 0.9; ease of swallowing by 0.9; aroma of 0.8; color of inner bread is 0.7; and crust color (a\* of 0.8; b\* of 0.8; and L of 0.8) The effectiveness index of sweet bread with the addition of improvers can be seen in Table 5.

Table 5. Effectiveness index value of sweet bread

Treatment	Effectiveness Value
Control (+480 ml)	0,352
Control (+510 ml)	0,733
Control (+540 ml)	0,69
Native (0,125%+ 480 ml)	0,418
Native (0,5%+510 ml)	0,332
Native (1%+540 ml)	0,352
Modification(0,125%+ 480 ml)	0,414
Modification (0,5%+510 ml)	0,586
Modification(1%+540 ml)	0,8

Based on Table 5, the effectiveness test of sweet bread with modification improver treatment (1%+540 ml) has the highest effectiveness value of 0.80 with characteristics: expand power 304.1±34.6%; density 0.17±0.00 g/cm<sup>3</sup>; H:D 0.17±0.02 (cm: cm); 5th day texture 86±28 g/10mm; and the fifth day's staleness is 1.06±0.28%; water content of 25.99±0.90%. Bread sensory test includes crust color a\* with a value of 7.29±0.68; b\* with a value of 1.28±0.45; and L with a value of 56.69±1.33; and sensory test (overall appearance with a value of 4.09; the color of the inside of the bread with a value of 3.62; aroma with a value of 3.97; texture with a value of 4.25; taste with a value of 4.25; and ease swallowed with a value of 4, 17; the color of the inside of the bread with a value of 3.62 received by the consumer with the value of preference.

### CONCLUSION

The modification improver application is very suitable for sweet bread products made from wheat flour, because in the modification improver the old fermentation causes the improver to have a shorter chain that has been converted into simple sugars so that during the proofing process it is used by the yeast as an energy source to increase the expand power. Based on the effectiveness test of sweet bread with modification improver treatment

(1%+540 ml) has the highest effectiveness value of 0.80.

## ACKNOWLEDGMENT

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## SOURSOP FRUIT POTENTIAL AS A SUBSTRATE IN NATA DE ANONNA PRODUCTION

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### ABSTRACT

Nata is a functional food that has an healthy effect because it contains vitamin C. Vitamin C is beneficial for the body. Nata derived from soursop fruit called nata de anonna, which is produced from the bacterial activity of *Acetobacter xylinum*. This study aimed to determine the potential of soursop fruit skin as a substrate for making nata de anonna. The research study show that the yield of nata from soursop fruit, soursop skin, and soursop pulp were 1.5 cm, 1 cm and 0.8 cm. This research concluded that the hump and soursop skin which become waste are part of the soursop fruit can be used as the substrate of nata de anonna.

**Keywords:** Soursop, nata de anonna, substrate

### INTRODUCTION

Soursop (*Annonamuricata L*) is a species of tropical fruit tree that belongs to the family Annonaceae. The Annonaceae family has about 119 species (Ross and Victor 2010). Soursop is a fruit plant that is very popular and is also widely known by the public. This plant has amazing benefits for body health, so many are cultivating this plant. According to Prasetyorini (2014) the soursop (*Annonamuricata L*) received a lot of attention from the community because of the news about its efficacy in killing cancer cells. Generally, the use of soursop fruit in Serang still need to improve. Nowadays, soursop fruit is used as a dish in the form of soursop ice, juice, and syrup.

Pulp is the part of soursop fruit commonly used. The skin and the hump soursop are rarely used and are often disposed of as waste. According to Bora (2014), soursop has high polyphenol compounds, and contains lots of vitamin C. Phenol and flavonoid compounds in many plants act as antioxidants

because they have molecular structures that provide electrons to free radical molecules.

Nata is a gel-like substance, insoluble in water and formed on the surface of fermentation. Nata de coco is a type of nata that used coconut water as media. While nata de annona is a type of nata that used soursop fruit extract as media. Nata is rich of dietary fiber (Priyanto, 2011).

Acoording to (Hamad et al., 2017) Pineapple skin as a substrate of nata de pina is very potential. Pineapple skin is usually disposed as waste. Although, it shows great potency as raw material of functional food. This indicate by the high yield of 80.24% nata de pina, 1.11 cm thick and the high moisture content of 89%. This parameters showed the similarity of nata de coco and nata de pina.

Nata de annona made from soursop substrate. However, it is not clear which parts have the best potential to be used as substrate for nata de annona. The skin and the humps is by product of soursop and usually dispose as waste. But, the skin and the hump is potential as a substrate for nata de annona. This research

*Dewi Ratnasari et al.,*

was conducted to determine the potential of soursop skin and humps as a substrate of nata de anonna.

## MATERIALS AND METHODS

### Tools and Materials

The materials used in this study were soursop fruit (*Annonamuricata* L), granulated sugar (carbon source), urea (nitrogen source, and glacial acetic acid  $\pm$  96%, mineral water. Experiments carried out by comparing the part of soursop fruit as a substrate of nata de anonna. Soursop fruit parts are skin, pulp, and hump. The tools used in the study consisted of analytical scales, cooking pans, vegetable spoons/tablespoons (stirrers), filters, washcloths, gas stoves, plastic basins, plastic trays with a minimum height of 5 cm, rubber bands, newspaper, pH meters, thermometers, glassware (Hamad *et al.*, 2017).

### Methods

The stages of the research carried out can be seen in Figure 1. as follows:

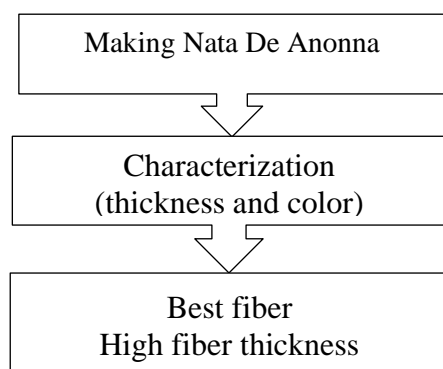


Figure 1. Steps of research

The stages of making nata de anona are as follows:

### Soursop Media Preparation

Soursop part was separated from the skin and pulp. the soursop part is mixed with water in a ratio of 1: 2 then blended and then separated with the pulp. As much as 500 ml of water was sterilized by boiling until boiling. (simmer for 5 minutes). Pour the boiled mixture into a 20x30x5 cm sterile plastic pan with a minimum height of 5 cm, then cover tightly with newspaper and let it sit a day until it is completely cold (Endria *et al.*, 2008).

### Seeds Adding and Storage

The media consisted of 17.5 kg of sugar as carbon substrate, 3 kg of urea as a nitrogen source and Glacial acetic acid (vinegar) as much as 20 ml to adjust the pH to 4.5. The

culture liquid was put in a 20 x 30 x 5 cm plastic baking pan as fermentation place. The nata starter, 60 ml of *Acetobacterxylinum*, was put into the culture liquid in a sterile condition. The culture was stored for 9 days in a cool and safe place (undisturbed and not rocking).

### Harvesting

Soursop water media (skin, hump, and pulp) that have formed nata de anona hydrogel fibers (pellicles) were washed and cleaned using flow water until the acid disappears. Nata de anonna colors was clear white. The cleaning process was done to relieve the smelly and rotten product due to the fungus growth. The cleaned nata de anonna was cut into dice form.

## RESULTS AND DISCUSSION

Nata de anonna is a type of nata that in its making uses soursop as a substrate. Soursop fruit is quite easy to find, so people are familiar with this fruit. All parts of soursop plants such as fruit, leaves, seeds, and stems can be used for health because they contain antioxidants, anticancer, and antiviral (Wullur *et al.*, 2012). Every 100 g of edible soursop contains 0.07 mg of B vitamin, 20 mg of vitamin C, 2.54% sucrose, 5.05% dextrose and 0.04% levulose (Sukarmin, 2010). The content of vitamin C is very good for the body because it functions as an antioxidant. Antioxidants are very good for increasing endurance (Hermawan and Leksono, 2013).

To produce solid, thick, supple, and translucent nata masses, it is important to consider the temperature of incubation (fermentation), composition and pH or acidity of the medium, besides the use of a starter (starter) is also important (Rizal, 2013).

### Growth of Nata Layer Thickness

The growth of nata with variations substrate of the soursop (skin, pulp and hump) in can be seen in Table 1.

Table 1. Nata layer thickness produced from the soursop section

No.	Part of soursop	Layer thickness(cm)
1.	Pulp	0.8
2.	Hump	1.5
3.	Skin	1

Nata de anonna from soursop hump is thicker than nata de anonna from soursop skin

and soursop pulp. It is 15 mm. The thickness of nata from fruit pulp has the lowest thickness of only 8 mm. The difference in thickness shows that, as a substrate, the soursop corm has a higher carbohydrate content than the other parts. The pulp part has a high moisture content, while the skin is the hard part. Soursop fruit contains numbers of vitamins and fiber. The vitamins contained was A, B, and C. In addition soursop fruit also contains 2.54% sucrose, dextrose 5.05% and 0.04% levulose (Radi, 1997).

Based on the results (Table 1), the thickness differences indicate that there is an interaction between *Acetobacterxylinum* and the different substrates. The thickest got from the hump substrates. It means that the interaction of bacteria with the substrate and other ingredients have a good interaction. According to Effendi et al., (2011) the thickness of the nata layer is influenced by the sucrose content in the liquid substrate media. The high of the sucrose content in the water substrate media correlate the high of the thickness of the nata layer. The addition of sugar to the media is useful as a food source for the growth of *Acetobacterxylinum* to produce layer of cellulose (nata) through the fermentation process (Malvanie et al., 2014).

Nurhayati (2005) said that seven-day fermentation provides sufficient nutritional and oxygen aeration requirements, this caused an increase in the number of bacterial cells and making the cellulose layer heavier. In addition, in the fermentation process, the dynamics of the growing population of *Acetobacterxylinum* bacteria are difficult to predict. This happens due to environmental conditions that cannot be controlled properly during the fermentation process. According to Suparti (2007) nata thickness is also influenced by the age of the bacteria. The age of the bacteria affects the final result, the older the culture used, the lower the yield (thickness) in the fermentation.

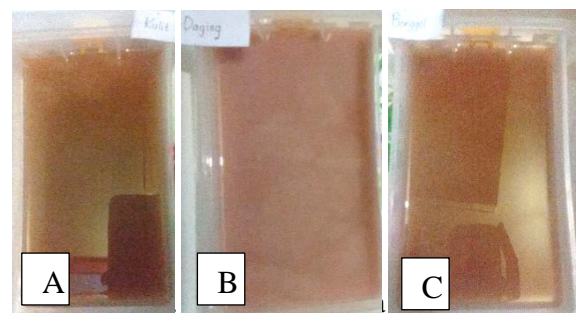
*Acetobacterxylinum* is a cell that produces cellulose or microbial cellulose. These bacteria are gram negative, aerobic, and can produce cellulose (Malvanie et al., 2014). *Acetobacterxylinum* synthesizes and produces the cellulose fibrils that come out of the pores of the cell membrane. During the fermentation process, the cellulose will bind other cellulose to form layers or pellicles. This layer will float

on the surface of the media so that oxygen can diffuse into the media. Oxygen is needed for the growth, development and formation of cellulose pellicles (Hamad et al., 2017).

The layer or pellicle is often referred as nata. Nata is an extracellular polysaccharide layer (cellulose) formed by capsule-forming microbes. This layer has a rubbery texture, resembles a gel and floats on the surface of the liquid (Iguchi et al., 2000). The growth of *Acetobacterxylinum* is influenced by several factors including nutrient content including the amount of carbon and nitrogen, the acidity level (pH), temperature, and air (Malvanie et al., 2014). These factors support the bacteria to grow, develop and produce a layer of cellulose.

### **Color**

The results of color analysis of the nata products produced can be seen in Figure 2.



According to the result study, it is known that the color of the skin, pulp, and hump substrate have almost the same color, namely brown. However, the three different substrate have different color. Nata also has a color that is not much different, namely white with a little murky. The most visible nata color is rather white found on the hump soursop. This is influenced by the amount of glucose contained in the substrate. According to (Edria et al., 2008) the color of nata is influenced by the interaction of sucrose with nutrients in mineral water, many interactions between sucrose and nutrients make the color of nata not bright (less white).

In the soursop hump, there is a natural sugar compound so that the mixing of sugar and substrate produce high glucose content. It also generate caramelization because of boiling process. This will affect the color of the resulting nata. Based on Figure 1, it shows that the nata color of the skin, pulp, and hump soursops substrate were not significantly

*Dewi Ratnasari et al.,*

different, showing almost the same color. The texture produced on the skin is slightly hard, the hump produce soft texture, and the pulp has a soft texture.

## CONCLUSION

This research can be concluded that soursop hump and soursop skin which are usually disposed of as waste have the greatest potential in making nata de anona. Nata de anona thickness produced from soursop skin produces nata which has a thickness of 1.12 cm with a slightly hard texture, 0.74 cm hump with a soft texture and 0.20 cm pulp with a soft texture. Part of soursop meat contains most of the water which has smaller nutrients than other parts so that it produces thin nata. This parameter shows that all parts of soursop fruit can be processed for making nata de anonna

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# ANALOGUE SAUSAGE FORMULATION OF TEMPEH-WHITE OYSTER MUSHROOMS (*Pleurotus ostreatus*) WITH THE ADDITION OF CARRAGEENAN

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## ABSTRACT

Sausage is a food product that is processed as a variety of food. Tempeh and white oyster mushroom (*Pleurotus ostreatus*) contain many nutrients needed by the body. In addition, carrageenan also contributes to the nutritional content of the product. This research was conducted to determine the chemical and sensory characteristics of sausage that are rich in fiber and received by consumers. This study used completely randomized design method (CRD) which consists of two factors. The first factor was the substitution of tempeh and white oyster mushroom with a ratio of 50:100; 75:75; 100:50. The second factor was the addition of carrageenan, including 4, 6 and 8 g. The chosen ratio was the treatment of a comparison of 75 g of tempeh and 75 g of white oyster mushroom with the addition 6 g of carrageenan. The chemical properties of the analogue sausage was: 14% protein, 11.22% fat, 44.24% moisture, 6.02% crude fiber, 2.37% ash and 27.29% total carbohydrate. While for sensory test color was 2.96 (light brown), flavor of tempeh 2.45 (enough flavor), flavor of mushroom 1.74 (enough flavor), texture 2.55 (chewy enough) and overall preference 2.45 (most preferred).

**Keywords:** Carrageenan, sausage, tempeh, white oyster mushroom

## INTRODUCTION

Sausages have been created from both meat and fish, as the main ingredients. But behind the delicacy, it turns out that sausages can have a negative impact on health like high cholesterol. According to Sabudi (2016), meat sausage is not good if consumed in excess, as it has much fat in the process of making that it can accumulate cholesterol in the body which is able to cause various kinds of diseases. Therefore sausages are developed with the main non-animal ingredients so that it has enough nutrients to consume which is good for consumers as it does not have bad impact for highly consuming it.

Tempeh is a food that has a fairly good nutritional content. Tempeh has a high protein and isoflavone that good for health. Tempeh is a soy-based food that has a high protein as one

of the content, which is good for body. Tempeh is easily obtained at a relatively cheap price. Some of the important components in tempeh that are beneficial to health are amino acid, unsaturated fatty acids, and isoflavones (Haron *et al.*, 2009). Some of the benefits of tempeh are low in fat, so it is good for diet, fiber content that is good for digestion, and lowers blood lipids.

Oyster mushrooms have a delicious taste, good nutritional value, and beneficial to health. White oyster mushrooms have high protein content, according to Djarijah and Djarijah (2001), white oyster mushrooms have a protein content of 10.5-30.4%. The results of the Schneider *et al.*, (2011) study shows that consuming 30 grams of oyster mushrooms for 21 days would have a positive effect on blood lipid profiles.

**Rohmawati S et al.**

Carrageenan is a seaweed resin extracted with water or an alkaline solution of certain species from the class of Rhodophyceae (red algae). It is known that the content of seaweed fiber is around 33.50% dry weight (Hernawati *et al.*, 2013). The substitution of oyster mushroom-tempeh will affect the level of food fiber in sausage analogues of oyster mushroom-tempeh. The application of adding carrageenan in making tempeh sausages is to be an emulsifier and also expected to contribute as a source of fiber. This study is conducted to examine the effect of carrageenan concentration and substitution of oyster mushroom-tempeh on food fiber content so that the analogue sausage of oyster mushroom-tempeh can produce the best characteristic. Test parameters include protein content, fat content, moisture content, ash content, crude fiber content, total carbohydrate content and sensory include color, tempeh flavor, oyster mushroom flavor, texture, and overall preference.

## **MATERIALS AND METHODS**

### **Tools and Materials**

Supporting cooking utensils, mortars, spatulas, Shimadzu AUX 320 digital scales, crucibles, hypertherm muffins, memmert oven, laboratory fume hoods, desiccators, electric stove, distillation flask, condenser, electrothermal, kjeldahl pumpkin, pipette measuring, erlenmeyer, waterbath, soxhlet tube, clamp, filter paper.

Tempeh, oyster mushroom from farmer in Sragen middle of Java, carrageenan, seasoning, sulfuric acid, aquadest, sodium hydroxide, hydrochloric acid, petroleum ether, xylene, anti-froth, asbestos, potassium sulfate, and alcohol 95%.

### **Methods**

#### **Experimental Design**

This study uses factorial completely randomized design with two factors: Ratio of tempeh with white oyster mushrooms (50:100; 75:75; 100:50 g) and the addition of carrageenan (4; 6; 8 g), it is obtained 9 combination treatment of each treatment with duplicates. The data obtained are analyzed by variance test at a significant level of 0.05. If there is a significant difference continued by the Tukey Test to find out the significant

difference between treatments at a significance level of 5%.

### **Sausage Production**

Tempeh was diced and weighed according to treatment (50; 75; 100 g). The mushrooms were sorted and weighed according to treatment (100; 75; 50 g) and then blanched it for 5 minutes. Tempeh and white oyster mushrooms were mashed using food processor. White tempeh and oyster mushrooms then mixed with 1.5 g sugar, 1.5 g salt, 1.5 g pepper powder, 0.5 g coriander powder, 2 g garlic, 0.5 g nutmeg powder, 5 g vegetable oil, and ice water 7 g. Mixed ingredients were added with carrageenan according to the treatment (4, 6, and 8 g). Materials were put into the sausage sleeve and steamed it for 30 minutes. Sausages were ready to be served.

### **Data Analysis**

The analysis consisted of chemical and organoleptic analysis. Chemical analysis were protein content (Sudarmadji *et al.*, 1997), fat content (AOAC, 1995), water content (Baedhowie and Pranggonowati, 1982), crude fiber level (Apriyantono *et al.*, 1989), ash content (Sudarmadji *et al.*, 1997), and total carbohydrate levels by difference (Kartika *et al.*, 1988). Organoleptic analysis of the scoring test method (Kartika *et al.*, (1988) consisted of color, tempeh and mushroom flavor, texture, and overall preference.

## **RESULTS AND DISCUSSION**

### **Chemical Analysis**

Chemical analysis results are shown in Table 1. It consists of protein, fat, water, crude fiber and ash content.

#### **a. Protein Content**

The lowest protein content was found in the treatment of the ratio of 100 grams tempeh and 50 grams white oyster mushroom with the addition of 4 grams of carrageenan which was 13.138%. The highest protein content was found in the treatment of 50 grams of tempeh and 100 grams of white oyster mushroom with 8 grams of carrageenan addition that is 16.161%. The sausage formulation containing much of white oyster mushrooms had higher protein content than the sausage formulation which contain a little white oyster mushroom. This is because the oyster mushroom protein is higher than the protein in tempeh. The



ingredients for making sausages also affect the sausage protein content.

According to Hudaya (2008), carrageenan protein content is 1.26% and according to Witanto (2013) carrageenan protein content is 2.27%, more than the tapioca flour protein content of 0.5-0.7%. This research of tempeh-oyster mushroom sausage had lower protein content than the Ambari study (2013) which stated that tempeh sausage made with the addition of 20% oyster mushrooms had a protein content of 14.67%. It is also known from the study that the more mushroom added, the higher the protein content. This is in accordance with the results of oyster mushrooms study in the treatment of making sausage in which the higher ratio of mushroom, the more the protein content will be. This protein content of tempeh – white oyster mushroom sausage meet national Indonesian standard (SNI) 01-3820-1995 which was over 13%.

#### **b. Fat Content**

The lowest fat content is found in the treatment of 50 gram tempeh and 100 gram oyster mushroom with the addition of 8 gram carrageenan of 9,642%, the highest fat content is found in the treatment of 100 gram tempeh and 50 gram white oyster mushroom with the addition of 4 grams carrageenan 14,971%. According to Suryati (2010) the fat content in oyster mushroom ranged from 1.08-9.4%, whereas according to Cahyana *et al.*, (1999) the fat contained in tempeh is 8.8 grams. The ingredients of sausage making that contribute to the fat content of sausages are carrageenan and the addition of vegetable oils. Fat content in oil is 15%.

According to Karyani (2013), carrageenan contains 1.60% fat content. Hudaya (2008), stated that the fat content of carrageenan is 0.13%, less than the fat content of tapioca flour which is 0.2%. The results of the study are smaller than Simanjuntak *et al.* (2016) which stated that in the treatment of mushroom:tempeh as much as 75:25, the fat content is 15.283%, and it is stated that the more tempeh added, the fat content would increase.

#### **c. Water Content**

Water in food will effect the damage to the food. Tabrani (1997) said that food damage is caused by chemical, microbiological,

enzymatic processes or a combination of them. All three processes require water. The lowest water content is found in the treatment of 100 grams tempeh and 50 grams of white oyster mushroom with the addition of 8 grams carrageenan which is 38.768%, the most water content is in the treatment of 50 grams of tempeh and 100 grams of white oyster mushroom with the addition of carrageenan 61.099%. The more oyster mushrooms, the higher the sausage water content. According to the analysis of Agro Industry Center / BBIA (2014) the oyster mushroom water content is 91.8%, and the tempeh water content is 43.30% (Bastian *et al.*, 2013).

#### **d. Crude Fiber Content**

The lowest crude fiber content is in the treatment of 100 grams of tempeh and 50 grams of white oyster mushroom with the addition of 4 grams of carrageenan which is as much as 5.435%, while the highest content of fiber content is in the treatment of 50 gram tempeh and 100 gram white oyster mushroom with the addition of carrageenan 8 gram which is as much as 6.787%. The more mushrooms will increase the fiber content. In addition, carrageenan also contributes to increase the fiber content in sausage. Hernawati *et al.*, (2013) also stated that the addition of carrageenan could increase the fiber content of a product. Larasati *et al.*, (2017), said that the addition of carrageenan had no significant effect ( $\alpha > 0.05$ ) on sausage fiber content. carrageenan fortification of 0% is 0.06%, carrageenan fortification of 2% is 0.12%. The fiber content obtained from the analysis of white oyster mushrooms sausage was in accordance with Ambari's *et al.* (2014) which stated that the Independent sample t-test result showed that the crude fiber content of selected sausage formula was significantly different ( $\alpha < 0.05$ ) with fiber content. This indicates that the addition of oyster mushrooms can significantly increase crude fiber levels in the final sausage product.

#### **e. Ash Content**

The ash content in a food product shows the amount of mineral content. The ash content of a food can reflect the quality of a food related to the presence of certain metal contaminants (Faridah *et al.*, 2006). The lowest ash content is in the treatment of 100 grams of tempeh and 50 grams of white mushroom with



**Rohmawati S et al.**

the addition of 4 grams of carrageenan which is equal to 1.778%. While the largest ash content is found in the treatment of 50 grams of tempeh and 100 grams of white oyster mushroom with the addition of 8 grams of carrageenan which is equal to 3.175%.

Besides, carrageenan also contains ash content, so the addition of carrageenan will affect the results of sausage ash content analysis. Karyani (2013) stated that the results of the average carrageenan ash content are 0.06%.

Table 1. Chemical properties of sausage

Ratio of Tempeh - Oyster Mushroom	Carrageenan (gram)	Chemical Test				
		Protein Content	Fat Content	Water Content	Crude Fiber Level	Ash Content
				(%)		
50:100	4	16,161 <sup>g</sup>	9,985 <sup>a</sup>	61,099 <sup>c</sup>	6,296 <sup>cd</sup>	2,624 <sup>abc</sup>
	6	16,187 <sup>g</sup>	9,860 <sup>a</sup>	60,312 <sup>c</sup>	6,414 <sup>d</sup>	2,835 <sup>bc</sup>
	8	17,707 <sup>h</sup>	9,642 <sup>a</sup>	57,476 <sup>bc</sup>	6,787 <sup>e</sup>	3,175 <sup>c</sup>
75:75	4	14,662 <sup>d</sup>	11,841 <sup>b</sup>	46,841 <sup>ab</sup>	5,769 <sup>b</sup>	2,316 <sup>abc</sup>
	6	14,877 <sup>e</sup>	11,223 <sup>ab</sup>	44,242 <sup>a</sup>	6,068 <sup>c</sup>	2,370 <sup>abc</sup>
	8	15,489 <sup>f</sup>	10,578 <sup>ab</sup>	44,118 <sup>a</sup>	6,210 <sup>cd</sup>	2,393 <sup>abc</sup>
100:50	4	13,138 <sup>a</sup>	14,971 <sup>c</sup>	40,322 <sup>a</sup>	5,435 <sup>a</sup>	1,778 <sup>a</sup>
	6	13,291 <sup>b</sup>	14,536 <sup>c</sup>	38,832 <sup>a</sup>	5,574 <sup>ab</sup>	1,956 <sup>ab</sup>
	8	13,524 <sup>c</sup>	13,714 <sup>c</sup>	38,768 <sup>a</sup>	5,620 <sup>ab</sup>	2,158 <sup>abc</sup>

Note: Value with different notation in the same column has a significant differences at 5% (Tukey test)

**Organoleptic Test**

The most preferred sausage for consumers is the treatment of 75 grams of tempeh and oyster mushrooms of 75 grams

with the addition of carrageenan 6 grams. The results of organoleptic sausage test is shown in Table 2.

Table 2. Organoleptic test results of sausage

M	R	Sensory test				
		Color	Tempeh Flavor	Mushroom Flavor	Texture	Total Preference
		(gram)				
100:50	4	2,620	2,127 <sup>a</sup>	2,153	1,107 <sup>a</sup>	1,903
	6	2,647	2,243 <sup>a</sup>	1,863	1,500 <sup>a</sup>	1,833
	8	2,743	2,377 <sup>ab</sup>	1,857	1,643 <sup>ab</sup>	1,580
75:75	4	2,833	2,443 <sup>ab</sup>	1,743	2,333 <sup>bc</sup>	2,057
	6	2,957	2,453 <sup>ab</sup>	1,743	2,553 <sup>c</sup>	2,453
	8	3,047	2,513 <sup>ab</sup>	1,657	2,707 <sup>cd</sup>	2,417
50:100	4	3,293	2,847 <sup>ab</sup>	1,517	2,910 <sup>cd</sup>	1,913
	6	3,507	3,097 <sup>ab</sup>	1,343	3,273 <sup>de</sup>	1,880
	8	3,560	3,467 <sup>b</sup>	1,223	3,853 <sup>e</sup>	1,823

Note:

M (Substitution of tempeh - white oyster mushroom)

R (Carrageenan)

1. Color: If the value is higher, the color is getting darker
2. Tempeh Flavor: If the value is higher, the flavor of tempeh is getting stronger
3. Mushroom Flavor: If the value is higher, the flavor of the mushroom is getting stronger
4. Texture: If the value is higher, the texture is getting thicker
5. Total Preference: If the score is higher, the panelists will like it more

Value with different notation in the same column has a significant differences at 5% (Tukey test)

The darkest brownish color which is 3,560 is found in the treatment of 100 grams of tempeh and 50 grams of white oyster mushroom with the addition of 8 grams of

**a. Color**

carrageenan. For the assessment of the brightest light brown color sausage of 2,620 is found in the treatment of the comparison of 50 grams of tempeh and 100 grams of white oyster mushroom with the addition of 2 grams of carrageenan. The difference in the ratio of tempeh and mushroom produces different color levels. The color formation of tempeh and white oyster mushrooms is caused by the process of caramelization and maillard reaction. Brownish color that appears is the reaction between carbohydrates and amino acids. During heating, the carboxyl group will react with amino group or peptide so that glycosylamine is formed. These components are polymerized to form dark colored components "melanoidin" which cause discoloration in the product, ie the product will become brownish (Larasati *et al.*, 2017). The addition of carrageenan is also one of the factors that causes the changing color to become brownish, so that if the addition of carrageenan increases, the color of the sausage will be darker.

#### **b. Tempeh Flavor**

The panelists' assessment of tempeh flavor ranged from 2.127 to 3.467. The sausage formulation which has a very strong tempeh flavor is the formulation with a comparison of 100 gram tempeh and 50 gram of white oyster mushroom which is 3.46 while the lowest tempeh flavor is at a comparison of 50 grams of tempeh and 100 grams of white oyster mushroom which is 2.217. The more tempeh added, the tempeh flavor will be stronger. Astuti (2009) stated that the typical aroma formation in tempeh is caused by the degradation of components in tempeh during the fermentation process.

#### **c. Oyster Mushroom Flavor**

The panelist's assessment of white oyster mushroom flavor ranged from 1.57-2.153. The lowest value of white oyster mushroom flavor is found in the treatment of 100 grams tempeh and 100 grams white oyster mushroom which is 1.57, the highest rating of white oyster mushroom flavor is found in the treatment of 50 grams of tempeh and 100 grams of white oyster mushroom which is as much as 2,153. The more oyster mushrooms added, the stronger the oyster mushroom flavor. The substitution of tempeh makes the flavor of the

white oyster mushroom in making sausage formulations diminish.

#### **d. Elasticity**

The texture value (elasticity) of the sausage ranges from 1,107-3,853. The lowest elasticity value is found in the treatment of 50 grams tempeh and white oyster mushroom 100 grams with the amount of carrageenan 2 grams which is as much as 1,107, while the most elastic sausage texture value is found in the sausage formulation by treating the comparison of 100 grams of tempeh and 50 grams white oyster mushroom with carrageenan 8 grams which is 3,853. The more carrageenan added, the thicker the sausage will be. The addition of tempeh will make the product solid, so that the formulation of more tempeh and a lot of carrageenan, will tend to make the sausage dense. The addition of carrageenan in large quantities results in the texture of the sausage being too hard and less liked by the panelists.

#### **e. Total Preference**

The level of panelist's preference ranged from 1,580-2,453. In Table 2, the lowest level of consumer preference is found in the treatment of a comparison of 50 grams of tempeh and 100 grams of white oyster mushroom with the addition of 8 grams of carrageenan which is 1.580, while the highest level of panelists preference for sausages is in the treatment of 75 grams of tempeh and 75 grams white oyster mushrooms with the addition of carrageenan 6 grams which is 2,453. Tempeh-white oyster mushroom Sausage formulation with the addition of carrageenan for the most preferred by panelists is the treatment of 75 grams of tempeh and oyster mushrooms of 75 grams with the addition of carrageenan 6 grams which is 2,453. On the addition 4 grams of carrageenan the preferred level is 2,057 and at the addition of 8 grams is as much as 2,417.

Panelists do not like the sausage formulation that contained too much mushroom. It may be because the white oyster mushroom has a distinctive and pungent odor. The smell of mildly fishy mushrooms is also a factor for panelists to dislike. However, in many tempeh substitutions, panelists also dislike. This is probably due to the smell and the texture of tempeh which become harder.

**Rohmawati S et al.**

The difference in the addition of carrageenan gives a different level of preference, since the addition of carrageenan which slightly causes the texture of the sausage to be soft and the addition of too much carrageenan will make the sausage texture harder.

## CONCLUSION

In accordance with the objectives of the study, which is to know the chemical characteristics, sensory properties, fiber-rich sausage formulations and preference by consumers, the best formulation is used in the treatment. The best result of Analogue sausage formulation of tempeh-white oyster mushrooms with the addition of carrageenan research is obtained from a combination of 75 grams of tempeh and 75 grams of white oyster mushroom and the addition of 6 grams of carrageenan. In these treatment, chemical characteristics are known; protein content 14.877%; fat content 11.223%; moisture content 44,242%; crude fiber content 6.068%; ash content 2,370%; total carbohydrate content 27.288%, while organoleptic analysis is known to be 2,957; flavor tempeh 2,453; mushroom flavor 1,743; texture (elasticity) 2,553; total preference is 2,453.

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## PHENOLIC COMPOUND OF FRESH AND BOILED SEA GRAPES (*CAULERPA SP.*) FROM TUAL, MALUKU

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### ABSTRACT

*Caulerpa* sp. commonly found in Tual, Southeast Maluku sea. *Caulerpa* sp. is generally consumed by coastal communities as raw food such as salads or processing food such as boiled *Caulerpa* sp. Nutrient information and total phenolic content of *Caulerpa* sp. during boiling has not been reported. The aim of this study is to determine bioactive components and total phenolic content from *Caulerpa* sp. as a result of the boiling process. The boiling process was carried out for 5 minutes at 90 °C. The boiling process causes flavonoids loss in *Caulerpa* sp. Total phenolic content after boiling process decreased by 11.76 mg GAE/g extract. Total phenolic of fresh *Caulerpa* sp higher than the boiled *Caulerpa* sp.

**Keywords:** *Caulerpa* sp., phytochemicals, boiling, total phenolic content

### INTRODUCTION

Maluku Province is geographically bordered by North Maluku province. In the east, it bordered by Southeast Sulawesi and Central Sulawesi, and in the south it bordered by East Timor and Australia. It is an archipelago province with an area of 712,479.65 km<sup>2</sup> consisting of 93.5% of the water area and 6.5% of the land area. The number of islands in Maluku reaches 1,340 islands. Tual City is a division of the Southeast Maluku district with an area of 19,088.29 km<sup>2</sup> with of 98.67% sea area and 1.33% land area. The main commodity in Tual city is seaweed (BKPM, 2012).

Seaweed production in Maluku region reached 6.3 million tons in 2013 which included seaweed from the *Caulerpa* genus (2015 MPA). The people inform that *Caulerpa* sp. is used for consumption. *Caulerpa* sp. is generally consumed by coastal communities as raw food such as salads or processing food such as boiled *Caulerpa* sp. The communities considers that boiling process can kill the

bacteria that might be found in *Caulerpa* sp., and it is done for five minutes.

Nurjanah *et al.* (2016) said that green seaweed *Caulerpa* sp could be as cosmetic material. Nurjanah *et al.* (2018) also stated that green seaweed *Caulerpa lentilifera* has high fiber content. *Caulerpa lentilifera* is potentially to produce low sodium salt and antioxidant (Nufus *et al.* 2017). Saputra *et al.* (2011) stated that seaweed *Caulerpa racemosa* is biogas material because it has high carbohydrate content. High and low content of carbohydrate affect biogas pressure, the higher the carbohydrate, the greater the biogas pressure was obtained. Adriani (2015) stated that *Caulerpa racemosa* contains flavonoid compounds that show antibacterial activity, because it produces endophytic fungi that can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria.

Phenolic compounds are a group of chemicals compounds in plants. The group of phenolic compounds has a role as antioxidant which can reduce the risk of heart disease,

blood vessels, and cancer. Many phenolic compounds are found in fruits, green vegetables, and nuts. Phenolic compounds act to protect body cells from free radical damage by binding to the free radicals so that they can prevent inflammation in the body cells (Winarti, 2010).

Phenolic compounds include various compounds from plants that have aromatic rings with one or two hydroxyl groups. Some groups of polymeric materials in plants such as lignin, melanin, and tannins are phenolic compounds. Phenolic is a aromatic compounds, so that it show strong uptake in visible spectrum regions (Harborne, 1987). Cho *et al.* (2010) stated that the total phenolic content of fresh seaweed *Caulerpa lentillifera* with methanol extract is 6.7 mg GAE/g extract. But, Maulida (2007) implies that the amount of total phenolic content is 8.95 mg GAE/g extract.

*Caulerpa sp* regards as sources of phenolic compounds. The total content of phenolic compounds *Caulerpa sp.* during the boiling process needs to be considered, because this process can affect the physical and chemical materials conditions. Analysis of phenolic content of boiled *Caulerpa sp.* have not been reported, so that an analysis is needed to determine the total phenolic content of *Caulerpa sp.* after the boiling process. This study aims to determine the bioactive components qualitatively and the total content of phenolic from fresh and boiled seaweed *Caulerpa sp.* in a temperature of 90°C for 5 minutes.

## **MATERIALS AND METHOD**

### **Materials and Tools**

The main ingredient was seaweed *Caulerpa sp.* The ingredients used for extraction were methanol and filter paper. The materials for proximate testing are hexane (Emsure) solvent, concentrated H<sub>2</sub>SO<sub>4</sub> (Emsure), aquades, NaOH (Emsure), H<sub>3</sub>BO<sub>3</sub> (Emsure), HCl (Emsure). Reagents for phytochemical analysis are sulfuric acid, dragendroff reagent, meyer, Wagner, chloroform (Emsure), anhydra acetate, concentrated sulfuric acid, magnesium, amyl alcohol, alcohol, ethanol, 5% FeCl<sub>3</sub>, 2N HCl (Emsure). The materials for analysis were Folin-Ciocalteau (p.a) reagents, gallic acid

(Yalong), ethanol (Emsure), aquades, and sodium carbonate (Yalong).

The sample preparation tools are knives, cutting boards, containers, and analytical scales. The proximate analysis tools are porcelain saucer, desiccator, oven, furnace, soxhlet tube, kjeldahl tube, flask, and erlenmeyer. The extraction tools are orbital shaker (WiseShake), rotary evaporator (Buchi Rot. R-205), and the phenolic analysis tools is Ultra Violet-Visible (UV-Vis) spectrophotometer (Hitachi U-2800).

### **Sample Preparation**

Samples were taken from Tual waters, Maluku. Part of the sample taken is all. Samples were taken fresh, then packaged using plastic bags, plus sea water and then put into a jar, sent from Maluku to the Bogor Agricultural Institute, Bogor using air transportation modes. Fresh samples last for one week after being taken from the waters. Arriving in Bogor, the samples were left at room temperature, then prepared. Fresh seaweed is cleaned of dirt that is still attached, then weighed. Samples were divided into two namely, *Caulerpa sp.* fresh and boiled. The boiling temperature and time used refers to Putera's research (2015), which is the best boiling treatment at 90 °C and the boiling time 5 minutes. Boiling uses mineral water, with a sample and water ratio of 1: 4 (w / v). The water is heated first until it reaches a temperature of 90 °C, then *Caulerpa sp.* put in boiling water and counted up to 5 minutes. The next step is proximate analysis, phytochemical analysis and total phenolic compounds. The flow chart of the research procedure can be seen in Figure 1.

### **Bioactive Compound Extraction**

The fresh *Caulerpa sp.* was mashed by mortar, then it is weighed. The boiled *Caulerpa sp.* was drained, mashed using a mortar, and weighed using scale. *Caulerpa sp.* was weighed as much as 100 g and put in the 500 mL of erlenmeyer. It is added 200 mL of methanol. The samples that have been submerged with methanol, then macerated by using an orbital shaker for 1x24 hours. The filtrate was then evaporated by using a rotary vacuum evaporator in 40 °C. The extraction was repeated three times.

**Phytochemical Analysis (Harbone, 1987)**

Phytochemical analysis was conducted to determine the presence or absence of bioactive components in fresh and boiled *Caulerpa sp.* which contains phenolic content. Phytochemical tests include alkaloid test, steroid / triterpenoid test, flavonoids, and phenolic hydroquinone.

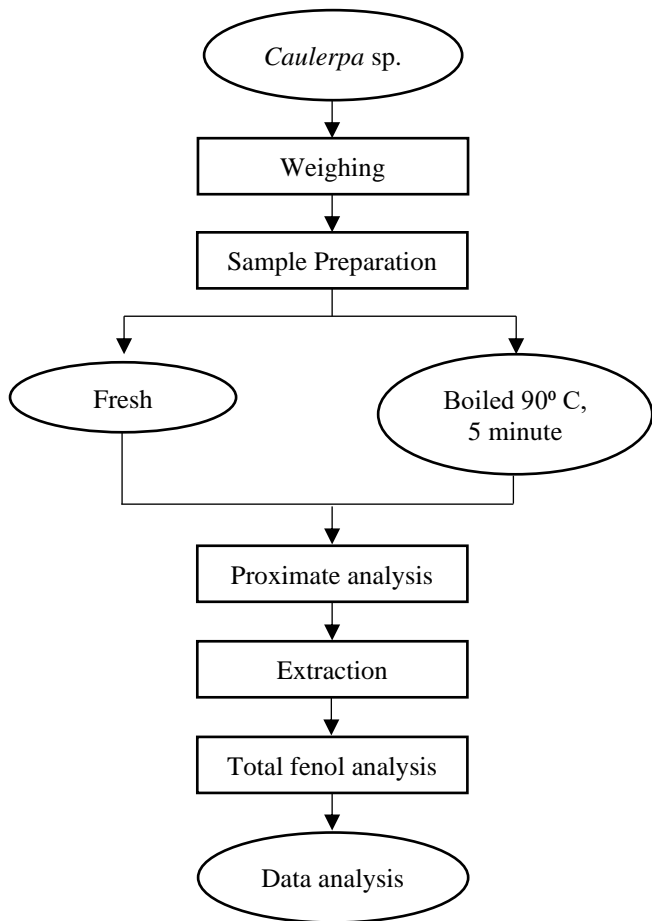


Figure 1. Flow chart

**Total Phenolic Content (Modified Andarwulan, 2000)**

Determination of the total phenolic content begins with making a blank solution and a gallic acid solution as a standard. A blank solution was made by piping 2 mL of 96% ethanol into 10 mL test tube. Gallic acid solution was made by preparing stock of solutions with a concentration of 100 ppm in 100 mL, 10 mg gallic acid dissolved in 50 mL 96% ethanol in a 60 mL extract bottle. The solution dilutes in a 10 mL test tube, with concentrations of 10, 30, 50, 70, and 100 ppm with a volume of 2 mL each.

The extract sample of *Caulerpa sp.* was weighed as much as 9.5 mg and dissolved with 2 mL 96% ethanol in a 10 mL test tube. Blank solution, standard solution or sample were

added 5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent 50% (v / v) and left it for 5 minutes. The solution was added 1 mL of sodium carbonate solution 5% (b / v) and homogenized and incubated in room temperature and in dark condition for 1 hour. Test the tubes before incubating them in aluminum foil to make the incubation effective in dark condition. The next stage after the incubation process was homogenized the solution then measured the total phenolic content using the Hitachi U-2008 UV-Vis spectrophotometer at a wavelength of 725 nm.

**Data Analysis**

Total phenol compounds were analyzed descriptively. Analysis of the data used for proximate values is a paired t test. This analysis aims to see whether there is an average difference from the *Caulerpa sp.* Sample. fresh and boiled against proximate values.

Hyphotesis:

H<sub>0</sub>: There is no difference in the proximate value of *Caulerpa sp.* fresh and boiled

H<sub>1</sub>: There is a difference in the proximate value of *Caulerpa sp.* fresh and boiled

Paired t test statistics are:

$$t = \frac{\bar{d}}{s_d/\sqrt{n}}$$

Noted :

$\bar{d}$  = the average difference between the value of fresh and boiled proximate

$s_d$  = standard deviation from the difference between fresh and boiled proximate values

n = the number of samples

**RESULT AND DISCUSSION**

**Morphology and Proximate of *Caulerpa sp.***

*Caulerpa sp.* is a green seaweed from the *Caulerpaceae* family in which the habitat attaches to the substrate of sand or rocks. *Thallus* in *Caulerpa sp.* forms roots and *ramuli*. *Ramuli* forms small dots which regularly close over ± 3-5 cm of branching. The *Caulerpa sp.* condition used is fresh, but some parts of the *ramuli* are damaged. Tampubolon *et al.* (2013) stated that *Caulerpa lentillifera* has *thallus* with round propagate branches and the branches are like wine branch. The morphology of *Caulerpa sp.* can be seen in Figure 2.





Figure 2. *Caulerpa sp.*

*Caulerpa sp.* is used by coastal communities as consumption so that it is necessary to know the chemical and nutrient content of *Caulerpa sp.* Chemical composition of fresh and boiled *Caulerpa sp.* are presented in Table 1.

Table 1. Chemical composition of *Caulerpa sp.*

Chemical Composition	Fresh	Boiled
	<i>Caulerpa sp.</i> Wet basic (%)	<i>Caulerpa sp.</i> Wet basic (%)
Water content	77,57±0,19	79,17±0,23
Ash content	1,18±0,15	1,02±0,01
Fat content	0,32 ± 0,01	0,37 ± 0,03
Protein content	3,84±0,04	3,63±0,06
Carbohydrate content by different	17,08±0,11	15,65±0,21

Water content in fresh *Caulerpa sp.* is  $77.57 \pm 0.19\%$  and boiled *Caulerpa sp.*  $79.17 \pm 0.23\%$ . The t-test analysis result showed there are differences in the water content of fresh and boiled *Caulerpa sp.* ( $\alpha \leq 0.05$ ). The difference of the water content between fresh and boiled *Caulerpa sp.* is due to the tissue in boiled *Caulerpa sp.* can absorb water, so the water content of boiled *Caulerpa sp.* has increased. Aisyah *et al.* (2014) stated that the changes in the water content after boiling process is due to network matrices in vegetables which tend to absorb water, so that the water content is relatively higher than fresh vegetables. Kumar *et al.* (2011) stated that the water content of *Caulerpa racemosa* obtained from Indian waters is 91.53% and Ma'ruf *et al.* (2013) stated that the *Caulerpa racemosa* water content from Jepara waters is 92.37%.

The result studies show that the water content of fresh *Caulerpa sp.* is  $77.57 \pm 0.19\%$ . The water content value is quite low compared to the previous studies. This difference is due to the lengthy of transportation process, and

the condition of the sample is slightly open, so that the water in the sample is evaporated.

The ash content of fresh and boiled *Caulerpa sp.* are  $1.18 \pm 0.15\%$  and  $1.02 \pm 0.01\%$  respectively. The t-test analysis results show that there are not any differences in the ash content of fresh and boiled *Caulerpa sp.* ( $\alpha > 0.05$ ). The boiling process affect the ash content in the material, because the minerals contained in *Caulerpa sp.* dissolve in boiling water. The boiling process also affect the ash content of the material, because of the influence of water vapor that comes out during the boiling process. Nurjanah *et al.* (2014a) states that the ash content of the genjer plant changes after steaming for 3 and 5 minutes. The minerals contained in the genjer plant emerge along with the discharge of water during steaming, so that the ash content of the genjer plants after steaming changes.

The fat content of fresh and boiled *Caulerpa sp.* are  $0.32 \pm 0.01\%$  and  $0.37 \pm 0.02\%$  respectively. The t-test analysis results show that there is no difference in the fat content of fresh and boiled *Caulerpa sp.* ( $\alpha > 0.05$ ). The fat content of *Caulerpa sp.* is low due to the high water content in *Caulerpa sp.*, so that the fat content decreases. Kumar *et al.* (2011) stated that the fat content of fresh *Caulerpa racemosa* from Indian waters is 2.64%. McDermid and Stuercke (2003) stated that the fat content of fresh *Caulerpa lentillifera* obtained from the Hawaiian coast is relatively high in 7.2%.

The fat content value result from this study has differences with the previous research. The difference is caused by different environmental conditions, which are generally the harvest season and habitat of the biota. Wong and Cheung (2000) stated that the fat content in seaweed is low. The low fat content is caused by a high enough water content, so that proportionally the fat content will decrease.

The protein content of fresh and boiled *Caulerpa sp.* are  $3.84 \pm 0.04\%$  and  $3.63 \pm 0.06\%$ , respectively. The results of the t-test analysis show that there are differences in fresh and boiled *Caulerpa sp.* protein content ( $\alpha \leq 0.05$ ). Boiling process affects the protein content which are due to the protein dissolves in the water that emerge from the material. Matanjun *et al.* (2009) stated that the fresh

**Nur Janah et al.**

*Caulerpa lentillifera* protein content are  $10.41 \pm 0.26\%$ . This difference is caused by differences in habitat from the material used. Ratana and Chirapart (2006) stated that the different protein content in seaweed is caused by differences in species, season, and geographical conditions, as well as the amino acid content.

The difference in protein content after boiling is due to the presence of volatile nitrogen in seaweed proteins, which will evaporate because of heating process (Febriyanti 2011). The boiling period and temperature play a role in changing the nutritional content of food. The higher of the temperature and the less of boiling period will reduce the food nutritional content (Widyati 2001). Megayana *et al.* (2012) stated that the nutrient content that exists in the environment has an influence on the chemical composition of organisms living.

Carbohydrate content (by difference) of fresh and boiled *Caulerpa sp.* are  $17.08 \pm 0.11\%$  and  $15.65 \pm 0.21\%$ , respectively. The t-test analysis results show that there are differences in carbohydrate content of fresh and boiled *Caulerpa sp.* ( $\alpha \leq 0.05$ ). The changes in carbohydrates content of boiled *Caulerpa sp.* is caused by the nature of these carbohydrates which can dissolve in water. Carbohydrates content generally have links with fiber in a material. Kumar *et al.* (2011) stated that the carbohydrate content of fresh *Caulerpa racemosa* from Indian waters is 4.5%. Ma'ruf *et al.* (2013) stated that the high fiber content is caused by high polysaccharides in seaweed cells, which function as dietary fiber and functional fiber.

#### **Phytochemicals from *Caulerpa sp.* Seaweed Extract**

The yield of extracted fresh *Caulerpa sp.* is as much as 8.03% and yield of boiled *Caulerpa sp.* is 6.36%. Bioactive components in *Caulerpa sp.* are mostly soluble in polar solvents. The amount of yield affects the solubility properties of bioactive components. Kusumawati *et al.* (2008) stated that yield is the percentage of raw material that can be used. The larger yield indicates that the raw material has a greater chance to be used compared to raw materials which have a low yield value.

Plants contain many phenol compounds. These phenolic content tend to dissolve in polar solvents (Harborne 1987).

The yield of extracts fresh *Caulerpa lentillifera* seaweed obtained from Teluk Betung, Lampung using methanol is 3.06% (Maulida 2007). This yields compare to the previous studies have far-reaching differences. This difference is expected because the method used, previous research using multilevel extraction methods and in this study using a single extraction.

Phytochemical analysis is carried out on fresh and boiled *Caulerpa sp.* to identify the chemical content as a first step in study the bioactive component. The compounds found in *Caulerpa sp.* tested qualitatively based on color changes or deposits formed in response to the given reagent. The presence of bioactive components *Caulerpa sp.* presented in Table 2.

Table 2. Chemical composition of *Caulerpa sp.*

Test	Seaweed <i>Caulerpa sp.</i>		Color change	
	Fresh	Boiled	Fresh	Boiled
Alkaloid				
Meyer	-	-	-	-
Wagner	-	-	-	-
Dragendroff	-	-	-	-
Steroid	+	+	Blue	Blue
Triterpenoid	-	-	-	-
Flavonoid	+	-	Yellow	-
Fenol hydroquinon	+	+	Bluish green	Green
Saponin	+	+	Foaming	Foaming
Tanin	-	-	-	-

Description:

(+) = Detected, (-) = Not detected

Phytochemical analysis showed positive results for the presence of steroid compounds in fresh and boiled *Caulerpa sp.* Steroid compounds are soluble in lipids and have potential as antibacterial compounds. The presence of steroid compounds in *Caulerpa sp.* according to Ahdyanti (2009) is fresh *Caulerpa racemosa* contains steroid and triterpenoid compounds.

Rosyidah *et al.* (2010) stated that steroid / triterpenoid compounds have activity as antibacterial compounds. Steroid / triterpenoid compounds can inhibit bacterial growth with an inhibitory mechanism for protein synthesis because it accumulates and causes changes in the constituent components of bacterial cells. Steroid / triterpenoid compounds are easily soluble in lipids, these

factors can cause steroid / triterpenoid compounds easily penetrate bacterial cell walls.

Fresh *Caulerpa sp.* shows positive results on flavonoid compounds and boiled *Caulerpa sp.* has no flavonoid compounds detected. The boiling process can eliminate flavonoids. It is because flavonoid compounds dissolve in boiling water or because of an oxidation process occurs. The results of Lusivera (2002) study suggest that the boiling process resulted in negative flavonoid compounds in the binahong leaves. The flavonoid content in the binahong leaves decreased by 78%.

The presence of sugar bound to flavonoids causes flavonoids to dissolve easily in water (Markham 1988). Flavonoids are compounds that show biochemical activity, namely antioxidants, antiviral, antibacterial, and anticancer. Flavonoids are a group of phenolic compounds with chemical structures C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> (Vermerris & Nicholson 2006). Sabir (2005) stated that flavonoid compounds able to inhibit bacterial growth. The inhibiting mechanism of these bacteria is the hydroxyl group found in the structure of flavonoid compounds causes changes in organic components and nutrient transport. Eventually, this lead to the emergence of toxic effects on bacteria.

Fresh and boiled *Caulerpa sp.* show positive results for phenol hydroquinone compounds. Phenolic compounds are found in plants. They are soluble in polar solvents. Phenolic compounds function as antioxidants. Nurjanah *et al.* (2014b) stated that water spinach plants contain phenolic compounds which are soluble in polar solvents. Phenolic compounds detected in water spinach have antioxidant activity.

Phenolic compounds tend to be soluble in water because these compounds bind to sugar as a glycoside and are usually found in cell vacuoles (Harbone 1987). Chen and Blumberg (2007) stated that phenolic compounds can reduce the risk of some chronic diseases because it characterize inflammatory, antioxidant, carcinogenic detoxification, and anti-cholesterol.

Fresh and boiled *Caulerpa sp.* show positive results on saponin compounds. Saponins generally dissolve in water and are

slightly soluble in polar solvents. Complete hydrolyzed saponins produce sugar and one non-sugar fraction. Nurjanah *et al.* (2014a) stated that fresh genjer plants contain saponins and reducing sugars. The boiling process does not change the saponin and sugar contents. Koche *et al.* (2010) stated that the primary part of plant metabolism includes reducing sugars, amino acids, proteins, and chlorophyl.

The bioactive components in the material have various benefits. Nurjanah *et al.* (2009) showed phytochemical component analysis of sea leech flour extract is alkaloid compounds, saponins, steroids and phenols which were play a role in reducing total cholesterol and low density lipoprotein (LDL). Ruiz *et al.* (2005) stated that saponins function as antimicrobial compounds, anti-inflammatory and have low toxicity.

Groups of phenolic compounds include phenolic acids, simple phenols, flavonoids, anthocyanins, lignin, and tannins (Vermerris and Nicholson 2006). The results of phytochemical analysis in fresh and boiled *Caulerpa sp.* indicate the presence of phenolic compounds. Fresh and boiled *Caulerpa sp.* are positive against flavonoids and phenol hydroquinone compounds.

### **Total Phenolic Compound**

Phenolic compounds are compounds that are widely found in plant species. They are consist of aromatic groups that absorb strongly in the spectrum of UV light. Reagent used to determine the presence of phenolic compounds in a material is Folin Ciocalteu (Harborne 1987). The total phenol analysis is conducted to determine the amount of phenol found in fresh and boiled *Caulerpa sp.* The total phenolic content of fresh *Caulerpa sp.* is  $28.56 \pm 1.97$  mg GAE / g extract and in boiled *Caulerpa sp.* is  $16.8 \pm 1.53$  mg GAE/g extract. The boiling process causes the total phenolic content of *Caulerpa sp.* decreases. It is because the total phenolic content of *Caulerpa sp.* is oxidized or dissolved in cooking water.

Aisyah *et al.* (2014) stated that the total phenolic content in vegetables after boiling process decreased by 13-16%. The boiling process will result in a total loss of phenolic compounds which can occur in two ways: dissolving in water during boiling and

*Nur Janah et al.*

oxidation. Cho *et al.* (2010) stated that the total phenolic content of fresh *Caulerpa lentillifera* with methanol extract is as much as 6.7 mg GAE / g extract and Matanjan *et al.* (2008) stated that the total phenolic content in *Caulerpa lentillifera* is 42.85 mg GAE/g extract.

There are high differences between the total phenolic content result from this study compared to the existing literature. This difference is due to environmental factors and differences in *Caulerpa sp.* habitat. Reyes and Luis (2003) stated that sunlight is one of stressors that can increase biosynthesis of phenolic compounds in plant tissues.

The total phenolic compounds are influenced by sunlight, habitat, harvesting time, herbivore presence, and plant health conditions (Fithriani 2009). Phenolic compounds can be oxidized in alkaline solutions or because of the enzyme polyphenol oxidase activity that forms orthosemiquinone radicals that are reactive and react with amino compounds (Pratt 1992).

Prior *et al.* (2005) stated that the Folin-Ciocalteu method provides an estimated total phenolic compound value of the total phenolic content analyzed. Phenolic compounds are bioactive components that function as antioxidants. Jimenez-Escrig *et al.* (2001) stated that phenolic compounds are medically known as antitumor, hypo-allergenic and anti-inflammatory compounds.

Phenolic curcumin compounds from turmeric and phenolic catechins from tea are protective against gastric and intestinal cancers (Arnelia 2006). The role of phenolic compounds in the fisheries is to improve the characteristics of catfish surimi gel. The phenolic compound is obtained from dried tea leaf extract (Wijayanti 2012). Andayani *et al.* (2008) stated that research in plants reported that many plants contained large amounts of antioxidants. The effects of antioxidant was caused by the presence of phenolic compounds such as flavonoids and phenolic acids. Compounds that have antioxidant activity are phenolic compounds which have hydroxy groups substituted in ortho position and para -OH and -OR groups.

## CONCLUSION

The water content after boiling process has increased by 1.6%, while the protein

content and the carbohydrate content after boiling process have decreased by 0.21% and 1.43% respectively. Bioactive components found in boiled *Caulerpa sp.* are steroids, phenol hydroquinone, and saponins. The boiling process with a temperature of 90°C for 5 minutes causes the loss of flavonoids which is one of the groups of phenolic compounds. Total phenolic compounds during the boiling process decrease as much as 11.76 mg GAE / g extract.

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## BIODEGRADABILITY OF NANOCOMPOSITE MADE FROM PVA, ZnO NANOPARTICLES AND STEARIC ACID

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### ABSTRACT

Nanocomposite film has been developed from PVA polymer added with ZnO nanoparticle and stearic acid to enhance its performance. Preparation of nanocomposite film was using the optimum film formulation with ZnO nanoparticle and Stearic acid composition 3.4% (w/w PVA) and 6.6% (w/w PVA), respectively. The biodegradability of the resulting film was evaluate using soil burial test and statistically analyze using t-test. The result show that the rate degradation of optimum nanocomposite film (1.3785 %/day) was not significantly difference ( $p>0.05$ ) compare with control (1.4885 %/day).

**Keywords:** Nanocomposite, PVA, ZnO nanoparticles, stearic acid, biodegradable

### INTRODUCTION

Packaging play an important role for obtaining food products with attractive appearance as well as to protect products during storage and distribution. Plastic packaging, that widely used at this time, is contributor to the problem of instability in environmental ecosystems, because it is non-biodegradable. Based on data from the Ministry of Environment, each individual produces around 0.8 kilograms of garbage per day (15% in the form of plastic waste) (Gusmayanti, 2010). Nanocomposite film appear as one of the environmentally friendly packaging alternatives and expected to reduce the amount of use of non-biodegradable packaging.

One of many biopolymers that has been studied intensively as nanocomposite packaging is PVA (polyvinyl alcohol) because it can form films well, water soluble, easy to process, non-toxic, biocompatible and biodegradable (Chandrakala et al, 2012). On contrary, PVA has poor barrier properties against water vapor, so it needs to be combined with fillers that can improve the characteristics

of produced film. An alternative of nano-sized fillers that potentially improve the barrier properties of PVA film is ZnO nanoparticles (ZnO NPs). ZnO NPs have large surface area to volume ratio and antimicrobial properties, so it can used as active packaging (Esmailzadeh et al, 2016), increase surface reactivity, thermal, mechanical, stable to heat and recognized as safe by FDA (Sharon et al, 2010).

Another alternative to overcome the low retention of PVA to moisture is by adding fatty acids. Stearic acid (SA) is chosen because of its abundant availability, affordable price, and does not easily interact chemically with other elements. There have been many studies that have developed film making with the addition of fatty acids including corn starch (Jimenez et al, 2011) , soybean protein concentrates (Caba et al, 2012), starch (Nobrega et al, 2012; Schmidt et al, 2013), cassava starch (Chiumarelli et al, 2014), soybean protein isolates (Wang et al, 2014), *Lepidium perfoliatum* seed gum (Seyedi et al, 2015).



This study aims to prepare the optimum formulation of PVA-based nanocomposite films with the addition of ZnO nanoparticles and stearic fatty acids as well as evaluate to biodegradability using soil burial test.

## MATERIALS AND METHODS

### Tools and Materials

The equipment that are used including hot plate stirrer, Memmert oven and dessicator, digital ultraturax IKA T-25, Pyrex glassware, teflon, petri dish, digital scales and other supporting tools.

The main materials used in this study were 17K type commercial PVA (polyvinyl alcohol), inorganic zinc oxide (ZnO) nanoparticle, and stearic acid, aquades, and other chemical such as KCl was laboratory grade.

### Methods

#### Preparation of Nanocomposite Film

Nanocomposite films was prepared using casting solvent method according to Chandrakala et al. (2013) with slight modification. A 5 grams of polyvinyl alcohol was dissolved in 95 ml of aquades and homogenized using hot plate stirrer at 120°C for 30 minutes. Subsequently, the PVA solution was mixed with 3.4% ( $w/w$  PVA) ZnO NPs and 6.6% ( $w/w$  PVA) stearic acid, and tween 80 which had been melted first at the temperature of 70-80°C. Then, the completed solution was poured into teflon crucible and dried in a vacuum oven at 40-45°C for 3 hours. The dried film was packed with aluminum foil and stored on the desiccator (RH 75%, saturated  $KCl_{(aq)}$ ) prior to analysis.

#### Biodegradability Analysis (Azahari, 2012)

A nanocomposite films was buried in soil with a depth of 3 cm from the ground. The soil is placed in a pot and stored outdoors to get the actual conditions in the environment. Calculation of degradation is determined every time interval (7 days) for 42 days.

#### Statistic Analysis

The statistical analysis used in this study is t-test performed by SPSS version 22.0 software to compare the differences between control and nanocomposite film.

## RESULTS AND DISCUSSION

Biodegradable plastic is plastic used as a conventional one, but will be broken down

by the activity of microorganisms become water and carbon dioxide after being used up and disposed of into the environment. Biodegradable plastics are polymers that can change into biomass, H<sub>2</sub>O, CO<sub>2</sub> and or CH<sub>4</sub> through stages of depolymerization and mineralization. Depolymerization occurs because of extracellular enzymes work (consisting of endo and ekso enzymes). Endo enzymes break the internal bonds in the main polymer chain randomly, and the enzyme ekso breaks the monomer units in the main chain in sequence. The parts of the oligomer formed are transferred into the cell to be mineralized. The mineralization process formes CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>, water, salts, minerals and biomass. The definition of biodegradable polymers and the final products formed can vary depending on polymers, organisms, and the environment (Anonymous, 2005).

To determine the biodegradability of bioplastics made, soil burial test is carried out with the aim to determine the rate of degradation samples with some variations so that it would be predictable how long the samples would decompose by microorganisms in the soil. This method is done by burying the sample in the soil controlled by physical and chemical properties then calculating the residual weight fraction of the sample in each time unit (gram / 7 days).

Tests is carried out on two samples, which are the optimum sample with 4 replications and controls. Sample mass reduction was weighed every seven days for 42 days. The sample was weighed every after removed from the soil to weigh it in a dry state. The average results are then plotted weight residue (%) againts time (days) of nanocomposite films. The results as shown in Figure 1.

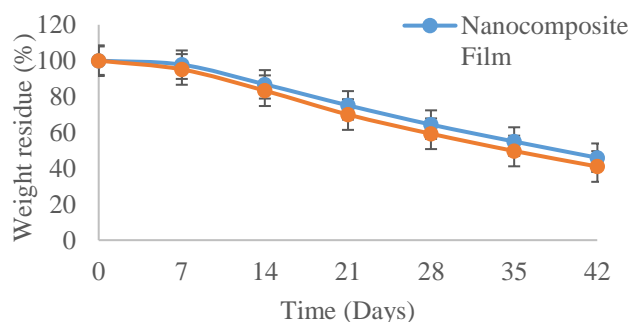


Figure 1. Biodegradability of PVA nanocomposite films, ZnO nanoparticles and stearic acid

Table 1. Rate of degradation of sample

Sample	Rate of degradation	t <sub>50</sub> (50% weight residue)
Control	1.4885 %/day	35 day
Nanocomposite film	1.3785 %/day	39 day

Based on Figure 2, it can be seen that degradation of control films is faster than



Day 1



Day 42



Day 1



Day 42

Figure 2. Biodegradability of nanocomposite films

Degradation occurs in two stages in the grave test in the soil: (a) water diffusion into film samples which results in swelling of the film that allows microorganisms to live in the film, (b) enzymatic degradation and other secretions causing severe decrease and damage to film samples. PVA have hydrophilic properties so that its solubility can also be another factor that causes decreasing in film weight (Guohua et al, 2006). Moreover, the addition of ZnO nanoparticles is not influence the biodegradability of nanocomposite film. This result might be the same as the addition of nano SiO<sub>2</sub> in PVA / starch-based films, which did not prove any significant effect on the film biodegradation. In addition, nano SiO<sub>2</sub> makes miscibility and compatibility increase and form a solid matrix structure, which in time will reduce the speed of infiltration of microorganisms. With increasing degradation time, the compactness of the film will be destroyed (Tang et al, 2008). On contrary, Hejri et al. (2013) reported that the addition of TiO<sub>2</sub> in the starch/PVA film formed many big

nanocomposite films, although the degradation value is not different at last day. The rate of degradation of the control film was -1.4885 %/day and 1.3785 %/day for the nanocomposite film. The control film need less day to gain 50% weight residue, while the nanocomposite was 4 days longer to half-part decomposed. However, this result was not significantly different (p>0.05).

cavities in the film surface observed by SEM. After the soil burial degradation, it was expected these holes become bigger and damage the structure of the films.

## CONCLUSION

The PVA-based nanocomposite was prepared using optimum formula with the addition of 3.4% (w/w PVA) ZnO nanoparticles and 6.6% (w/w PVA) stearic acid. The rate of degradation of nanocomposite and control film were -1.3785 %/day and -1.4885 %/day, respectively. This results was not significantly different (p>0.05).

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*Nur Janah et al.*

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# THE EVALUATION OF GOOD MANUFACTURING PRACTICES (GMP) IN FISH PROCESSING SME CENTER CASE STUDY OF BALIKPAPAN CITY

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## ABSTRACT

The application of Good Manufacturing Practices (GMP) is a parameter towards Hazard Analytical Critical Control Points (HACCP). The Center for Small and Medium Enterprise (SME) X in Balikpapan needs to evaluate its production process to improve the fisheries processing industry that fits the GMP criteria. This study aims to evaluate the application of GMP in fish processing SME Center. GMP evaluation carries out by looking at the production and building processes through interview and documentation. The evaluation result shows that the facilities and infrastructure of SME center need to be improved to meet the implementation of good GMP, since it has many critical and major deviations that need to be enhanced in terms of buildings and production processes. Based on the observations, it shows that there are 20 minor findings, 30 major findings, 28 serious findings, and 30 critical findings that are found. Deviations in the SME center is categorized as D grade, so they are not feasible for a production process.

**Keywords:** Fish processing, fish product, food safety, SME

## INTRODUCTION

Fish processing SME center in Balikpapan City experiences increasing development. The processed fish industry is a major factor in improving the economy of Balikpapan city. The development of SME center needs to be done to standardize the process of SME fish processing in order to maintain the quality and quantity. One way to improve the value of SME products is the quality practices. One important and fundamental quality practice is the application of Good Manufacturing Practice (GMP).

Good manufacturing practices (GMP) are the minimum sanitation and processing requirements needed to ensure the production of safe and healthy food. GMP is one of the basic pre-requisite programs or basic requirement program in implementing the HACCP system, which ensures the practice of preventing contamination that causes unsafe

product. GMP is divided into several sub-sections, each of which has detailed requirements and is related to activities at food processing facilities. These GMP subdivisions are personnel, building, distribution, and measurement of product defects (Katsuyama and Jantschke, 1999). The revised GMP in 1986 was officially announced by the FDA to meet the criteria set by the Federal Food, Drug and Cosmetic Act (FD & C Act) to achieve contamination-free food (Katsuyama and Jantschke, 1999).

GMP is not a new quality system known in Indonesia, as the Ministry of Health of Republic of Indonesia has introduced it since 1978 through the Minister of RI Decree No. 23/MenKes/SK/1978 dated January 24th, 1978 concerning Guidelines for Good Production Methods for Food (CPMB). Guidelines for implementing Good Food Production Methods (CPMB) explain the

procedures for producing food to be qualified, safe and suitable for consumption (Dirjen POM, 1999). GMP covers all basic principles and important requirements to produce food. This study purposes to evaluate the application of GMP in fish processing SME X center.

**MATERIALS AND METHODS**

This study was conducted at the SME X Center in Balikpapan City with the focus of industries are the fish processing (amplang and crab chips) and fruit processing (dragon fruit) industries. The data and information obtained from direct observations. Conformity assessment of GMP application is carried out on 13 elements, involving: location, building, sanitation facilities, production equipment, materials, processing, final products, laboratories, employees, packaging, labeling, storage, and maintenance. From each of these elements an assessment is carried out relating to the standard parameters set.

**Research Procedure**

This research method is data retrieval and chemical testing. The methods of collecting data are done by interview and direct observation referring to the regulation of the Ministry of Marine Affairs, namely the decision of the Minister of Marine and Fisheries No. Kep. 01 / MEN / 2007. Analysis of irregularities is carried out based on the classification of deviations by the Directorate General of P2HP 2007.

Technical constraints in the application of basic feasibility requirements result in incompatibility with existing regulations or deviations presented in Table 1. Preparation of deviations classification as follows (DG P2HP 2007):

1. Minor deviations, is deviation in which if it has no corrective action taken will affect food quality
2. Major deviations, is deviation in which if it is not corrected will cause potential affect to food security
3. Serious deviations, is deviation in which if it is not corrected will affect food security
4. Critical deviations, is deviation in which if it is not corrected will immediately affect food security

Rating	Value criteria			
	Total Deviation			
	Minor	Major	Serious	Critical
Grade A (Very good)	0 – 6	0 – 5	0	0
Grade B (Good)	≥ 7	6 – 10	1 – 2	0
Grade C (Enough)	NA	≥ 11	3 – 4	0
Grade D (Not Qualified)	NA	NA	≥ 5	≥ 1

Table 1. Value criteria of feasibility

**RESULTS AND DISCUSSION**

**Evaluation of GMP Implementation in Balikpapan SIKHPK**

GMP is a guideline for the food industry regarding how to produce good food and drinks. GMP is a popular guideline of most countries in the world, especially for industries in Indonesia through the decision of the Minister of Health No. 23/MenKes/SK/1978. This GMP of Minister of Health's decision includes: factory location and environment, buildings and rooms, final product quality, production equipment, raw materials, employee hygiene, processing control, sanitation facilities, labeling, packaging containers, storage, maintenance and sanitation programs, transportation, documentation/recording, product withdrawal, and laboratory and inspection.

The results of observation shows that the X fishery SME center building does not meet GMP standards by means of 20 minor findings, 30 major findings, 28 serious findings, and 30 critical findings. Architectural design layout is a critical deviation because it is not in accordance with KEP.01 / MEN / 2007 regulations, CHAPTER V. B, 2 points 1.3 and 1.4 which indicate that the SME area has to be clean, separated from the dull area and can prevent contamination. The SME area in X industrial center has not been separated from the dirty space. The storage section of the laundry is a pile of rubbish and unused items.

Reception space is a critical deviation as it is not in accordance with KEP.01/MEN/2007 Regulations, CHAPTER V. B, 3 which shows that the regulation that reception rooms



*Taufik Hidayat et al.*

must be clean and easily repaired, floors and walls made of material that is easy to clean. SME X does not have its own reception space.

Non-protected lamps considered as critical deviation because if it has no corrective action taken, it immediately affects food security. Water supply in SME X is not in accordance with the CAC / RCP 52-2003 guidelines, that is the availability of drinkable quality water.

### **Location and Environment of the Factory**

A good and healthy food industry should be located in a free of pollution area. The location of Balikpapan SME center is far from densely populated settlements and polluted factories. The area is free of puddles and the road to get to the location is good, however the construction of houses which is in the form of stilt houses is risky and easily get flooding while heavy rain. Besides, the buildings that are made on swampy land has a risk of landslides. In order to defense the building from flooding, a good drainage system is needed. The central location has a very small drainage system and the buffer is easily corroded so that a wider water channel should be made to facilitate running water in order to decrease congestion that cause flooding.



Figure 1. Location of SME



Figure 2. The appearance of Balikpapan SME

There are still many empty locations that will be used for constructing the next stage of production houses. This can be seen in Figure 1. The appearance of SME center buildings is shown in Figure 2, the appearance of SME center buildings built on swamps is shown in Figure 3, and the appearance of processed fisheries and agricultural production house is shown in Figure 4.



Figure 3. The appearance of SME center buildings on swamps area



Figure 4. House of processed fisheries and agricultural production in Balikpapan

### **Buildings and Processing Rooms**

In general, the factory layout is in accordance with the process sequence, but there are several processes carried out in the same space. Placement of processing equipment looks unorganized. There is a buildup of items in the processing room. The processing room must also be designed in such a way as to facilitate the cleaning and maintenance process. The angle of meeting between the walls, and the wall with the floor is in the elbow shape instead of curving, making it difficult to clean. The walls are not tiled yet so they easily dirty. The production room floor is made of cement tiles which are very difficult to clean and to drain water, and else there is a drainage hole that can cause slippery when production process involves wet raw materials. The absence of partition between production spaces, and the incompatibility of buildings with production



sequences and similarity of facilities has failed to meet the GMP feasibility element.

The ceiling construction is made of durable and brightly colored internal material. The ceiling has a height of 3 meters from the floor. The building has a wooden door and some rooms left untidy. The windows have ventilation for air circulation. Hence the air circulation in the frying chamber is not yet visible. The heat of air temperature causes the cooking room door to open during the production process. This should not be done since it increases the chance of contamination during the production process. The room should be equipped with an air circulation regulator.

Lighting uses a bulb lamp that has no buffer to support. The lamp is used only in the packaging room and the intensity is not enough to illuminate during the process. The blower used is very similar to the shape of a fan. This is not in accordance with production house standards and should be replaced with a room blower that has higher in speed because the production process uses high temperatures as Heating Ventilating Air Conditioning (HVAC). For building doors, it is also seen that there is only one door as the entrance and exit gate, so the additional one needs to be added so that it does not depend on one door only.

Drying places need to be made in the form of rack. The thing that also needs to be considered in the building is the manufacture of Waste Water Treatment Plant (WWTP) channels. WWTP for the fish and fruit processing industry must be differentiated so that the handling of sanitation and hygiene will be easier. The conditions found in production houses can be seen in Figure 5 to Figure 6, Figure 7, and Figure 8.



Figure 5. Ceiling and lighting



Figure 6. Ventilation and window of SME center



Figure 7. Blower



Figure 8. Floor

### **Sanitation Facility**

Water source comes from well water. The use of water for processing and sanitation or cleaning is not differentiated. Well water is directly used for processing and has not been tested for its quality. According to Yunita and Dwipanti (2010), if turbid water used and there is absence of regular water checks can result in cross contamination of food products.

The location of toilet is outside the production room. There is one toilet that can be used for all total 6 employees. Sanitation of the building has been equipped with a sink to wash hands in the processing room, but hand washing soap, towels or other means are not provided to dry hands and covered trash cans. Shoe rinse (foot dipping) is provided. The dressing room is separate from the production room but is integrated with the storage space for raw materials (flavor, packaging, etc.). According to Anggraeni *et al* (2019), the provision of sanitation facilities that have not been maximal, can cause cross contamination of products so that the hygiene is not maintained.

The central processing unit does not yet have a liquid waste processing facility. Therefore it is necessary to build an IPAL of at least 3. According to Yunita (2008), processed wastewater is usually treated first, in which the waste is flowed directly into the septic tank and then sucked into the pump and into a one meter depth reservoir coral, gravel, palm fiber, sand, gravel with a thickness of 20 cm each and the upper part planted with plants that can bind poisons, i.e. walini plants. These plants function to bind poisons, and the layers that are formed serve to filter out impurities. Filtering causes the water that comes out of the reservoir to be clear and harmless, then it directly flows into the river. Solid waste is not processed but is immediately disposed of to landfill (final disposal site) or burned. Sanitation facilities in production houses such as water tanks, and sink are shown in Figure 9 and Figure 10.



Figure 9. Water tank



Figure 10. Sink

### **Production Equipment**

The equipment in the packing room should consist of a freezer and sealer, then equipment as a container for the product that must be maintained from hygiene. All equipment is washed immediately after being used by clean water and soap (detergent). Hot water is used for the final rinsing after washing the equipment. The process of cleaning or washing processing facilities including equipment is a routine process that is very important to ensure the quality and safety of food products produced by a food producer.

At the SME center, packaging room is separated with freezer. Freezers in SME are only used for storage of raw materials. SME of fish processing does not provide freezers for storage of fish crackers, because the fish products that is produced are dry. However, SME that process fruit need to provide a freezer as storage product because the products produced are wet (brownies, fruit chili, syrup). The space for the packaging process still looks empty therefore the need for production room must be completed (Figure 11).



Figure 11. Packaging room

In addition, production equipment should be made of strong materials, durable, non-toxic, easy to move or disassemble so that they are easy to clean and maintain and facilitate monitoring and control of pests (National Agency of Drugs and Food Control, 2012). Cleaning equipment is absolutely done as a form of treatment. This condition is related to the remnants of the previous production material which contains microbes to shorten the shelf life of the product, thereby reducing the quality of the product from the next production process. The main machines and equipment needed in the manufacture of products are: scales, pulper (fruit crushers), screener (filter), homogenizer (mixing material), pasteurizer, bottle sterilizer (bottle sterilizer), and freezer (refrigerator). Supporting equipments needed are: plastic containers (containers), buckets, stainless pans, knives, and other kitchen utensils needed in the preparation process of the material (especially washing and stripping).

### **Employee Health and Hygiene**

The cloth must be changed every day to prevent contamination to the product. Employees working in the processing center unit are in a healthy condition, but no periodic checks are carried out. This greatly affects the health of employees processing. Employees who are sick do not handle the production, and

there is no recording of employee health. Some hygiene practices that have not been carried out by employees in the processing unit include the use of work clothes when carrying out production, namely laboratory suits and masks, and special slippers worn in the packaging room (sterile room) during the process of filling products into packaging containers and the clothes are not used when out of the packaging room. Employees have used masks during the packaging process, washed their hands after doing a job or process, did not eat, spit, sneeze, and did not smoke while doing production.

### **Storage**

The storage space for raw materials and final products needs to be separated. The storage space for raw materials also differentiates between wet raw materials and dry raw materials. The storage system is first in first out (FIFO), both for storing raw materials, and storing end products.

Raw materials must be stored in a warehouse in clean room conditions, smooth air circulation, and not in direct contact with floors, walls and ceilings. Food additives must be stored on a shelf in the processing room. Wet raw material that is easily damaged is immediately stored in the freezer in the processing room. Specific records of storage (using a card system that contains; name of material, date of receipt, origin of material, number of receipts, date of warehouse exit, final remaining packaging, date of inspection, and inspection results) have not been carried out. SME only makes a simple record of the number of entering and leaving items.

The raw material stored in the production room is the raw material used only for 3 production times. The final product is stored separately from the processing room. The condition of the storage room is quite clean and there is a record of products entering and leaving.

The warehouses in processing industry should be divided into two, which are: material warehouse and product warehouse. Meanwhile the raw material warehouse needs to be divided into wet and dry warehouses. Ideally, the materials sent by suppliers are stored in material warehouse and the products produced are stored in the finished goods warehouse that

has been provided by the SME Center management according to the warehouse standard. Based on observation, the raw materials and finished products are stored in each SME warehouse.

In addition, warehouse conditions will greatly determine the quality of the product produced. In materials, warehouse conditions with extreme temperature changes will cause damage to the material. This condition will be very detrimental to business actors. According to Purnomo and Adiono (1987) in Handajani (1996), the conditions of storage temperature affect the total number of microbes because temperature affects the metabolism and growth of microorganisms. While low air humidity can accelerate the transpiration process so that it can cause considerable weight shrinkage during storage process. Similarly, the product storage room, temperature and humidity must be kept as optimal as possible to maintain product shelf life.

### **Quality of Final Products**

Organoleptic examination is carried out on the product before being sent to market. Chemical, physical, and microbiological examination of products must be done in the laboratory, as well as in a planned (periodic) manner. The organoleptic inspection procedure is carried out in a sampling manner for each product that is ready to be marketed, then the product is stored in the laboratory. Storage of this product is done to find out how the condition of the product that has been circulating in the market.

### **Laboratory and Examination**

The product processing unit does not have a special laboratory for testing the end products. SME Center in Balikpapan City has to have a laboratory (currently there is no testing laboratory). The final product inspection is carried out periodically in the laboratory so that the products quality entered in the market is maintained.

### **Packaging**

The type of packaging used is polypropylene-based packaging which is lightweight, easy to form, large tensile strength, not easily torn and also food grade. Packaging is supplied from the Surabaya area.

### **Product Description or Labelling**

The information stated on the label consists of product name (trademark), product expiration date, producer name, product composition, product net weight, PIRT number and halal certification logo from MUI (Indonesian Ulama Council). However, all of these products are not uniform, all are listed, some are not halal labels, some have number of P-IRT number, and some only include trademarks and manufacturers. In the future, all the skills in the integrated center in Balikpapan can be uniform in terms of labeling on the packaging. Suggestions for improvement from the author for labeling are to include the number of nutritional adequacy (AKG) and the inclusion of SNI so that SME products can enter in the modern retail and supermarkets.

### **Transportation**

Product distribution tools must be available. Distribution equipment used is a four-wheeled vehicle, such as a private car to guarantee the product remains good and distribution process can be maintained. Shocks when distributing make the products in cool boxes vulnerable to damage. The vehicle cooling system (AC) must be turned on frequently and the hygiene of the car is maintained. Car cleaning management must be regulated by SME and submitted to each employee who distributes the product.

### **Management and Supervision**

Existing buildings must be well maintained, especially in the production room. Transportation equipment used for distribution requires periodic maintenance. The inside of production room should be needy. Monitoring of the effectiveness of the sanitation process activities both employee sanitation, tools, and space is very important.

### **Preparation of Material (Pre-Process)**

In order to produce safe and quality products, the process of preparing materials for both raw and auxiliary materials is an absolute quality control procedure. The raw material of fish and fruit received must be off from contamination, both in physical, chemical and microbiological. Raw materials can be the main source to endanger the safety of the products produced. The condition of raw

materials that is not suitable for consumption will produce inappropriate products even though they have been processed. Supervision of auxiliary material especially food additives is also mandatory. The use of food additives must be chosen from the safest type for consumption. It is recommend that in the process of storing each type of material is labeled or coded to avoid the occurrence of errors in its use. Chemical additives are placed in special storage cabinets to make them safer.

In general, it can be said that the stages of material preparation have fulfilled most of the stipulated requirements despite the fact that there are still many shortcomings. Raw materials received by processing unit do not fully meet the quality standards set. Fish supplied to SME are the fresh one that has been cleaned. SME only accept clean fish. However, fish supply is not sustainable due to the scarcity of raw materials. This blocked out the production process. The raw material for the fruit is also seasonal, as so every season changes, SME produces processed products with different fruits. Based on observation, the stored fish and fruit are not labeled, so there is no first in first out system in this pre-process.

### **Production Process of Fisheries and Agriculture**

The production process generally includes 3 groups of activity components, involving preparation and handling of materials (including raw materials and auxiliary materials), processing, and packaging up to storage (National Agency of Drugs and Food Control, 2012). The sorting process (sorting of fish and fruit) has been carried out in order to separate the rotten or damaged material to be spared of contamination against other high-quality materials. The fruit sorting process has to pay attention to the fruit maturity level, while the fish sorting process needs more attention to its freshness which can be seen from the organoleptic results. This is related to the quality and yield produced. Field observation shows that fish and fruit have been handled well.

According to Thaheer (2005) the production process and the health and hygiene of workers involved in the processing process must be considered. This becomes very essential to ensure the safety of the products

produced and prevent the spread of disease through foods. According to the P2HP Directorate (2010), there are three groups of people who are not allowed to be involved in the food processing process, which are: sufferers of respiratory infections, people with digestive infections, and sufferers of skin infections. These three types of diseases can be transferred to other people through food that is processed or served by the sufferers. The hygiene of workers must be considered to prevent contamination of food products handled. Therefore, workers must follow adequate sanitation procedures, including: washing hands before work, wearing an effective hair cover, wearing a mask covering the mouth and nose, may not smoking, eating and drinking around the processing area. These regulations have been stipulated in the SME center, yet there are some workers who are lack of discipline in their implementation.

### **Enviromental Conservation**

The efforts to implement green factory have also been done in surrounding. The by-product of fish and fruit processing is used by several people as flour and feed. For the fruits, farmers can use its by-product as providers of rootstock seeds. Skin and pulp have been used as animal feed. Environmental conservation can be endeavored also by the use of ultrafiltration membranes for purifiers and transforming brackish water into drinkable water as an effort to reduce the use of groundwater that exceeds the limit.

### **CONCLUSION**

SME Center still needs a lot of improvements in terms of buildings and production processes since it has D grade and not feasible for an industry. Buildings that are far off from flooding and pollution need to be considered. Clean water and waste management must also be noticed while the production process of GMP training for employees of SME center X Industry needs to be done to gain quality products.

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## HAZARD ANALYSIS AND CRITICAL CONTROL POINTS IMPLEMENTATION IN AMPLANG PROCESSING

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### ABSTRACT

Amplang is a products produced from fish in the form of crackers. Amplang is produced by SMEs (Small and Medium Enterprises) in Balikpapan. The quality standards of processing amplang in SMEs can be improved analyzed hazard and critical control point in the amplang processing. The research method is survey research with data collection conducted by interviews, field observations, and FGDs. Based on observations, the SME's quality control team needs to be determined. Food hazards are divided into raw material and production process hazards. There are four processes that consider as Critical Control Point that are thawing, frying, draining, and packaging.

**Keywords:** Amplang, HACCP, quality, SME

### INTRODUCTION

Amplang is a products produced from fish as raw material in the form of cracker. This food is from Kalimantan. This product can support tourism in Kalimantan. Various types of materials can be used as raw materials such as fish and crabs. Generally, amplang is processed by SMEs in cities of Kalimantan. This SMEs practically did not implement the GMP yet, thus SME still has not paid attention to food quality and food safety. SMEs didn't implement the correct method of production so that the products have different quality each production. There are still 1% of SMEs that pay attention to the GMP.

This encourage authors to build HACCP plan for amplang production. HACCP is a quality assurance system to identify, assess and control potential hazards as well as a control system that focuses on prevention. HACCP emphasizes quality control and food safety (Muhandri *et al.*, 2012, Thompkins, 2009). The HACCP approach is used because HACCP has become an accepted food safety standard internationally (Muhandri *et al.*,

2012). The HACCP study on amplang production is important because the sanitation and hygiene in the SME processing was still poorly implemented, HACCP document as control process has not build yet, encouragement of consumers and local government to produce similar quality of product for each production, and becomes an responsibility for SMEs to maintain food safety according to Law No. 7 of 1996.

Procedure of HACCP planning consist hazard analysis to determine many kinds of contaminant that possibly appear in the raw materials, processes, and products. Contamination of raw materials, processes, and products happen because of several things such as unclean procedures, worker rotations, and contamination of microorganisms from the air during processing (Novotny *et al.*, 2004). One of raw material used for produced amplang was fish or crabs. Contaminant in fish or crabs can be microbial pathogen, heavy metals, chemical residu and grovel from fish environment.



Microbial pathogens grow easily in the fisheries commodities because these commodities contain protein and water in the high amount. Fish contains water of 11.72-12.48%, ash 21.83-22.62%, protein 59.24-63.59%, and fat 1.20-1.91% (Asikin & Kusumaningrum, 2018). Crab contains 47.5% protein and 11.20% fat (Karim, 2005 in Katiandagho, 2012). Microorganism contamination can be prevented by apply of sanitation and hygiene in every step of the processing. So that, proper processing techniques and knowledge of production processes are needed to avoid hazards and quality degradation (Citaesmi & Wahyuni, 2018). Processing technique and production knowledge was one of the important point in the HACCP planning. This prevent potential dangerous of biological, chemical, and physical hazards as well as reducing the risk of hazard events by controlling the critical points in each production processes.

The aim of this study was to build HACCP planning in the amplang production. The HACCP consist of several steps including the preparation of the HACCP team, identification of product description and consumers, establishment of process flow diagrams, identification of hazard and CCP, and determination of control actions, corrections, monitoring, and verification procedures .

## **METHOD**

The results were obtained by conducting survey and observation in the amplang production house located in Balikpapan city. Balikpapan's SME center (Sentra Industri Hasil Perikanan dan Kelautan Teritip) was chosen as object study because local government will design this center as a pilot of SME's center in Indonesia. This study was expected to help SME to produce uniform quality of product beside to assure of food safety. The data collection was done by direct observation, indepth interview, and FGD. Direct observation and in-depht interview was done to analyze amplang processing from purchasing until distribution.

Focus group discusstion was done to verify the flow processing diagram and to explain the correct GMP implementation. The selection SME as a sample was done by

purposive sampling. SMEs that have P-IRT and Halal certificates were selected as object study. The data analysis method was descriptive method and literature study from several previous research about fish-processed and HACCP implementation.

Hazard or contaminant on amplang production was reviwed based on Standar Nasional Indonesia and existing condition. Identification of Critical Control Points (CCP) was carried out by implementing the CCP decision tree (Fig. 1) and using some questions that are (Lee & Hathaway, 1998):

Q: Could the hazard be present in or on the product at unacceptable levels at this step?

A: Yes (give reasons and go to next Q),

No (not a CCP; proceed to next identified hazard.

Q: Is there a control measure available at this step that would prevent unacceptable levels of the hazard?

A: Yes (this step is a CCP),

No (not a CCP and go to next Q),

Q: Is there a control measure available at a previous step that would significantly contribute to preventing unacceptable levels of the hazard at this step?

A: YeS (retrospectively assign the previous step as a CCP),

No (if the answer to Q also was no, consider whether addition of further steps could control the hazard or whether redesigning the process is necessary for ensuring the availability of a control measure). At the end one should proceed to the next identified hazard (Lee & Hathaway, 1998).

## **RESULT AND DISCUSSION**

The implementation of HACCP in fish processing industries includes evaluating the basic feasibility and evaluating the plan for implementing the HACCP program. Basic requirements in implementing HACCP include Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedure (SSOP) (Standart National Organization, 1999). Based on the survey results, there are some SME that have not implemented the GMP.

The HACCP system refers to the Codex Alimentarius Commission guidelines in the "Guidelines for Application of Hazard

Analysis Critical Control Point System" which consists of stages which are assembling the HACCP team, describing the product, identifying intended use, constructing process flow diagram, listing all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control

identified hazards, determining Control Points, establishing Critical limits for each CCP, establishing a monitoring system for each CCP, establishing corrective actions, establishing verification procedures and establishing documentation and records keeping (WHO & FAO, 2009).

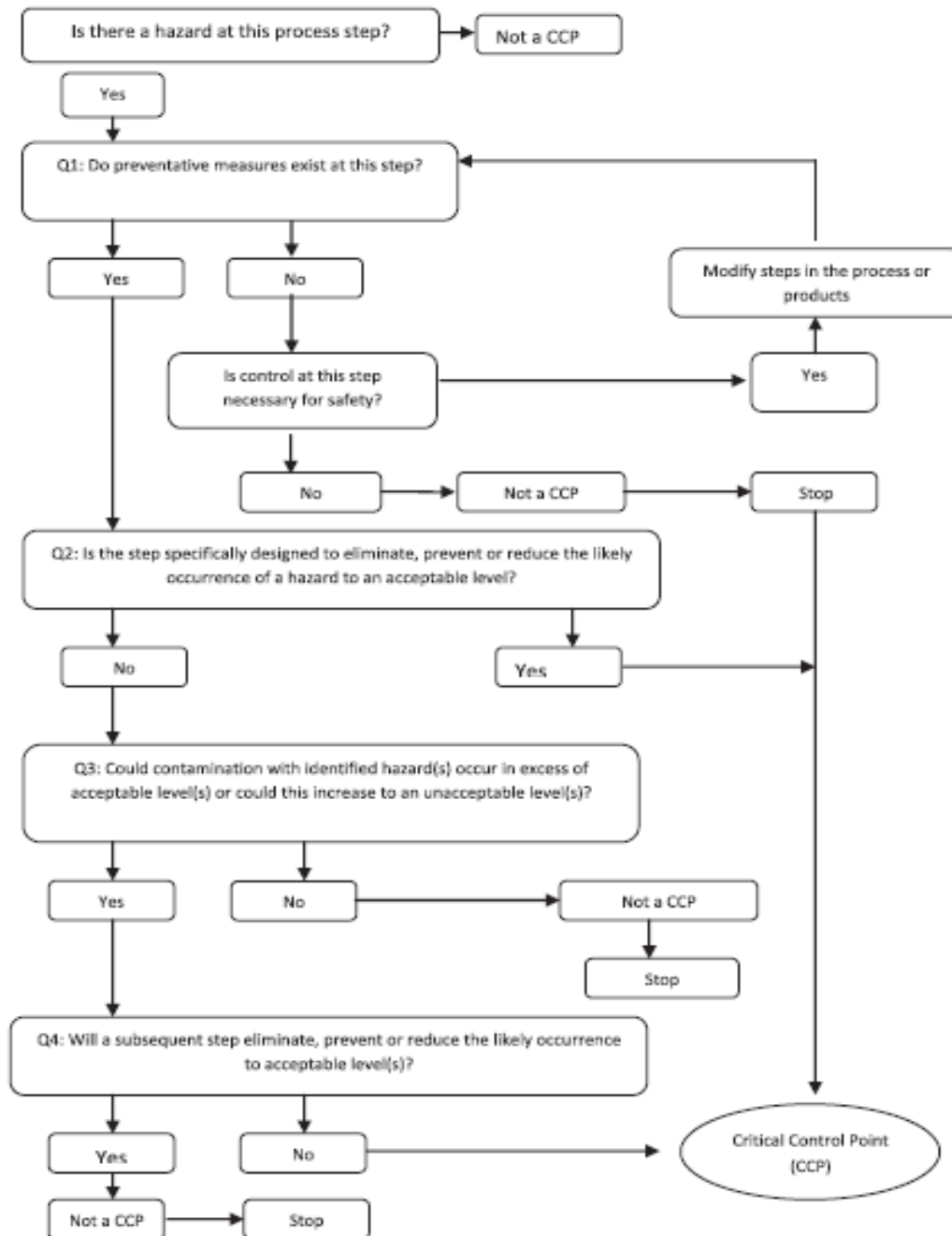


Figure 1. Process Step a Decision Tree (Horcner *et al.*, 2006 in Ramalingam *et al.* 2013)

**Assembling the HACCP Team**

The team quality assurance in the SME must be determined and this team must understand the HACCP principle. This situation does not found in the SME where quality assurance only follows the flow *Food SciTech Journal Vol. 1 (1) 2019*

diagram and SOP determined by bussiness leader. Based on survey, there were not HACCP team in the SME beside it was found that employee never participate in the HACCP training. Infact, the business owner acts as quality and sanitation control, and head of

**Zulfatun Najah et al.**

marketing and production. The basic knowledge required for the members of HACCP team includes understanding the severity and hazard of pathogens and their toxins, understanding the principles and the concept of HACCP, ability to develop a flow diagram, ability to identify hazards and CCPs, ability to prescribe precautions for avoiding infections (microorganism destruction or suspension), and ability to propose solutions for hazardous food (Tzouros & Arvanitoyannis, 2000).

According to Pierson and Corlett (1992), the HACCP team may include not more than six members but all members should be qualified to identify possible hazards; recognize the severity of these hazards; propose predictive measures, critical limits, and monitoring and verification procedures; propose corrective actions; seek sources of information for the development of the HACCP plan; and validate the system.

**Product Discription**

Main products produced are amplang from fish and crabs. There are several types of fish that are used such as cork fish, flat fish, mackerel fish, etc. These depends on supplier’s supply. Besides, SME also produced fish chips, fish crackers and abon. The description of Amplang can be seen in Table 1. Product description is a complete description of the product regarding composition, physical / chemical structure, treatment, packaging, storage conditions, and durability and distribution methods (Wicaksani & Andriyani, 2017).

However, SME does not display chemical or physical structures. The physical structure were taste, form, or other organoleptic displays. The chemical structure including AW, pH. Even the product does not include nutritional adequacy figures.

**Arrange the Production Process**

The amplang processing can be seen in Fig 2. This flowchart has been verified by the business owners by FGD. This processing had also socialized to the workers by displaying the flowchart on the production room wall according to the standard HACCP process where the production process is known by the whole workers and the workers work according to the flowchart. The construction of a flow diagram is important as it helps to

identify all the aspects of the production process that influence product quality (Mead, 2000). The flow chart must be verified by the HACCP team to test the accuracy of the process flow diagram. The flow diagram can be modified if there is changeable processes and condition (Handayani, 2012).

Table 1. Product description of amplang

Product description	Product specifications
Raw material	Various types of sea fish and crabs, flour, seasoning, oil.
Process stages	Purchasing, washing, storing, thawing, crushing, mixing, cutting, frying, draining, packaging, packing, storing
Packaging	Primary packaging was using PP plastic, secondary packaging was using carton. For products 100 - 250 grams was packed using PP. For products 1 kg was packed with PP and cartons.
Storage and transportation	Raw materials (fish and crabs) were stored at -10 until -5 <sup>o</sup> C. The product were stored at 25 <sup>o</sup> C. The transportation of fish / crabs was carried out by pickup trucks, where the fish / crabs are stored in containers that are given ice grains. Transporting amplangs to outlets was done by car.
Label	Product name, company name and logo, product weight, expired date, P-irt logo, and halal logo, composition, Balikpapan logo, and original Indonesian’s product logo.
Product utility	Amplang is a product that can be consumed directly by consumers without further processing.
Consumer	Products are snacks of a kind of crackers intended for all kinds of consumer. This product was positioned as gift
Shelf life	The amplang has been labeled expired with a shelf life of 3 months from production.

The processes begin with raw materials handling. SME gets raw materials from fishermen or suppliers. In this process, QC officer should check raw material's quality. Quality testing can be done by examine the visual appearance of raw materials. Preferably, if the raw material did not meet the SNI, it was returned to the supplier. Infact, SME receives all fish supplied because the the scarce of fish

or crabs. Then, fish or crabs were immediately washed by the employee and then stored in the freezer. Storage is carried out by adhering to the FIFO principle (first in first out). Fish or crabs that will be processed were half thawed then grinded. The batter mixed with flour and seasoning then formed, cutted and fried. Frying was done by submersed products into oil with periodically stirring to avoid burning.

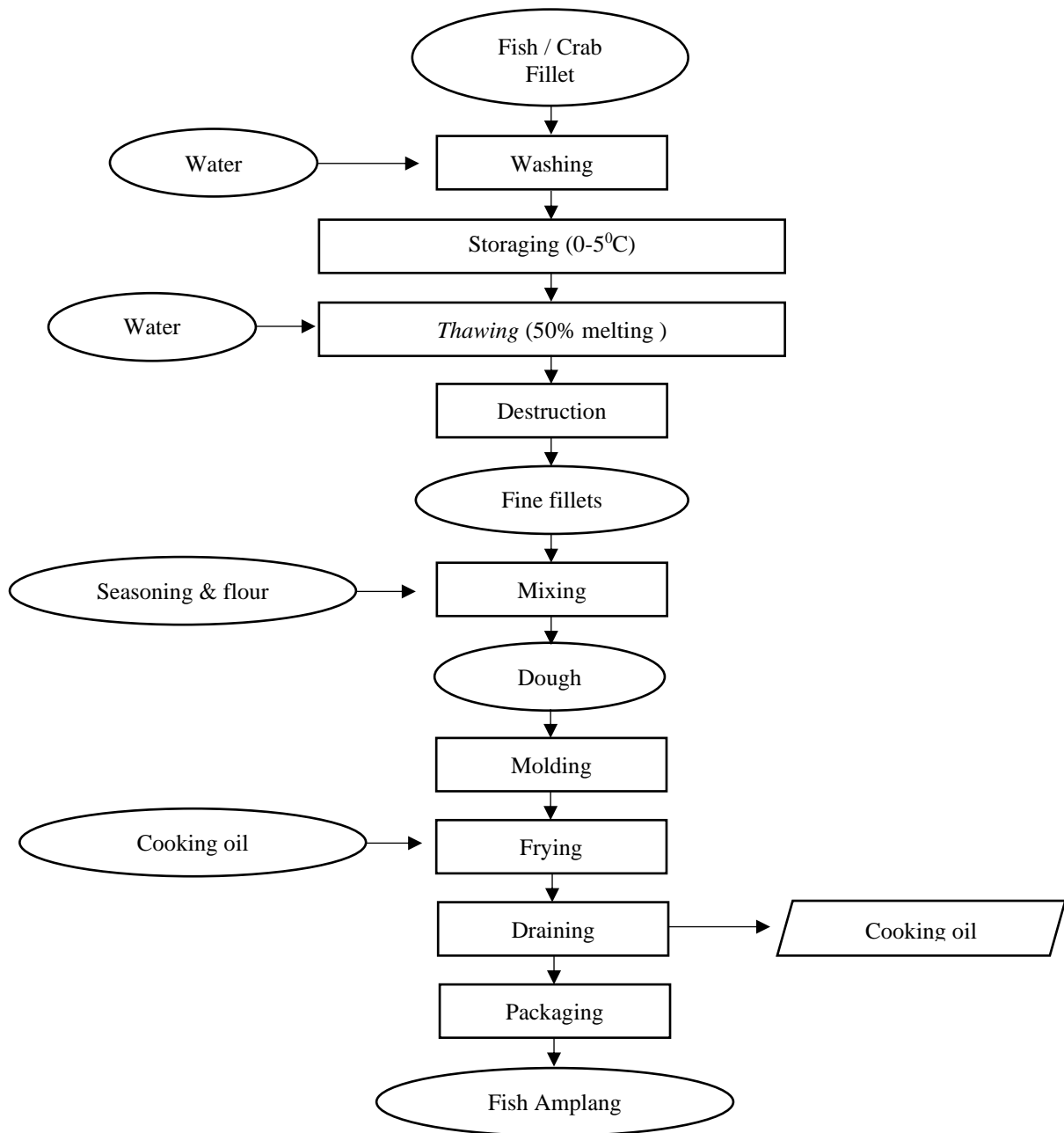


Figure 2. Production process of amplang

**Application of HACCP Principles in the Production Process**

**a. Hazard Identification**

The first principle of HACCP is identification of hazards that may arise during

production process (Wicaksani & Andriyani, 2017). In the HACCP framework, the term hazard refers to any agent in, or condition of, food that is unacceptable because of its potential to cause an adverse health effect.

**Zulfatun Najah et al.**

Hazard analysis was defined as the evaluation of the severity of a hazard and its probability to occur. Its application results in the determination of critical control points in a production line where several parameters of the product can be measured and corrective actions might be implemented (ILSI, 1997).

Contamination of hazards in food may occur in any step of processing. To prevent hazard contamination, manufacturers need to apply hazard analysis. Possible hazards arising from the processing of amplang can be seen in Tables 2 and 3.

Table 2. Hazard analysis of raw material

No	Raw material	Hazard type		Cause	Control action
1	Fish	Physical	Foreign materials such as sand	Contamination from the environment, containers and transportation equipment	Based on SNI 2729-2013 Certified supplier Frozen Storage
		Chemistry	Formalin, chemical residue	Fishries live, preservation technique.	Thrown away
		Biology	Parasites, bakteri patogen	Delivery temperature above -0°C	Cool storage and distribution (GMP/SSOP), SNI 2729-2013
2	Tapioca Flour, Salt, Flavoring, Flavoring (powder ingredients)	Physical	Foreign hazard such as dust, hair.	From suppliers and from packaging.	Selection of certified materials (CoA
		Physical	Foreign sand or worm	Contamination from water sources	Using ground water, PDAMs, or filtering water. Law 416/MENKES/PER/IX/1990)
3	Water	Biology	Pathogenic bacteria	Improper water treatment	Use bacterial killers such as ozone or UV in water installation pipes. Law 416/MENKES/PER/IX/1990)
		Chemistry	Heavy metal. Brackish water type	Inaccurate contamination from water sources and water purification	Using ground water, PDAMs, or filtering water. Law 416/MENKES/PER/IX/1990)
		Physical	Foreign objects such as dust, hair, skin	Stripping less clean and not through washing	Choosing certified suppliers, SSOP/GMP procedure
4	Garlic	Chemistry	Heavy metal	Location of cultivation	SSOP/GMP procedure
		Physical	Foreign materials such as sand	Contamination from environment, containers and transportation equipment	SSOP/GMP procedure
		Physical	Foreign objects such as dust, hair, skin	Stripping less clean and not through washing	Choosing certified suppliers, SSOP/GMP procedure

Physical hazards identified in fish and crabs were sand or dust from the sea/ponds or contamination during the transportation process. In addition, fish skin, fish scale, or crab shell carried on flesh can be regarded as hazard. SME's only receive raw materials in the form of fish/crab flesh. This physical hazard can be removed by cleaning in flowing water. The biological hazard in fish/crabs can be parasites and bacterial pathogens (SNI 2729, 2013; Novotny et al., 2004). In amplang processing, physical hazard can be gravel, dust

ot hair. Physical hazard in flour can be dust or hair because of processing or environment.

Some pathogens transmissible to human through contact with fish and aquatic environment are *Mycobacterium spp*, *Streptococcus iniae*, *Photobacterium damsela*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Erysipelothrix rhusiopathiae* (Novotny et al., 2004). Foodborne pathogens associated with fish and fish products are *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Escherichia coli*, *Aeromonas spp.*,

Salmonellosis, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum*, *Clostridium perfringens*, *Campylobacter jejuni* (Novotny *et al.*, 2004).

This hazard appears in the food because of unhygiene processing and sanitation or environment Chemical hazards in fish/crabs can be in the form of formaldehyde or chemical residues because of fish lives's environment (Dewi *et al.*, 2018). Possible chemical hazard in the fisheries product are Pb, Cd, As, Sn, and

Hg (SNI 2729, 2013) According to Dewi *et al* (2018), there are still heavy metal of Pb and Cd residues in some fishes from rivers in Balikpapan. Referring to the SNI 7387 2009 regarding the maximum limit of heavy metals in food, the heavy metal content in fish is high and meets the permitted requirements (Dewi *et al.*, 2018). Acrylamide can be a chemical hazard in the amplang production process because of over cook/ burning.

Table 3. Review of hazard identification on amplang processing

No	Process	Possible Type of hazard		Reason	Control action
1	Purchasing	Physical	Gravel, dust, hair	Not clean raw material	SSOP/GMP
		Chemistry	-	-	-
2	Thawing	Biology	Parasites, bacteria pathogen	The temperature of the distribution of fish / crabs is more than 5°C.	Choosing suppliers that have been certified HACCP or have CoA
		Physical	Dust	Air circulation	Using blower in the production house. According to GMP/SSOP.
		Chemistry	Metal	The water used was brakish. Oxygen accelerates meat decomposition.	Using ground water, PDAMs, or filtering water. According to GMP/SSOP.
3	Grinding	Biology	Bacteria pathogen	The temperature and time of thawing more than 5°C for 24 hours, contamination from water, hand contamination of workers who do not use gloves	According to GMP/SSOP. HafI thawing was implemented to keep cool condition.
		Physical	Gravel, hair, dust	Not clean fish	GMP/ SSOP procedure
		Chemistry	Oxygen	Oxygen exposure accelerates meat decomposition	GMP/SSOP procedure. Grinding was carried out when half meat had melted
4	Mixing	Biology	Bacteria pathogen	Contamination from equipment, worker and air.	GMP/SSOP procedure
		Physical	Gravel, hair, dust	the processing did not apply GMP and SSOP properly	GMP/SSOP procedure
		Chemistry	-	-	-
5	Rounder (shapping)	Biology	Bacteria pathogen	Contamination from equipment, worker and air.	GMP/SSOP procedure
		Physical	Gravel, hair, dust	The environment was not clean, the processing did not apply GMP and SSOP properly	Workers use sanitation tools during the production process such as masks, gloves, headgear, lab clothes, work shoes

				(GMP/SSOP Procedures). Cleaning the production house	
	Chemistry	-	-	-	
	Biology	Bacteria pathogen	Contamination from equipment, worker and air.	Using stainless steel tools GMP/SSOP procedure	
	Physical	-			
6	Frying	Chemistry	Acrylamide	Burning	The frying process is carried out for a maximum of 25 minutes. Burning product was thrown away.
		Biology	Toxin from bacteria pathogen		
7	Draining	Chemical	Rancidity	The oil was oxidized, the oil is not perfectly drained, there is still oxygen in the packaging	Using spinner until t properly drain
		Physical	Dust, hair	Environment and worker	GMP/SSOP procedure
8	Packaging	Chemistry	Chemical material	Migration from packaging material.	GMP/SSOP procedure Product was properly drain.
		Biology	Bacteria pathogen		Using clean packaging. GMP/SSOP procedure

## b. Determination of Critical Control Points (CCP)

The principle of HACCP is the determination of CCP. The critical control point in the amplang processing is purchasing, mixing, frying and sorting. The emergence of a biological hazard during the production process is caused by the process of transportation, handling and storage that is not in accordance with GMP. Material handling was the first processes. This process has a hazard potential, where fish are contaminated with sand, parasites, bacteria, chemicals and unfavorable conditions in fish conditions, thus affecting product quality. Fish temperature should be lowered as soon as possible to 0°C after capture and that temperature should be carefully maintained. Capture, handling, and transportation are responsible for a great release of adrenaline and cortisol in fish tissues causing shortening of time for onset of rigor mortis, which softens fish texture and enhances the ease of penetration by pathogens. Increase of microbial load is prevented by gentle treatment of animals during transport and transfer to stunning, while a well-controlled cold chain keeps it that way (Sigholt *et al.*, 1997).

Thawing was CCP 1. In this process, thawing was done until half frozen's meat was melt. Some of biological hazard such as *E coli* may appear in this process if improper thawing was done by worker. Besides, improper thawing process can cause meat oxidation and decomposition (Cutting & Spencer, 1968). According to the observation, thawing processed used water to accelerate melting process, but if water was unclean, it may causes hazard. Water for the production is odorless, tasteless, non-turbid, microbiological, chemical and physical content in accordance with Annex II Permenkes No. 416 / Menkes / Per / IX / 1990. Thawing should be properly and thoroughly carried out, so that cooking could sufficiently lower the microbial population even at the center of each food portion (Tzouros & Arvanitoyannis, 2000).

Frying was determined as CCP 2. Frying determines the quality of the product. Frying is done by dipping the product into hot oil. The product is fried until it color changes and it may not burn. Burning will causes acrilamide appearance in the product. Acrilamide was a group og chemical hazard. This component is carcinogen and a neurotoxin (Ubaoji & Orji



2016). Acrylamide is a contamination generated during cooking as a consequence of the Maillard reaction, derived from the reaction between the free amino acid asparagine with reducing sugars or other carbonyl compounds (Ubaoji & Orji 2016).

In draining processes, the product is drained and aerated until it is warm so the air become contaminants. This can trigger the risk of hazard due to bacteria in the container and pollution from the surrounding. The drained process should be carried out in a closed place and the air is not contaminated with anything. Tools used for draining should be cleaned frequently (Pratidina *et al.*, 2018). This stage is designated as CCP 3. Packaging was determined as CCP 4. Products are packed on plastic according to the weight. Based on the observation, products will repackage if netto product improper. This process is also critical of the emergence of hazards so that this process needs to pay attention to GMP. Packaging must properly done. Migration from the plastic material to the product may happens because of heat and contact time (Bhunia 2013).

Beside, the plastic is stored in clean and dry environment to prevent biological contamination.

**c. Establish Critical Limits, Monitoring Proceures and Corrective Action**

Critical limits are control that are carried out to eliminate hazards or reduce them to safe limits. The criteria used as critical limits are temperature, time, RH, pH, Aw, chlorine content, textures and visual appearance (Afrianto, 2008). Determination of CCP for each process can be seen in Table 4.

The amplang production’s critical limit is described in each CCP. Temperature, time and contaminants are the main factors that significantly affect product safety and quality. Monitor action is carried out based on the origin of the contaminant. In the monitoring process, every contaminant must be documented and carried out to evaluate the process and avoid similar events in the future. Critical limit, monitoring procedures, and corrective actions can be seen in Table 4.

Table 4. Determination of CCP and Critical Limits

CCP	Process	Critical limits	Reference standard	Corrective action
CCP 1	Thawing	The maximum thawing temperature is 5°C to prevent bacteria growth. ( <i>E.coli</i> <2 APM/mL).	Thawing should be done quickly because it prevent meat oxidation and decomposition (Citraresmi & Wahyuni, 2018; Cutting & Spencer, 1968)	Thawing was maintain until half meat melted to keep meat in cool condition (meat are not fully melted).
CCP 2	Frying	Frying is done until the amplang turns yellowish.	Burning (over frying) will causes acrilamide appearance in the product (Ubaoji & Orji, 2016)	Product was fried in 20-25 minutes until product turn yellowish. If burning happens, the product must be discarded/thrown away.
CCP 3	Draining	Oil draining was done properly. Tools used should be cleaned frequently.	Products that still contain oil cooking will cause rancidity Processing was done according to GMP/ SSOP procedur.	Product was spinned until oil was removed, so that rancidity can be prevented. It better for using spinner equipment According to the SSOP/GMP
CCP 4	Packaging	Free of foreign matterial (workers hygiene and sanitation)	GMP/ SSOP procedur.	Accroding to Procedur SSOP, Reject product.

**d. Establish a HACCP Process Verification Procedure**

Internal verification is carried out by a team specifically designated to maintain product quality (SME quality management).

Verification should be done twice a year (every 6 months). Verification is carried out by checking the suitability of SOP manual documents with actual HACCP practices in the field. External verification is carried out by the

HACCP system certification body within a year.

#### **e. Make Documents**

Documentation of amplang production process includes every record of the CCP, critical limits, monitoring records, corrective actions taken against non conformities and responses to the auditor's verification process. Documentation is carried out in the process of monitoring. Documentation was shown to food safety auditors when the SME held an external audit.

#### **CONCLUSION**

Amplang is a fishery and marine product produced from fish and crabs. Based on observation, membership of HACPP need to be determined. Food hazards are divided into raw material hazards and production process hazards. The hazard is distinguished into physical contamination which includes dust, gravel, and hair. Chemical hazards can be metals, heavy metals, acrilamide, and packaging material. Biological hazards include parasites and pathogenic bacteria originating from raw materials and arise due to less hygienic production. The CCP on processing is thawing, frying, draining and packaging.

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# SCOPE, POLICY, AND AUTHORS GUIDELINES FOR FOOD SCIEN TECH JOURNAL

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## Journal Scope

Food ScienTech Journal (FSJ) publishes high quality research articles on food sciences, food technology or its applications in food industry. The published articles can be in the form of research articles or short communications which have not been published previously in other journals(except in the form of an abstract or academic thesis/dissertation or presented in seminar/conference).

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A research article is an original full-length research paper which should not exceed 5.000 words (including table and figures). Research article should be prepared according to the following order: title, authors name and affiliations, abstract, keywords, introduction, materials and method, result and discussion, conclusion, acknowledgement (optional), and references.

### Short communication or Review

A short communication consists up to 3.500 words (including table and figures) and consists of title, authors name and affiliations, abstract, keywords, introduction, materials and method, result and discussion, conclusion, acknowledgement (optional), and references. A short communication should contribute an important novelty for science, technology, or application.

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The introduction states background of the research supported mainly by the relevant references and ended with the objectives of the research.

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