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Utilization of Papain Enzymes on the Production of Virgin Coconut Oil

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Abstract - *Coconut is a tropical plant commonly found in Indonesia which can be used to make virgin coconut oil that has many benefits for the human body. Virgin Coconut Oil can be applied in the cosmetic, culinary and health industries. VCO contain less calories compared to other fats as it can accelerate the healing of diseases and obesity. One of method that can be used to produce VCO is by enzymatic process. VCO produced from this enzymatic process has advantages, among others, the fat content in VCO does not change much so that its properties are maintained, it is not easily rancid. One of the enzymes that can be used in the process of making VCO is papain enzyme. The purpose of this study was to determine the most influential variable on the making of virgin coconut oil. The research method used is factorial design 2^3 with variable temperature, enzymatic time, and concentration of papain enzymes. The research data were processed using minitab19 software. The result will be analyzed for water content and free fatty acid. The results obtained from the study, the most influential effect on water content and free fatty acids were time and enzyme concentration variables, while the most influential interaction variables were fermentation time and temperature. The optimum water content obtained is 0.18% and free fatty acids are 0.28% which is in accordance with APCC and* SNI 7381-2008. *The optimum combination of independent variables that can produce optimum water content and free fatty acids is papain enzyme concentration of 3gr, temperature 33°C, and fermentation time of 24 hours.*

Keywords: *virgin coconut oil; papain enzyme; factorial design; enzymatic*

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INTRODUCTION

Coconuts are widely used by the people of Indonesia, both those that have been processed previously or are still in the form of coconuts as daily consumption.

Coconut tree is a tropical plant commonly found in Indonesia. This fruit has many benefits for society such as in the culinary, cosmetic, and health industry (Ghani et al., 2018). All parts of the coconut fruit can be used as food. One of them can be converted into coconut milk which is commonly used as an ingredient in Indonesian cuisine. However, apart from being used as a food ingredient, coconut milk can also be processed into Virgin Coconut Oil (VCO) (Rachmayanti et al., 2020).

Virgin Coconut Oil (VCO) is oil produced from coconuts. The manufacturing process is carried out at low temperatures and does not use organic chemicals and oil solvents (Srivastava et al., 2018). VCO has low water content and free fatty acid content, smells good, and has a long shelf life (Kappally et al., 2015). Silaban et al., (2014) said that when compared with other vegetable oils such as palm oil, soybean oil, corn oil and sunflower oil. VCO products contain about 64% medium chain saturated fatty acids consisting of more than 50% lauric acid (C12), 6–7% capric acid (C10), and 8% caprylic acid (C8) (Sulistio & Poeloengan, 2009).

VCO is an oil that has many benefits for the human body (Deen et al., 2021). In the field of Health, VCO can function to increase the human body's resistance and accelerate the healing of disease and obesity (Hardi et al., 2021).

Making VCO can be done in various ways, one of which is by enzymatic method (Rohman et al., 2021). The process of making VCO enzymatically can take advantage of the help of various enzymes (Yan et al., 2021). VCO produced from this enzymatic process has advantages, among others, the fat content in VCO does not change much so that its properties are maintained, it is not easily rancid because the fatty acid composition does not change much and the yield is high (Fajrin, 2012). One of the enzymes that can be used in the process of making VCO is papain enzyme derived from papaya fruit (Mamboya, 2012).

Enzymes are proteins that catalyze chemical reactions and affect the rate of reactions but do not take part in the reactions. Enzymes act as biocatalysts (Worku, 2018). The main components of enzymes are protein molecules (polypeptides) (Moeksin et al., 2008).

Papain enzyme is an enzyme found in papaya latex or leaves which is a type of proteolytic enzyme, namely an enzyme that catalyzes the reaction of breaking down polypeptide chains in proteins by hydrolyzing their peptide bonds into simpler compounds such as dipeptides and amino acids (Tigist et al., 2016). This hydrolysis reaction causes the emulsion system to become unstable so that the bound oil becomes separated from the emulsion system and clumps together (Winarno, 1989).

In the process of making VCO enzymatically, one of the things that really affects is the number of enzymes used in the process of making VCO itself. The purpose of this study was to determine the most optimum variable on the making of virgin coconut oil.

METHODOLOGY

Materials and Tools

The materials used in this study were coconut milk, papain enzyme, water, NaOH 0.1N, pp indicator, 96% ethanol. While the tools used include analytical balance, basin, filter cloth, glass beaker, centrifuge, burette, stative, spoon, oven, measuring cup, Erlenmeyer, and plastic bottles.

Method

Preparation

At the preparing stage, the ingredients that need to be prepared are coconut fruit and papain enzymes. The coconut used is old coconut which is sold in the market for the coconut cream, while the papain enzyme used is the extracted papain enzyme which is sold in the market as a meat tenderizer. At the stage of making coconut cream, fresh grated coconut is mixed with a little water, then squeezed to obtain thick coconut milk. This is repeated up to 5 times in order to obtain maximum coconut milk. The obtained coconut milk is then deposited for 1 hour so that the cream and water are separated.

Fermentation of Virgin Coconut Oil

150mL coconut cream then added with papain enzyme as much as 3 gr/mL and 5 gr/mL then fermented at 29ºC and 33ºC, and 24 hours and 48 hours. Coconut milk that has been fermented will separate and form 3 layers. The coconut oil is then taken and put into a centrifuge to separate the oil from the water

Water Content

Measurement of water content in this study using the oven method (evaporation). The empty of porcelain cup was heated in the oven for 15 minutes and cooled in a desiccator for 15 minutes then weighed to a constant weight. After that, 5gr of the sample was put in a porcelain cup. The cup and its contents were heated in an oven at a temperature of 105-110ºC for 3-5 hours. Then the cup was transferred to a desiccator for 15 minutes. After cooling, it was weighed again, then dried again for 30 minutes and repeated again until it got a constant weight (Sudarmaji et al., 1984).

Free Fatty Acid

Weigh 10gr of sample into a 250mL Erlenmeyer. add 50mL of 96% ethanol. Add 3 drops of PP indicator and titrate with 0.1 N NaOH until it's color pink.

RESULTS AND DISCUSSION

In this study, the 2^3 factorial design method was used by running the experiment 8 times to determine the effects on the process variables used and the optimum conditions obtained were more precise because they included interaction factors. The most influential variables in this study can be identified by factorial design analysis using the Minitab19 application which calculates the main effects and interaction effects on the water content and free fatty acids produced.

Factorial Design Analysis of Water Content

Analysis of research results used to determine water content with independent variables using factorial design analysis on the Minitab 19 application. This analysis aims to determine the most influential variable in producing water content in the study.

Table 1. Main effects and interaction effects of factorial design water content

Term	Effect
Papain (gr)	0.09250
Temperature (ºC)	-0.00250
Time (hr)	0.12250
Papain (gr)*Temperature (ºC)	0.02250
Papain (gr)*Time (hr)	0.02750
Temperature (ºC)*Time (hr)	0.05250

The calculation of the main effects and interaction effects in this study uses the Minitab19 application. From the table above shows the main effect on water content in this study is time with a value of 0.1225. While the most influential interaction effect is temperature and time, which is 0.052.

Table 2. Model summary of the water content factorial design

S	R-sq	R-sq(adj)	R-sq(pred)
0.0035355	99.98%	99.84%	98.55%

The summary model results obtained from the analysis using the Minitab 19 application, obtained an R-square value of 99.84%. This R-square value can determine whether the experimental design made has explained the variables used. Yulianto et al., (2018) explained that the closer the R value to 1, the better the model is in predicting a response. In the summary model results using the Minitab application, it shows that the results of the water content are influenced by the independent variables in the study

Table 3. Analysis of Variance factorial design water content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	0.055175	0.009196	735.67	0.028
Linear	3	0.047138	0.015713	1257.00	0.021
Papain (gr)	1	0.017113	0.017113	1369.00	0.017
Temperature (^oC)	1	0.000012	0.000012	1.00	0.500
Time (hr)	1	0.030012	0.030012	2401.00	0.013
2-Way Interactions	3	0.008037	0.002679	214.33	0.050
Papain (gr)*Temperature (^oC)	1	0.001012	0.001012	81.00	0.070
Papain (gr)*Time (hr)	1	0.001512	0.001512	121.00	0.058
Temperature (^oC)*Time (hr)	1	0.005512	0.005512	441.00	0.030
Error	1	0.000013	0.000013		
Total	7	0.055188			

From table 3. We can see P value <0.05 indicates that the variable has a significant effect on the results of the water content, while the P value> 0.05 means that the variable has no significant effect. From the results of the data analysis, a Pareto diagram can be made that can identify which variables have the most significant effect in determining the water content of the experimental results.

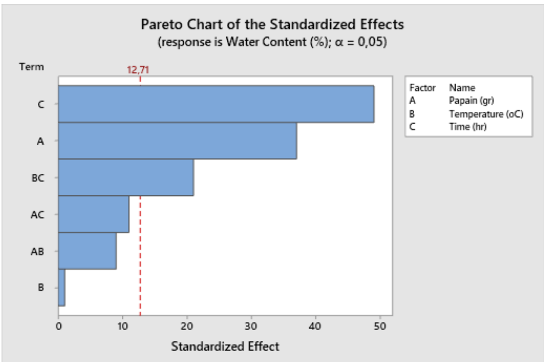

Figure 1. Pareto chart of the Standard effect of water content

From figure 1. it can be seen that time has the most significant effect on the results of water content in the experiment. As for the interaction variable that gives the most significant influence in determining the results of the water content, namely the interaction between temperature and time.

Factorial Design Analysis of Free Fatty Acid

Table 4. Main effects and interaction effects of FFA factorial design

Term	Effect
Papain (gr)	0.08500
Temperature (^oC)	-0.00500
Time (hr)	0.12000
Papain (gr)*Temperature (^oC)	0.01500
Papain (gr)*Time (hr)	0.02000
Temperature (^oC)*Time (hr)	0.05000

For the calculation of the main effects and interaction effects in this study using the Minitab19 application. The table above shows that the main effect on free fatty acids in this study was time with a value of 0.12. While the most influential interaction effect is temperature and time, which is 0.05.

Table 5. Model summary of the water content factorial design

S	R-sq	R-sq(adj)	R-sq(pred)
0.0141421	99.60%	97.19%	74.27%

From the model summary table above, the results obtained from the analysis using the Minitab19 application, obtained an R-square value of 99.6%. This R-square value can determine whether the experimental design made has explained the variables used. Yulianto et al., (2018) explained that the closer the R value to 1, the better the model is in predicting a response. The results of the summary model using the Minitab application show that the results of free fatty acids are influenced by the independent variables in the study.

Table 6. Analysis of Variance factorial design of free fatty acids

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	0.049550	0.008258	41.29	0.119
Linear	3	0.043300	0.014433	72.17	0.086
Papain (gr)	1	0.014450	0.014450	72.25	0.075
Temperature (ºC)	1	0.000050	0.000050	0.25	0.705
Time (hr)	1	0.028800	0.028800	144.00	0.053
2-Way Interactions	3	0.006250	0.002083	10.42	0.223
Papain (gr)*Temperature (ºC)	1	0.000450	0.000450	2.25	0.374
Papain (gr)*Time (hr)	1	0.000800	0.000800	4.00	0.295
Temperature (ºC)*Time (hr)	1	0.005000	0.005000	25.00	0.126
Error	1	0.000200	0.000200		
Total	7	0.049750			

From the table of analysis of variance obtained the value of the experiment. Where the value shows if the P value <0.05 indicates that the variable has a significant effect on the results of free fatty acids produced, while the P value >0.05 means that the variable has no significant effect. From the results of the data analysis, a Pareto diagram can be made which can identify which variables have the most significant effect in determining the free fatty acids of the experimental results.

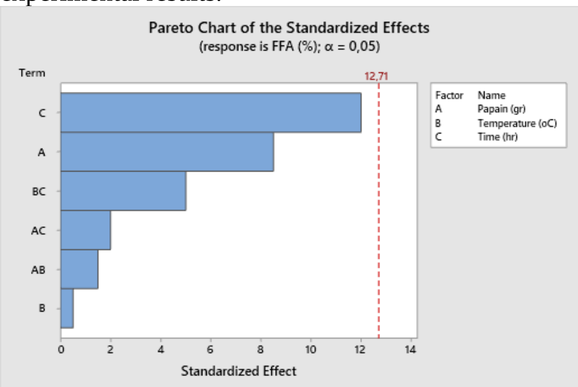

Figure 2. Pareto chart of Standard Effects of Free Fatty Acids

From the Pareto diagram of free fatty acids above, it can be seen that the fermentation time is the most influential variable on the results of free fatty acids in the experiment. However, this variable does not provide a significant value according to the hypothesis of the resulting free fatty acids. Likewise, the interaction variables of temperature and fermentation time have the main influence in producing free fatty acids, but they do not provide a significant value according to the hypothesis of free fatty acids.

Table 7. Optimization Response Table

Solution	Papain (gr)	Temperature (ºC)	Time (hr)	Water Content (%) Fit	FFA (%) Fit	Composite Desirability
1	3	33	24	0.18125	0.285	0.988560

Table 7. shows the optimization response of the study. The critical value for VCO optimization is obtained by the ratio of papain enzyme concentration of 3gr; fermentation temperature 33ºC; With a fermentation time of 24 hours, the water content was 0.18% and free fatty acids were 0.28%.

Water Content Analysis

Table 8. Water Content of Virgin Coconut Oil

Parameter	VCO Experiment	APCC Standard VCO	SNI 7381-2008
Water content (%)	0.18	Max 0.5	Max 0.2

Based on the results obtained from the study, the optimum water content obtained was 0.18%. This result is still in accordance with the APCC Virgin Coconut Oil which is a maximum of 0.5% and SNI 7381-2008 a maximum of 0.2%. In a study conducted by Perdani et al., (2019) the results of the water content in the manufacture of VCO using crude papain enzymes were obtained as 0.13%. The results of the water content in this study were better than our study. This can be happen because in a study conducted by Perdani et al., (2019) the process of breaking the coconut cream emulsion was more effective than that carried out by our research, so the ability to separate the bonds between oil and coconut milk was more perfect, so that the oil layer was easily separated from coconut curd and water which causes lower water content (Sastono & Verdial, 2008).

The water content of the VCO greatly affects the quality of the oil produced (Choe & Min, 2006). The greater the value of the water content, the worse the quality of the oil produced because high water content can cause rancidity and make the shelf life of the oil short (Aji, 2016). The water content of this study was influenced by the enzymatic time and the concentration of enzymes used. The longer the enzymatic time, the higher the water content produced. In addition, the increase in the concentration of papain enzymes will also increase the value of the water content in the oil. The increasing water content of VCO can be caused by the process of breaking down the coconut cream emulsion does not take place effectively due to the high concentration of enzymes so that the ability to separate the oil bonds with coconut cream residues and water is not perfect (Sastono & Verdial, 2008).

Free Fatty Acid Analysis

Table 9. Free Fatty Acid of Virgin Coconut Oil

Parameter	VCO Experiment	APCC Standard VCO	SNI 7381-2008
Free Fatty Acid (%)	0.2	Max 0.5	Max 0.2

Free fatty acids are an influential indicator in determining oil quality because they are related to oil damage that occurs as a result of hydrolysis and oxidation reactions (Muharun, S.TP, 2014). These free fatty acids are formed from the chemical reaction of hydrolysis which is accelerated in the presence of water in the material (Harni & Putri, 2014). The optimum result obtained from the research is the free fatty acid content of 0.28%. Previous research by Pramitha & Juliadi, (2019) made VCO by fermentation obtained free fatty acids of 0.11. Meanwhile, in the study of Effendi et al., (2012) who made VCO using the bromelain enzyme, the free fatty acids obtained were 0.25%. Both our research and previous studies have found that the free fatty acid levels are in accordance with the free fatty acid levels according to the APCC, namely a maximum of 0.5% and SNI 7381-2008 a maximum of 0.2%.

In previous studies, the results of free fatty acids were better than our research. This is influenced by the optimum research variable, namely the fermentation time. Increased levels of free fatty acids can occur due to the hydrolysis reaction of the oil. The hydrolysis reaction of coconut oil will occur quickly under the influence of heat, water, acidity, and a catalyst in the form of enzymes. If this hydrolysis reaction lasts longer, the more levels of free fatty acids will be formed (Pramitha & Juliadi, 2019). Therefore, the longer the fermentation time, the higher the free fatty acids produced. It is also influenced by the concentration of enzymes used. The more papain enzymes used, the more FFA will produced. This is because the more enzymes used in fermentation, the greater the hydrolysis of triglycerides that occur due to damage to oil or fat (Effendi et al., 2012).

In addition, the water content also plays a role in determining the value of the acid number. The higher the water content, the faster the hydrolysis of coconut oil which will produce free fatty acids (Raharja & Dwiyuni, 2008).

CONCLUSION

The study used a 2^3 factorial design analysis using the Minitab19 application. The results showed that the most influential effect on water content and free fatty acids was the time variable, while the most influential interaction variables were fermentation time and temperature. The optimum water content obtained is 0.18% which is in accordance with the APCC VCO, which is a maximum of 0.5% and SNI 7381-2008 a maximum of 0.2%. The optimum free fatty acid produced is 0.28% which is also in accordance with the APCC VCO standard, which is a maximum of 0.5% and SNI 7381-2008 a maximum of 0.2%. The optimum combination of independent variables that can produce optimum water content and free fatty acids is papain enzyme concentration of 3gr, temperature 33ºC, and fermentation time of 24 hours.

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