

The Optimization of Addition of Bromelain Enzyme Catalyst on the Fermentation of Coconut Milk to VCO (Virgin Coconut Oil) Using Tempeh Yeast

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Abstract - Coconut (*Cocos nucifera*) is one of the most abundant tropical plants in Indonesia. It is proven that in 2020 the total area of coconut plantations in Indonesia reached 3,377,376 hectares. Virgin Coconut Oil (VCO) is one of the diversified coconut products with high commercial value. In this study, the production of Virgin Coconut Oil (VCO) was carried out by fermenting tempeh yeast and with the help of the bromelain enzyme as a catalyst. This is because the bromelain enzyme is a type of protease enzyme that acts as a protein breaker in the oil contained in the coconut milk emulsion. Research has shown that the concentration of the bromelain enzyme affects the yield of VCO. Virgin Coconut Oil (VCO) with the addition of 6 grams of bromelain enzyme, 30 hours of fermentation time, and the use of 2 grams of tempeh yeast resulted in the highest yield value of 9.2% and free fatty acids (FFA) of 0.21%. This is because the higher the bromelain enzyme concentration, the more it will bind to protein as a substrate. In addition, the longer the fermentation, the more hydrolysis that occurs in the oil, so that the fermentation time also affects the concentration of free fatty acids of VCO. Analysis of fatty acid content was carried out using the GCMS method and showed that the highest fatty acid content was lauric acid at 54.27%. While the physical characterization of the resulting VCO is in accordance with SNI 7381:2018, which has a distinctive smell of coconut aroma, a distinctive taste of coconut oil, and is colorless.

Keywords- coconut, Virgin Coconut Oil, bromelain enzyme, fermentation

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1. Introduction

Indonesia is one of the countries with abundant coconut (*Cocos nucifera*) commodities. This is evident from the area of coconut plantations in Indonesia which reached 3,377,376 ha in 2020 [1]. However, the problem with this coconut product is that the products on the market are always supplied as primary products without being processed into secondary or tertiary products [2]. This is the cause of the selling value of coconut in the market is still not maximized. Recently, the use of coconuts has become more varied, where one of the many

benefits of coconut fruit diversification products is Virgin Coconut Oil (VCO).

Virgin Coconut Oil (VCO) is coconut oil that is processed with minimal heating and without chemical refining. The utilization of VCO is usually used as a raw material in the food, pharmaceutical, and cosmetic industries [3]. VCO has many health benefits, such as high lauric acid content, including Medium Chain Fatty Acid (MCFA). MCFA can stimulate insulin production to activate glucose metabolism so that it runs normally [4]. Based on the article[5], Virgin Coconut Oil (VCO) contains

medium-chain fatty acids that are easily digested and oxidized by the body, so it can prevent accumulation in the body. VCO contains very high antioxidants such as tocopherol and beta-carotene. Antioxidants are substances that are able to neutralize free radicals. In addition, antioxidants are compounds that can inhibit oxidative reactions by binding free radicals and potentially harmful molecules with high activity, so that in the presence of antioxidants they can suppress cell damage.

The process of making VCO can be done in various ways such as the salting process, the fermentation process, and the enzymatic process. The enzymatic method is a way of producing VCO using the bromelain enzyme, which helps break down protein-oil bonds in coconut milk emulsions. Bromelain is found in many parts of the pineapple. During this enzymatic process, pineapple extract containing the bromelain enzyme breaks down the protein layer of the coconut milk emulsion, so that the oil is completely separated from the water [6]. The fermentation method is a process carried out with the help of microbes to be able to decompose organic compounds into new products [7]. One of the microorganisms that are often used is *Rhizopus* sp which in making VCO can break the protein-oil bonds in coconut milk [8].

In this applied research, the process of making Virgin Coconut Oil (VCO) uses a combination method of tempeh yeast fermentation by adding the enzyme bromelain from pineapple as a catalyst. The purpose of this research is to find a more effective method of making VCO. In addition, to determine the effect of variable concentrations of bromelain enzymes, tempeh yeast, and optimum fermentation time to produce VCO with good quality and quantity.

2. Methodology

2.1. Materials and Tools

The tools used in this research are beaker, measuring cup, digital scale, filter cloth, pipette, glass funnel, burette, clamps and stands, stirrer, basin, plastic, bracelet catheter, spoon, plastic cup, watch glasses, centrifuge, cuvette, and erlenmeyer. The main ingredients used in this research are coconut and tempeh yeast obtained from the Semarang area, Central Java, then the bromelain enzyme was obtained from an online chemical store. While other supporting materials such as alcohol (95%), PP indicator, and 0.1 N NaOH were obtained from the Industrial Chemical Engineering Technology Laboratory, Diponegoro University.

2.2. Methods

In this applied research, there are fixed variables including the ratio (coconut: aquadest) which is 1:1, incubation time of 2 hours, cream volume 500 mL, centrifugation time 10 minutes, and centrifugation speed 100 rpm. Meanwhile, to determine the independent variables used factorial design method 2^3 where there are 3 independent variables and each variable is taken 2 points. For more details, the variation of independent variables in this study can be seen in Table 1 below.

Table 1. Variation of Independent Variables with Factorial Design Method

Run	Bromelain Enzyme (gr)	Fermentation Time (hr)	Tempeh Yeast (gr)
1	3	24	2
2	6	24	2
3	3	30	2
4	6	30	2
5	3	24	4
6	6	24	4
7	3	30	4
8	6	30	4

The process of making Virgin Coconut Oil (VCO) in this study was carried out in several stages, including preparing tools and materials, making coconut milk, fermenting coconut milk, and analyzing results.

Preparation of Tools and Materials

Before the process of making VCO is carried out, it is necessary to prepare the tools and materials needed in advance, with the aim that the implementation of the process of making Virgin Coconut Oil (VCO) can run smoothly.

Making Coconut Milk Cream

Choose coconuts that are old and then grated. The grated coconut is then extracted by adding aquadest in a ratio (coconut:aquadest) of 1:1. Then take the coconut milk by squeezing it using a filter cloth. Leave the coconut milk for 2 hours until two layers form, the bottom layer is water and the top layer is cream or kanil, then take the cream at the top using a spoon and a pipette.

Fermentation Process

Enter the coconut cream into a 500 mL beaker, then add the tempeh yeast and the bromelain enzyme according to the different variables and stir until dissolved and homogeneous. Cover the beaker using plastic and let stand according to the variable fermentation time 24 hours or 30 hours with a temperature of 28 - 30 °C. After reaching the fermentation time is complete three layers will be formed where

coconut oil is on the top layer, in the second layer there is blondo or kanil dregs and the bottom layer is water. Then separate the VCO from the water and blondo with a pipette and then do the purification by centrifugation for 10 minutes.

Analysis of the resulting VCO

Quality parameters that will be observed on VCO (Virgin Coconut Oil) obtained from the research results include VCO yield, water content analysis test, free fatty acid analysis test (FFA), GCMS analysis test, and organoleptic test.

Yield

VCO yield is calculated by comparing the volume of VCO with the volume of coconut milk used in the process. The VCO yield can be calculated by the following formula:

$$Yield (\%) = \frac{VCO\ volume}{Coconut\ milk\ volume} \times 100\%$$

Moisture Content

How to calculate the moisture in the VCO, first by cleaning the empty cup in the oven and then cool it in a desiccator. Weigh the VCO sample and the cup as much as 2 grams on a digital balance. Next, the VCO and the cup were put in the oven for 3 hours and at a temperature of 105°C. After finishing in the oven and then cooled in a desiccator. Weigh the VCO that has been in the oven until a constant weight is obtained. The value of the moisture content of VCO is calculated by the following equation:

$$Moisture\ content (\%) = \frac{a - b}{a} \times 100\%$$

a : Initial weight before oven (sample + cup)

b : Weight after oven (sample + cup)

Free Fatty Acid (FFA)

Determination of free fatty acids in VCO can be done by weighing up to ± 30 grams of sample in a 250 ml Erlenmeyer. Add 50 ml of neutral 95% ethanol to the sample and 3-5 drops of pp indicator. Titrate the sample with 0.1 N NaOH standard until a constant pink color is obtained (no change for 15 seconds). Then weigh the mass of NaOH used and calculate according to the following formula:

$$FFA (\%) = \frac{V\ NaOH \times N \times BM}{1000 \times Sample\ Weight} \times 100\%$$

N : Normality of NaOH

BM : 40 (Molecular Weight of NaOH)

GCMS Analysis

Analysis with GCMS was carried out to determine the fatty acid composition of Virgin Coconut Oil (VCO). The GCMS test was carried out at the Diponegoro

University Integrated Laboratory. GC analysis provided a chromatogram showing the retention time and relative abundance of components in VCO. The components separated by GC were analyzed by MS and the mass/charge curve (m/e) was generated as a function of relative abundance. To find compounds in Virgin Coconut Oil (VCO), it is done by matching the existing spectrum with the spectrum in the tool [9].

3. Results and Discussion

Research on the process of making VCO using a combination method between tempeh yeast fermentation and the addition of the bromelain enzyme obtained products that can be seen in Table 2. Determination of variables in this study using the Factorial Design method with three variables changing, including bromelain enzyme concentration, tempeh yeast mass, and fermentation time, in which each variable is taken 2 levels/point.

Table 2. Results of Making VCO with Variations in Bromelain Enzyme Concentration, Tempeh Yeast Mass, and Fermentation Time

Run	Results			
	Yield (%)	FFA (%)	Moisture Content (%)	GCMS
1	6.5	0.34	-	-
2	7.2	0.39	-	-
3	8.2	0.22	-	-
4	9.2	0.21	0.24%	Attached
5	8.9	0.33	-	-
6	9.1	0.32	-	-
7	7.4	0.30	-	-
8	7.2	0.35	-	-

3.1. Yield

In this study, the most influential variables can be identified using the Quicker Method. This method was used to calculate the main effect as well as the interaction effect on VCO yield [10]. Tables 3 and 4 below show the results of the calculation of the variables that most influence the yield value of VCO.

Table 3. Results Main Effects and Interaction Effects on Virgin Coconut Oil (VCO) Yield Value

Effect	Value
K	0.425 → Main Effect
T	0.075
M	0.375
kt	-0.025
km	-0.425
tm	-1.775 → Interaction Effects
ktm	-0.175

Table 3 above shows that the main effect in this study was the concentration of the bromelain enzyme with a value of 0.425 with the interaction effect of fermentation time and tempeh yeast mass of 1.775.

Table 4. Determination of Variables Affecting the Yield Value of Virgin Coconut Oil (VCO)

Effect Identity	P (%)	Effect
Tm	7.1428571	-1.775
Km	21.428571	-0.425
Ktm	35.714286	-0.175
Kt	50	-0.025
T	64.285714	0.075
M	78.571429	0.375
K	92.857143	0.425

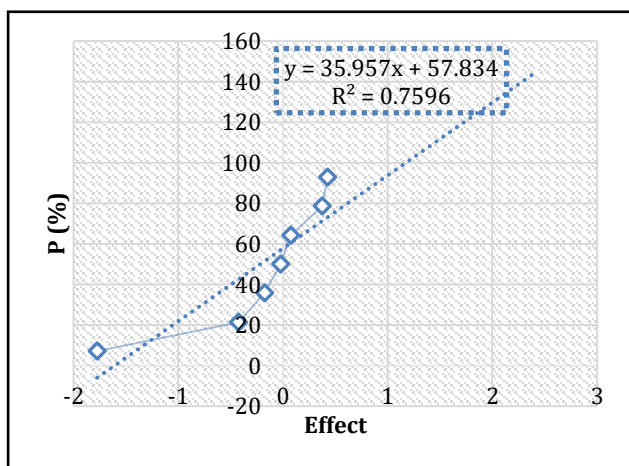


Figure 1. Normal Probability Plot Graph of VCO Yield Value for Design Factorial 2³

Based on Figure 1 above shows the Normal Probability Plot graph between the P value and the effect [11]. By activating the Trendline feature in Microsoft Excel, a regression (R²) of 0.7596 is obtained. This means that 75.96% of the total variation of the model can be represented by a regression equation that shows the relationship between VCO yield and the variables of the research process (bromelain enzyme concentration, fermentation time, and tempeh yeast mass) is $y = 35.957x + 57.834$. So it can be concluded that the variable that greatly affects the yield value of the obtained VCO is the bromelain enzyme concentration.

Based on the results of the analysis that has been carried out, it shows that the amount of VCO yield is influenced by the presence of the bromelain enzyme in the process of making the VCO. This is evidenced by the highest yield of VCO obtained with the addition of the enzyme bromelain 6 gr, fermentation time of 30 hours, and using 2 gr of tempeh yeast, with a yield value of 92

mL or about 9.2%. From these results, it can be said that the higher the concentration of bromelain enzyme added, the higher the yield. The addition of pineapple stems containing the bromelain enzyme to coconut milk during fermentation can result in higher VCO yields [6]. This may be because the higher the bromelain enzyme concentration, the more protein substrates will react with the bromelain enzyme. However, the increase in yield is not always proportional to the amount of enzyme added. The amount of enzyme added must be proportional to the amount of substrate because the enzyme activity reaches its maximum value at a certain enzyme concentration. This is also reinforced by other research which says that one thing that can speed up enzymatic reactions is by adding enzyme concentrations to certain substrates [12].

3.2. Free Fatty Acid (FFA)

The most influential variables on the content of Free Fatty Acids (FFA) which were analyzed through the calculation of the main effect and the interaction effect of the Quicker Method are presented in Tables 5 and 6, it is shown that the fermentation time is the main effect in this research where the value is 0.06625 while the interaction effect in this study is the fermentation time and the mass of the tempeh yeast with a value of 0.06125.

Table 5. Results Main Effects and Interaction Effects on the Value of Free Fatty Acids of Virgin Coconut Oil (VCO)

Effect	Value
K	0.00875
T	-0.06625 → Main Effect
M	0.04875
Kt	0.01125
Km	0.01125
Tm	0.06125 → Interaction Effects
Ktm	0.01875

Table 6. Determination of Variables Affecting the Content of Free Fatty Acids (FFA) Virgin Coconut Oil (VCO)

Effect Identity	P (%)	Effect
T	7.142857143	-0.06625
K	21.42857143	0.00875
Kt	35.71428571	0.01125
Km	50	0.01125
Ktm	64.28571429	0.01875
M	78.57142857	0.04875
Tm	92.85714286	0.06125

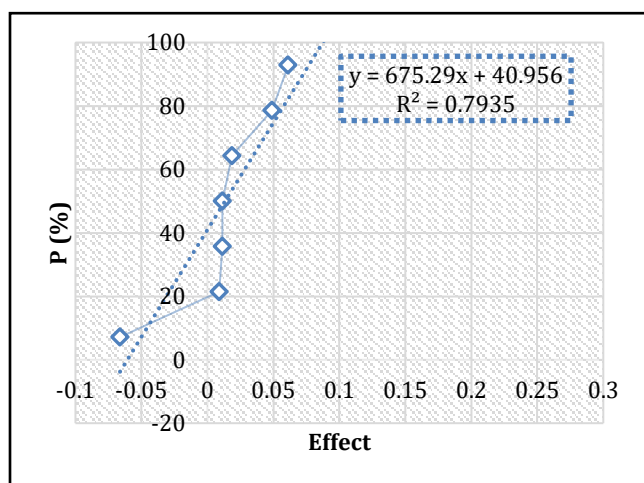


Figure 2. Normal Probability Plot Graph of Free Fatty Acid Content of VCO for Design Factorial 2³

Based on Figure 2 above shows the Normal Probability Plot graph between the P value and the effect [11]. By activating the Trendline feature in Microsoft Excel, a regression (R²) of 0.7935 is obtained. This means that 86.19% of the total variation of the model can be represented by a regression equation that shows the relationship between the content of Free Fatty Acids (FFA) and the parameters of the research process (bromelain enzyme concentration, fermentation time, and tempeh yeast mass) is $y = 675.29x + 40.956$. Therefore, on the free fatty acid content produced, it can be concluded that the fermentation time is the most influential variable on the value of Free Fatty Acids.

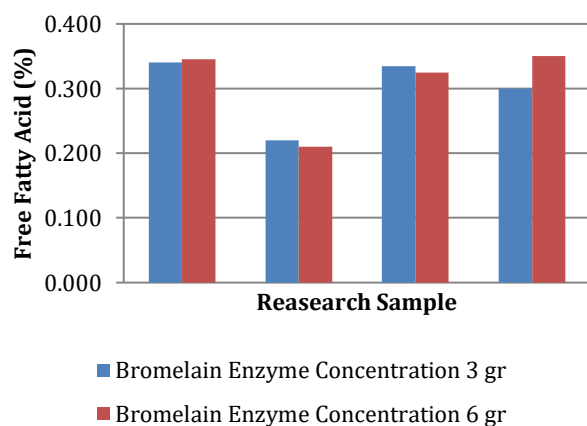


Figure 3. Effect of Bromelain Enzyme Concentration on Free Fatty Acid Content

Based on Table 1, it can be seen that the FFA content in VCO which has the highest value is produced by sample 8 with a bromelain enzyme concentration of 6 gr, fermentation time of 30 hours, and tempeh yeast 4 gr.

While the lowest FFA levels were obtained in sample 4 with a bromelain enzyme concentration of 6 grams, fermentation time of 30 hours, and tempeh yeast 2 grams. Based on Table 1 above, in addition to the bromelain enzyme concentration variable, the fermentation time also greatly affects the free fatty acid content contained in the resulting VCO which affects the quality of the VCO. Because the higher the value of the acid number, the lower the quality of the VCO.

3.3. Fatty Acid Content

This happens because the longer the fermentation is carried out, the greater the hydrolysis of the oil that occurs. Based on SNI 7381:2018 regarding Virgin Coconut Oil (VCO) for the parameter FFA value, which is a maximum of 2.0% [13]. Meanwhile, in the results of the study, the best free fatty acid (FFA) content and closest to SNI 7381:2018[13] is in sample 4 where the FFA value is 0.21%. Based on the article [14], the formation of free fatty acids is caused by the hydrolysis process in the oil. Then the cause of the hydrolysis of the oil is due to the presence of water in the oil, besides that the storage time also affects the hydrolysis of the oil. It is more likely that the oil will be hydrolyzed if it is stored for a long time [12].

Analysis of fatty acid content was carried out on VCO resulting from the best-operating conditions, namely in sample 4. The analysis was carried out using the GCMS method to determine the fatty acid content of VCO. Analysis with GCMS is considered effective because it does not require a longer retention time and the results can be proven accurate. GCMS test results data for VCO results are shown in Table 7.

Table 7. Results of Analysis of Fatty Acid Composition in VCO with GCMS Method

Fatty Acid Components	Structural Formula	Content (%)
Saturated Fatty Acid		
Caproic acid	C ₆ H ₁₂ O ₂	0.78
Caprylic acid	C ₈ H ₁₆ O ₂	11.99
Capric acid	C ₁₀ H ₂₀ O ₂	9.41
Lauric acid	C ₁₂ H ₂₄ O ₂	54.27
Myristic acid	C ₁₄ H ₂₈ O ₂	14.47
Palmitic acid	C ₁₆ H ₃₂ O ₂	0.21
Stearic acid	C ₁₈ H ₃₆ O ₂	2.73
Unsaturated Fatty Acid		
Oleic acid	C ₁₈ H ₃₄ O ₂	0.54
Linoleic acid	C ₁₈ H ₃₂ O ₂	4.82

Based on Table 7 above, it can be seen that the highest fatty acid content in the resulting VCO is lauric acid, which is 54.27%. The lauric acid component of VCO has many benefits, including smoothing and moisturizing the skin, removing dead skin cells, and strengthening skin tissue to prevent sagging and wrinkles [15]. Lauric acid also has other functions, including lauric acid is converted into monolaurin in the body. Monolaurin is an antiviral, antibacterial, and antigenic monoglyceride compound that is used by the human immune system to digest viruses, bacteria, and protozoa [14].

From the results of the GCMS analysis above, it can be said that the VCO produced contains 76.45% Medium Chains Fatty Acid (MCFA) consisting of 0.78% caproic acid, 11.99% caprylic acid, 9.41% capric acid, and 54.27% lauric acid. While the rest contain Long Chains Fatty Acid (LCFA).

In addition to analyzing the fatty acid content, moisture content testing was also carried out under the best-operating conditions. Testing the moisture content is very important because it affects the quality of the VCO. The higher the water content in the oil, the shorter its shelf life will tend to be [16]. The moisture content in the oil is related to the hydrolysis reaction. When fats and oils contain water, they are hydrolyzed to produce free fatty acids and glycerol, making the oil more susceptible to rancidity [17]. Based on SNI 7381:2018 [13] regarding Virgin Coconut Oil (VCO) for the moisture content parameter, which is a maximum of 0.2%. Meanwhile, from the results of testing the moisture content in the best sample, namely sample 4, it was obtained that the moisture content was 0.24%.

3.4. Organoleptic Test

In this research, organoleptic tests were also carried out to determine the quality of the VCO produced. The results of the organoleptic tests are shown in Table 8.

Table 8. Organoleptic Test Results on Virgin Coconut Oil (VCO) Produced

Sample	Organoleptic Test Results		
	Smell	Taste	Color
1	Coconut scent	Taste like coconut	Colorless and clean
2	Coconut scent	Taste like coconut	Colorless and clean
3	Coconut scent	Taste like coconut	Colorless and clean
4	Coconut scent	Taste like coconut	Colorless and clean

5	Coconut scent	Taste like coconut	Colorless and clean
6	Coconut scent	Taste like coconut	Colorless and clean
7	Coconut scent	Taste like coconut	Colorless and clean
8	Coconut scent	Taste like coconut	Colorless and clean

The VCO from the research results is compared with the standards set in SNI 7381:2018 Virgin Coconut Oil (VCO) [13] where the standard for the organoleptic test for the smell of VCO must have a normal smell, which is typical of fresh coconut oil and not rancid. VCO color standard has no color to pale yellow. In addition, for other standards, such as the maximum moisture content of 0.2%, the maximum free fatty acid content of 0.2%, and the lauric acid content of 45.1 - 53.2%. As for the test results in the research conducted for the organoleptic test of 8 samples, the results were the same which had a distinctive smell of coconut aroma, a distinctive taste of coconut oil, and was colorless. Then for the other tests in this study, the moisture content was 0.24%, the FFA content was 0.21% and the lauric acid content was 54.27%. From the results of the tests that have been carried out for the organoleptic test of smell, taste, and color, it is in accordance with the standard, while for the test of moisture content, free fatty acid, and lauric acid content, it is still not according to SNI but it is close. So it can be concluded that the effect of all variables does not increase the value of free fatty acids which can cause rancid odor in coconut oil, does not contaminate VCO which can cause foreign taste in coconut oil, and does not damage the pigments of coconut fruit so as to produce good VCO color quality.

4. Conclusion

Based on the VCO produced in this applied research, the best treatment was obtained namely, in sample 4 with the variable addition of the enzyme bromelain 6 gr, fermentation time 30 hours, and the addition of 2 gr tempeh yeast with a yield of 9.1%, the free fatty acid content of 0.21%, 0.24% water content, the largest fatty acid content is lauric acid 54.27%, and the results of the organoleptic test on VCO research results have a distinctive smell of coconut aroma, a distinctive taste of coconut oil and colorless. These results are close to the standard of SNI 7381:2018 Virgin Coconut Oil (VCO) [13] with the requirements for the maximum water content of 0.2%, a maximum free fatty acid of 0.2%, a

maximum lauric acid content of 45.1–53.2%, and for testing Organoleptic has a normal smell, which is typical of fresh coconut oil and is not rancid, has a distinctive taste of coconut oil, and has no color to pale yellow.

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