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Development of Coconut Oil Fermentation MethodS (Cocos Nucifera) Using Rhizopus SP Microbe

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Abstract: Generally, the manufacture of coconut oil still uses high temperatures so it requires a lot of energy or fuel. In this study, the manufacture of coconut oil was developed using the help of *Rhizopus sp* microbes with the fermentation method. The aim is to save fuel, little residue, low rancidity level, fragrant aroma, free of cholesterol-inducing compounds and to study the effect of microbial weight, fermentation time. The results obtained from the organoleptic test of coconut oil produced a savory taste, fragrant aroma and clear color, in accordance with SNI 7381:2008. The results of the chemical analysis test were pH 4, the free fatty acid value was 0.15%, the peroxide number was 2.97 mekO₂/Kg and the saponification number was 246 MgKOH/gr in accordance with SNI 01-2902-1992. Then the microbial test was found Kapang of 1 and Khamar of < 1, in accordance with the standard SNI ISO 2157-1 (BPOM).

Keywords: Fermentation, *Rhizopus sp*, Oil, Coconut

INTRODUCTION

The scarcity and high demand for cooking oil made from palm oil has recently become a topic that really needs attention for the people of Indonesia. On this basis, it is necessary to research the manufacture of oil from local basic ingredients, namely coconut oil. In addition, coconut oil is known as a functional food that provides health and nutritional benefits (Cristianti, 2015). Coconut oil can be used as an obesity drug (Dwijoseputro, 1990) multipurpose nutritional supplements such as vitamins, amino acids, antioxidants, antimicrobials, and antiviral compounds (Fardiaz, 1992). In addition to having advantages in the health sector, coconut oil has a very high lauric acid content ranging from around 50-53% compared to other vegetable oils. In the human body lauric acid (2004) will be converted into monolaurin which has antibacterial, antiviral, and antifungal properties (Ghani *et al.* 2018).

Thus, this is a factor in increasing the consumption of coconut oil. Coconut oil is made using 2 (two) methods, namely the dry method and the wet method (Arsa, 2004). To obtain coconut oil through the dry method, it is done by pressing copra (dried coconut meat), then filtering the resulting oil. Meanwhile, to obtain coconut oil through the wet method, it is carried out by heating, fermentation, acidification and the addition of enzymes (Asmoro, *et al.*, 2018; BSN, 1992). Coconut oil produced by dry method has good quality with a clear, clean color structure of coconut oil and a fragrant coconut oil aroma (BSN, 2008; Bawalan, *et al.*, 2006).

This is because copra (dried coconut meat) before the pressing stage will go through a drying process until it is made dry by using the hot sun or through the oven. However, making coconut oil through the dry method requires greater operational costs in the drying process and depends on weather conditions (BSN, 2008; Bawalan, 2006). Coconut oil produced by the wet method is usually simpler, namely by heating at high temperatures and for a long time. However, coconut oil obtained by high heating can change the structure of the oil and will produce coconut oil with a color that is not clear and brown in color, and the coconut oil produced is easily rancid (Buckle, 1988). In general, oil damage is caused by oxidation and hydrolysis processes. Fatty acids can be oxidized, causing a rancid odor. This new rancidity is the breakdown of hydroperoxides (Kadir, 2015). Thus, to get a quality type of coconut oil with maximum results, it can be done by utilizing the presence of microorganisms or known as fermentation (Ketaren, 1986). The advantages of the fermentation process compared to other methods are that it is practical and easy to produce coconut oil, saving fuel, little residue, low rancidity with longer shelf life, fragrant aroma, and free of cholesterol-inducing compounds (M. Asy'ari *et al.*, 2006). Based on the above background, it will be researched the process of making coconut oil by fermentation using *Rhizopus* sp. microbes contained in *Rhizopus* sp. as a mixture to speed up the process of chemical reactions. In this study, the pH value, free fatty acid value, peroxide number, saponification number, and microbial test were also tested.

LITERATURE REVIEW

Making coconut oil with a fermentation method that utilizes baker's yeast as a separating agent. The fermentation time varies from 6-30 hours which has yeast variations ranging from 0.5 to 2.5 gr. The results obtained are the amount of oil produced by reviewing the optimum conditions achieved (Ganjar *et al.*, 2016).

Making coconut oil with a fermentation method that utilizes tempeh yeast in the coconut oil extraction process. By using the variation weight of tempeh yeast and the length of time of fermentation which only has 2 variables, namely T1 and T2. The results obtained were in the form of coconut oil testing to determine the data, including specific gravity, water content and FFA (free fatty acids) values (Asmoro *et al.*, 2018).

Coconut oil was made using the type of microorganism *saccharomyces cerevisiae* found in tape yeast by determining the best conditions for the oil obtained through the fermentation process. The types of variables used in this study were fermentation time, variations of tape yeast, and the use of room temperature. The results obtained in the form of coconut oil analysis by knowing the analytical data in the form of FFA, saponification number, iodine value, and peroxide test (Utami *et al.*, 2008).

The process of making coconut oil can be done through two methods, namely heating and fermentation. The principle of making coconut oil by the heating method is that the cream obtained from coconut milk is heated to a certain temperature so that the oil is separated from the cream and the water evaporates due to heating. Making coconut oil with the fermentation method is done by adding microorganisms to help the process of separating the oil and protein components. The fermentation process will form three layers, namely the top layer in the form of oil, the middle layer in the form of blondo (protein), and the bottom layer in the form of water (Asy'ari, 2006).

From a process like this, the resulting oil tastes soft with a unique coconut smell. If the oil freezes, the color of this coconut oil is white. Meanwhile, if it is liquid, coconut oil is colorless (clear). Coconut oil is not easily rancid because of its high saturated fatty acid content so that the oxidation process does not easily occur. However, if the quality of the coconut oil is low, the process of rancidity will run earlier. This is caused by the influence of oxygen, the presence of water, and microbes that will reduce the fatty acid content in coconut oil into other components (Silaban, 2012). Physically, coconut oil should be clear in color. This indicates that it is not mixed with other materials and impurities. If there is still water content in it, usually there will be white lumps. The presence of this water will speed up the process of rancidity. In addition, the lump may also be a component of blondo that is not completely filtered out. This kind of contamination will directly affect the quality of coconut oil.

The fermentation method is another easy alternative, namely by using the addition of yeast as a starter for the process of breaking the coconut milk/cream emulsion so as to get the desired coconut oil (Muharun and Apriyantono, 2014). The advantages of the fermentation process in the manufacture of coconut oil are the simple method so that it can be produced practically, fuel efficient, little galendo residue, low rancidity, long shelf life, fragrant aroma, and free of compounds containing cholesterol (Uswatun, 2013).

Fermentation is carried out to reduce losses in the manufacture of coconut oil by dry and wet methods. This fermentation method is based on a simple biotechnology discovery that uses bacteria or enzymes to separate oil from carbohydrates and proteins contained in the endosperm cells of coconut seeds (Ganjar, 2016).

RESEARCH METHODS

The research method used in this research is the fermentation method using the microbial *Rhizopus* sp. The stages of the research carried out are as follows:

1. Sample Preparation Stage

The coconut meat is washed first to remove dirt, after washing the coconut meat is grated. The grated product is then filtered to take the coconut milk by flowing water into the grated coconut after the water is felt to seep then squeeze it using your hands so that the coconut milk comes out. After the coconut milk comes out then filtered again so that there are no impurities. The filtered coconut milk is then deposited for \pm 3 hours. This precipitation aims to separate the water at the bottom with the coconut cream at the top, this deposition occurs due to the difference in density between water and coconut cream. After that take the coconut cream.

2. Fermentation stage using *Rhizopus* sp. microbes with mass variations.
Enter *Rhizopus* sp. with mass variations of 1, 2, 3, 4 and 5 grams into bottles. After that, add coconut cream to each variation of the yeast mass, then let the mixture sit for 3 days.
3. Fermentation stage using *Rhizopus* sp. microbes with time variations.
Enter the *Rhizopus* sp. microbe of coconut cream then make variations of 1, 2, 3, 4 and 5 days of fermentation time. After that, the characterization of the sample test was carried out.
4. Samples Testing Stage
The results of the heating stage will then be tested in the laboratory to determine the pH value, free fatty acid value, peroxide number, saponification number, and microbial test.

FINDINGS AND DISCUSSION

1. Coconut Oil Making Process

This research activity aims to determine the effectiveness of coconut oil production using the fermentation method which is varied with the addition of yeast.

2. Coconut Oil Preparation Stage

The research begins with the selection of coconuts that have good quality with criteria including old coconut, brown in color, not yet sprouted, still contains coconut water, not rotten and can be found in the market together with other coconut groups. After that grate and strain the coconut meat using the MP-03 electric coconut grater machine to get coconut cream. Then deposit the coconut milk for \pm 3 hours to separate it from the water and unfiltered coconut meat, where the coconut milk is above and the water is below. This is due to the difference in the density of water and coconut cream because the density of water is greater than that of coconut cream. At this stage produce coconut cream in large quantities because the coconut is selected according to the criteria.

3. Fermentation Stage

When it has been separated, the water is removed and the coconut cream that is formed is fermented using *Rhizopus* sp. with a mass variation of 1, 2, 3, 4 and 5 g with a fermentation time of 72 hours. At this stage of fermentation aims to accelerate the process of making coconut oil with the help of microbes. Presentation of data in the form of tables and curves of the effect of the use of the amount of yeast on the amount of coconut oil products obtained:

Table 1. Physical Analysis

Mass of <i>Rhizopus</i> sp. (gr)	The volume of oil produced (mL)
0	19,5
1	16,5
2	19
3	18
4	30
5	22,5

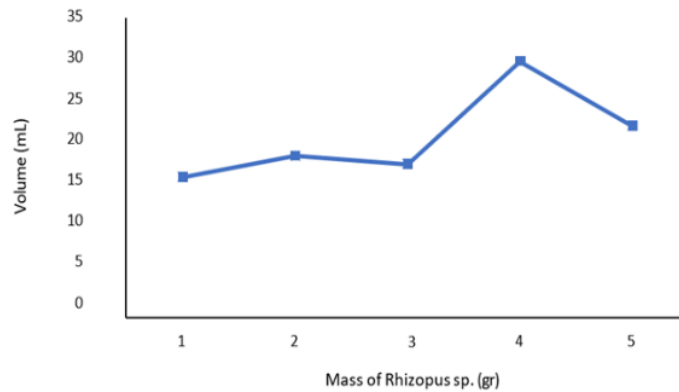


Fig. 1 The curve of the influence of the number of uses of Rhizopus sp. of coconut oil obtained

From the presentation of the curve above, the effect of the variation in the amount of yeast used on the amount of coconut oil product obtained is obtained. This is closely related to the fermentation process that takes place. The more the amount of yeast used in the fermentation medium (coconut milk), the more white tissue (Mycelium) is formed. Mycelium will form sporangium and its spores in large numbers, so that it can have a good impact on the process of fermentation being fast and the coconut oil obtained will be more and more. In addition, the results of fermentation are also influenced by the ratio between the amount of yeast used and the amount of coconut milk. This is due to the factor of the optimal number of yeast cells in extracting coconut milk nutrients so as to produce the optimal amount of oil as well (Debmandal, 2011). So, when viewed from the graph, the amount of coconut oil obtained using Rhizopus sp. with 4 grams to produce as much as 30 mL of coconut oil.

4. Sample Test

The oil samples obtained will then be tested at the Chemical Engineering Laboratory to determine the value of free fatty acids, pH, and organoleptic tests. The following are the observations obtained from the sample testing stage:

Tabel 2. Organoleptic

Mass of Rhizopus sp. (gr)	Flavor	Aroma	Color
0	Tasty	Fragrant	Clear
1	Good	Fragrant	Clear
2	Good	Fragrant	Clear
3	Good	Fragrant	Clear
4	Good	Fragrant	Clear
5	Good	Fragrant	Clear

Table 2 the organoleptic test shows that Rhizopus sp. meet the standards that have been determined by SNI 7381:2008, where coconut oil must have a fragrant aroma, coconut taste and clear color. The pH results obtained in samples of Rhizopus sp. 4 grams with a pH of 4.



Table 3. Free Fatty Acid Test

N _{NaOH}	V _{NaOH} (mL)	Sample Weight (gr)	%FFA	Average %FFA
0,1	1,8	5,05	0,71	
0,1	0,9	5	0,36	0,6
0,1	1,8	5	0,72	

The free fatty acid test was carried out 3 times. This aims to get more accurate results in producing coconut oil. The test is carried out by determining the value of free fatty acids in coconut oil. Free fatty acids are indicated by the acid number, the higher the acid number, the higher the amount of free fatty acids and indicates the coconut oil has poor quality.

Meanwhile, the lower the value of free fatty acids contained in coconut oil, the better the quality of coconut oil in its use (Elok, 2018). The value of free fatty acids obtained in *Rhizopus* sp. by 0.6%. Furthermore, it is analyzed based on the physical and chemical aspects.

The results of the sample test obtained coconut oil using *Rhizopus* sp. obtained a low value of free fatty acids, as well as the aroma, color and taste of coconut oil produced by fermentation using *Rhizopus* sp. according to the standard of SNI 7381:2008 where coconut oil must have a fragrant aroma, clear color and savory. Then coconut oil with fermented *Rhizopus* sp. tested again to determine the quality by using procedures according to SNI in the Laboratory of the Balai Besar Industri Agro, Bogor. The following are the results of the tests that have been carried out:

Table 4. Results of Coconut Oil Analysis Using *Rhizopus* sp

Parameter	Results	SNI 01-2902-1992	Unit
Free Fatty Acid (as Lauric Acid)	0,15	5	%
Peroxide Number	2,97	5	Mek O ₂ /kg
Saponification Number	246	245-265	Mg KOH/gram
Microbes		SNI ISO 21527-1 BPOM	
Kapang	1	20	Koloni/gr
Khamir	<1	20	Koloni/gr

5. Physical Analysis

Physical analysis results, on the color, aroma and amount of coconut oil produced. Coconut oil obtained from the fermentation of *Rhizopus* sp. 4 grams have a fragrant aroma and colorless (clear) and tasty. Organoleptic Analysis, pH and Free Fatty Acid. The results of fermented coconut oil were then tested for organoleptic (taste, aroma, color), pH test, free fatty acid test.

a. Organoleptic Test

In coconut oil samples using *Rhizopus* sp. produces a fragrant aroma, savory, and the color is clear.

b. pH test

pH is a factor that needs to be controlled because although in the fermentation process causes a decrease in pH, but for working efficiency the microorganisms used require an optimal pH. The pH test was carried out to determine the enzyme activity during the

fermentation process, when the pH decreased, the protein would coagulate and separate from the oil phase with protein. Each enzyme has a pH to carry out its activities, during fermentation the pH will continue to decrease until it reaches a pH of around 4-5 (Utami, 2008). From the pH test results obtained coconut oil fermented with *Rhizopus* sp. 4 grams obtained pH 4. This means that the pH conditions in each coconut oil sample that went through the fermentation process resulted in a higher yield of coconut oil. The higher the yield, the more coconut oil will be obtained (Mahesar, 2014). This condition occurs because the coconut cream is in an isoelectric state. Where, this situation causes the separation of oil with water (Mujdalipah, 2016). The magnitude of the pH value is caused by several other factors such as the type of coconut, the age of the coconut, temperature, duration of fermentation, and heating of coconut oil. Thus, coconut oil in each of these samples still contains water content which causes the pH to be high (Setiaji, 2006).

c. Free fatty acid test

Free fatty acids are a parameter to determine the damage of coconut oil due to the hydrolysis process by the interaction with water and enzyme activity (Sutarmi, 2005). In the free fatty acid test on coconut oil samples using *Rhizopus* sp. by 0.6%. The data is taken based on the results of calculations through tests carried out at the Chemical Engineering Laboratory.

6. Chemical and Microbial Analysis

From the results of the previous analysis, obtained coconut oil using *Rhizopus* sp. according to the criteria in terms of aroma, taste, pH and color. Furthermore, an analysis was carried out with a certified institution at the Balai Besar Industri Agro.

a. Free Fatty Acid

Free fatty acids are fatty acids that have been separated from triglycerides due to the hydrolysis process. These free fatty acids can be oxidized by autoxidation or by enzymes called lipooxygenases. Free fatty acids are also an indicator of oil freshness. This can be due to the free fatty acid content which can determine the resistance of the oil, the purity of the oil, and errors during the manufacturing process. In the hydrolysis reaction, the oil will be converted into free fatty acids and glycerol (Mansor, 2012). The presence of free fatty acids is usually used as an early indicator of oil damage. The results of the analysis of the number of free fatty acids of coconut oil using *Rhizopus* sp. by 0.15%. Meanwhile, according to the Standar Nasional Indonesia (SNI) for coconut oil (SNI 01-2902-1992) which explains that the maximum free fatty acid content of coconut oil is 5%. That is, the value of free fatty acids of coconut oil in this study is below the value of the SNI requirement. So, it can be categorized that the oil has good quality and is in accordance with standards.

b. Peroxide Number

The peroxide value is the most important value for determining the degree of damage to the oil. Unsaturated fatty acids can bind oxygen in the double bonds, thus forming peroxides. The presence of peroxide in the long term will result in the destruction of the vitamins contained in the oil. The higher the peroxide number, the easier the oil will go rancid. The results of the research that have been carried out show that the peroxide value of coconut oil using *Rhizopus* sp. of 2.97 mek Oxygen/kg. Based on the SNI coconut oil (SNI 01-2902-1992) is a maximum of 5 mek Oxygen/kg. Thus, the value of the peroxide

value of coconut oil in this study has met the requirements of SNI and indicates that the oil has good quality.

c. Saponification Number

The saponification number is the amount of KOH required to saponify 1 gram of fat and oil and alcohol in KOH to dissolve hydrolyzed fatty acids and facilitate the reaction with bases to form soap. Then the greater the value of the saponification number, the lower the molecular weight. Molecular weight that is too high will make the fat content more and if the fat content is too much the oil will freeze more easily. This can refer to a decrease in the quality of coconut oil. The results of the research that have been carried out show that the amount of saponification of coconut oil using *Rhizopus* sp. of 246 mg KOH/g. The number of saponification recommended by SNI 01-2902-1992 is 245-265 mg KOH/g. If the saponification number is in this range, it can be categorized as having good quality oil. From the results of the study, it was obtained that the number of saponification was in the specified standard range.

d. Microbes

Yeast is a microbe that is oval in shape and can reproduce itself through the formation of buds, yeast is commonly called yeast. Molds are microbes that look like fine threads commonly called hyphae, many hyphae are called mycelium, the way molds reproduce is to divide. In processed foods, the higher the number of molds and yeasts, the more damage the product will produce. The results of the research that have been carried out show that the microbes found in coconut oil using *Rhizopus* sp. consisting of yeasts and molds with yeast values of <1 colony/gr and molds of 1 colony/gr. Based on SNI ISO 21527-1 the maximum limit of microbial contamination in processed food which explains the determination of the maximum limit of microbial contamination in fat emulsion processed food, especially the water-in-oil emulsion type, is a maximum of 20 colonies/gr. Thus, the microbial test on coconut oil obtained results that matched the standard and did not exceed the maximum limit.

CONCLUSION AND RECOMMENDATION

From the results of the research that has been done, the conclusions from this study are as follows: The results of coconut oil from the fermentation process using the *bacteria* *Rhizopus* sp. (yeast tempe) produced as much as 30 ml, with the results of physical analysis of savory taste, fragrant aroma and clear color in accordance with SNI 7381:2008. In addition, the results of chemical quality analysis such as 0.15% free fatty acids, peroxide number 2.97 mek O₂/kg, and saponification number 246 Mg KOH/gram. These results meet the standard requirements for coconut quality based on SNI 01-2902-1992. Based on the results of the microbial test, 1 colony/gr, the Food and Drug Supervisory Agency (SNI ISO 2157-1) states that the microbe standard in coconut oil consists of molds and yeast should not be more than 20 colonies/gr, so coconut oil fermentation using *Rhizopus* sp. meet quality standards and are fit for consumption.

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