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Modification of the Fermentation Process and Papain Enzymes in The Manufacture of Virgin Coconut Oil Using Optimization of Response Surface Methodology, Central Composite Design

Jakfar [1,*], Husni Husin [1], Komala Pontas [1], Rizalman Mamat [2], Mohd Rosdi Salleh [3], Mirna Zulrika [1] and Ahmadi [1]

[1] Department of Chemical Engineering, Faculty of Engineering, Universitas Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia; husni_husin@che.unsyiah.ac.id (H.H.); komalapontas@usk.ac.id (K.P.); mirna63@mhs.unsyiah.ac.id (M.Z.); ahmadiahmadi840@gmail.com (A.)
[2] Faculty of Mechanical Engineering, University Malaysia Pahang, Pekan 26600, Pahang, Malaysia; rizalman@ump.edu.my
[3] Automotive Technology Center (AteC), Politeknik Sultan Mizan Zainal Abidin, Dungun 23000, Terengganu, Malaysia; rosdi.salleh@psmza.edu.my
* Correspondence: jakfar@che.unsyiah.ac.id

Abstract: This research focuses on optimizing fermentation modification and papain enzymes. In manufacturing virgin coconut oil using the response surface methodology (RSM), the experiment was carried out based on the Central Composite Design (CCD). Coconut oil yield, as a function of crude papain enzyme (CPE) mass (0–1 g) and fermentation time (12–60 h), was observed for 13 runs. The yield of virgin coconut oil with natural fermentation without the addition of crude enzyme papain is 19%, and with the addition of 1 g of the enzyme, it can reach a maximum of 27.7%. Optimal conditions were obtained at a mass of crude papain enzyme 993.5 mg, fermentation time 60 h respectively, yields: Banda Aceh virgin coconut oil (BAVCO) 28.4%, Pidie Jaya virgin coconut oil (PJVCO) 25.6%, and Bireuen virgin coconut oil (BVCO) 24.7%, quality of virgin coconut oil (VCO): water content (WC) 0.047%, free fatty acid (FFA) 0.01%, and peroxide (PN) 0.024% from each of the total mass of 300 g grated coconut, VCO modified by fermentation with the addition of CPE increased the yield. The quality of VCO in terms of WC, FFA, and PN meets the VCO quality standard. The optimization for desirability was 0.998, and the product had a transparent color, a distinct aroma, and did not have a putrid aroma.

Keywords: optimization; fermentation modification; papain enzyme; response surface methodology; central composite design

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1. Introduction

Coconut is a plant that can thrive in tropical areas such as Aceh Province, Indonesia. One of the most critical uses of coconut fruit is to extract oil. In general, there are two kinds of coconut oil manufacturing processes, namely wet and dry processes. Wet processes are usually on a small scale or in the home industry, and dry processes are on a large scale or industrial scale [1–3]. The wet process is carried out by heating the coconut milk extracted from grated coconut with water so that the oil is separated from the dregs. The disadvantages of this method are that there is still much residual oil in the dregs, the oil produced tends to have a rancid smell, the use of high temperatures, and excess fuel [3,4]. Another alternative for making coconut oil is a dry process without heating, namely pressing. This method is relatively more efficient but cannot produce oil optimally and eliminate rancidity. Therefore, this study was conducted to modify it by adding crude papaya enzymes (crude papain) from young papaya latex [2,5–8]. Papaya sap has enzymatic properties as a catalyst that can break down proteins [9–12]. Further exploration of the

potential of the papain enzyme for dissociating fermented coconut oil products showed that the papain enzyme was effective in enumerating *Listeria monocytogenes*. Furthermore, gelatinolytic activity and at least partial collagenolytic activity and stability under procedure conditions make papaya latex protease able to increase coconut oil yield [13–15].

Enzyme pre-extraction treatment of copra meal was introduced into the rural wet copra oil extraction process to investigate the possibility of enhancing the traditional process with enzyme treatment. The enzymes used in this study were a combination of proteases from *Aspergillus niger*, cellulase/hemicellulase preparations from *Trichoderma reseei*, amylase from *A. oryzae*, and pectinase from *A. niger* (all raw preparations). Finely ground copra meal samples were mixed with water and enzymes and incubated at 37 °C for 6 h or more. After treatment, the cake was extracted using a water flotation technique. An increase in yield of about 50%, relative to experimental controls, was observed, indicating the possibility of using an enzyme-assisted oil extraction method to improve traditional copra processing [10,16,17]. Existing coconut oil extraction methods include the cold extraction process, which has the problem of producing a poor yield [9,18,19]. In the hot extraction procedure, antioxidant capabilities are diminished if the temperature exceeds 40 °C [4,6]. Low-pressure extraction has greater capital costs [20]. Chilling, freezing, and thawing has significant operating costs since the temperature must be lowered below 0 °C [18,21]. Natural fermentation necessitates an extended extraction period [22]. The advantages of induced fermentation include its ease of processing and high yield, while its downsides are a decrease in the quality of coconut oil, a lengthy extraction time, and a fermented odor [18,23]. The method of centrifugation has the disadvantage of a low yield, while the advantage of enzymatic extraction is its high yield [24]. Modification of the extraction process by fermentation is expected to increase the quality and quantity of coconut oil.

This research lies on the raw material of coconut from Aceh, which was taken from Rieng Krueng Village, Meureudu District, Pidie Jaya Regency, and Rukoh Village, Syiah Kuala District, Banda Aceh City, Republic of Indonesia. The novelties of this research include the chemical composition of coconuts, and physical properties, such as smell, taste, and color. The place of the coconut grew, the weather, rainfall, and temperature. Previous studies have not used VCO from coconuts with different locations. The effects of enzymes and fermentation time for the three coconut types evaluate by using analysis of variance (ANOVA). Chemical composition varies from one location to another, as well as in other countries. The difference in oil content depends on the environment and where the coconut grows, and the type [25]. Likewise, crude papaya, as a source of papaya enzymes, was taken from Saree, Aceh Besar District, Aceh Province. It also has a different chemical composition compared to other places or other countries. This research is the first to use papaya enzymes in Saree Aceh Besar, Aceh province. The type of papaya used is weasel papaya which is entirely unknown. It is also a new modification that has never been done before.

Another difference lies in the modification of natural and enzymatic fermentation with experimental independent variables of enzyme mass (crude papain, 0–1 g) and curing time (12–60 h). The enzymatic fermentation modification technique uses multi-component variables and interactions of each. For each of these individual influences, the application of the response surface methodology (RSM) provides a highly efficient design and broad utilization of the optimized parameters. In addition, individual and interaction factors influence the choice of RSM as the preferred alternative. RSM, as a design technique, evaluates the effects of individual factors and interactions to achieve optimal conditions for a finite number of desired responses planned trial [26]. In this study, the optimal conditions for increasing the yield of virgin coconut oil from both Pidie Jaya and Banda Aceh locations and using crude papain enzyme from Saree Aceh Besar were optimized using RSM, CCD expert design version 13.

2. Materials and Methods

2.1. Production of Coarse Papain Enzymes

Papayas were obtained from Saree Village, Lembah Seulawah District, Aceh Besar District, Aceh Province, Indonesia. First, extraction of papaya latex was carried out by scratching the skin of the papaya fruit as much as four strokes with a depth of two mm, the sap that came out was accommodated in the reservoir, and then $NaHSO_3$ 0.7% fresh weight was added. Then the sap is dried in an oven at 60 °C. Finally, the dried papaya sap as a slab is made into flour using a blender and then sieved to obtain a 100-mesh size of coarse papain enzyme [7,11,13,27].

2.2. Virgin Coconut Oil Synthesis

Coconuts were obtained from Rhieng Krueng Village, Meureudu District, Pidie Jaya Regency, Rukoh Village, Syiah Kuala District, Banda Aceh City, Aceh Province, Indonesia. The first step involves the preparation of samples. The coconut flesh was grated with a grater machine, and then 300 g each was weighed as a sample. Next, each sample was added with crude papain enzyme flour (crude papain) with variations (0–1 g) and then stirred so that it was evenly distributed. Then it was put into an incubator at a constant temperature of 55 °C and varied fermentation time (12–60 h), then baked at 80 °C for 15 min to deactivate the enzyme, then pressed with a hydraulic press to produce an emulsion, then centrifuged to separate the oil from the dregs [4,18,23,28,29]. Process flow diagram as in Figure 1.

Figure 1. Flowchart of CPE process for VCO production.

2.3. Oil Quality Test

The determination of oil quality is based on SNI 3741:2013, which determines the water content through heating at 130 °C, the peroxide value via iodometric titration, and free fatty acids with alkalimetric titration.

2.3.1. Moisture Content

The weight loss that occurs while heating in the oven at (130 ± 1) °C is used as the basis for the calculation of the moisture content. As much as 5 g of oil is weighed in a beaker glass. Subsequently, the oil is heated in an oven for 30 min. The sample was then transferred to a desiccator and chilled to ambient temperature for 20 min before being reweighed until a constant weight was obtained.

2.3.2. Free Fatty Acid

As much as 10 g of oil is weighed and placed in an Erlenmeyer. A total of fifty milliliters of tepid ethanol and three droplets of phenolphthalein were added to the Erlenmeyer as an indicator. The sample was titrated with 0.1 N potassium hydroxide until a pink color emerged, and the amount of KOH consumed during the titration was written down.

2.3.3. Peroxide Number

The peroxide number is determined by reacting excess saturated potassium iodide with the existing peroxide in the oil. Using a thiosulfate solution of 0.1 N and a starch indicator, the amount of iodide released was titrated. The sample was initially placed in the Erlenmeyer and weighed up to 5 g. The Erlenmeyer was filled with 50 mL of glacial acetic acid-isooctane solution, and the mixture was agitated until homogeneous. Thirty milliliters of aquadest were added, and the container was promptly sealed. Shaken and titrated with a 0.1 N sodium thiosulfate solution until a mild yellow color was achieved. Afterward, 0.5 mL of a starch indicator was added, and the titration was repeated. Shake vigorously to remove all iodine from the solvent layer until the color diminishes to blue.

2.4. Experimental Design

Statistical design of experiments is an efficient method for developing experiments to produce valid and objective conclusions after analyzing the data. The two main applications of experimental design were screened, and the factors influencing the experiment were identified and optimized. Design Expert 13 (Stat-Ease Inc., Minneapolis, MN, USA) software was applied for regression, graphical analysis of the data, analysis of variance (ANOVA) in the regression model, fitting the model, and 3D response surface plots.

3. Results

From Table 1, the maximum yield of coconut oil from Banda Aceh and Pidie Jaya was obtained at a mass of 1 g of the enzyme, and a fermentation time of 60 h, respectively, 27.7%, 25.2%, and 24.5%. The minimum yields obtained under conditions without the addition of enzymes for 12 h were 19.6%, 18.5%, and 18.3%, respectively. The experimental design model used is Design Expert 13, type response surface methodology, subtype randomized, design type: CCD.

ANOVA was used to examine the effect of individual interactions with the mass of the papain enzyme and stopping time on the results of BAVCO, PJVCO, and BVCO, WC, FFA, and PN. by comparing the F-value and p-value at the confidence level $\geq$ 95%. The mathematical equation for the effect of each of these variables, both individually and interactively, on the response variable at each p-value listed in the ANOVA as listed in Table 2 and the mathematical rental model, intercept, coefficient of each individual variable, and quadratic interaction, listed in Table 3.

Table 1. CCD and the response of different parameters at various fermentation conditions.

Run	F_1 A: X_1 (g)	F_2 B: X_2 (h)	R_1 Y_1 (%)	R_2 Y_2 (%)	R_3 Y_3 (%)	R_4 Y_4 (%)	R_5 Y_5 (%)	R_6 Y_6 (mg O_2/g VCO)
1	0.6	36	23	23.1	25.1	0.124	0.014	0.014
2	1	12	22	21.3	25	0.026	0.012	0.008
3	0.6	36	23	22.4	23.1	0.124	0.014	0.014
4	0.6	12	21	20.1	23.5	0.126	0.016	0.007
5	0.5	60	27.5	25.1	24.8	0.201	0.015	0.024
6	0.6	36	23	22.5	23.1	0.124	0.014	0.014
7	0	60	19	19.1	18.7	0.124	0.012	0.012
8	0.6	36	23	23.1	23.1	0.124	0.014	0.014
9	1	60	27.7	25.2	24.5	0.011	0.01	0.024
10	0.6	36	23	22.9	21.5	0.124	0.014	0.015
11	0	12	19.6	18.5	18.3	0.354	0.01	0.008
12	1	36	23.6	22.5	21.8	0.011	0.012	0.01
13	0.5	36	22.8	21.8	20.8	0.1242	0.016	0.015

Factors (F); Responses (R); Papain enzyme mass (A: X_1); Fermentation time (B: X_2); BAVCO yield (Y_1); PJVCO yield (Y_2); BVCO yield (Y_3); Water content (Y_4); Free fatty acid (Y_5); Peroxide number (Y_6).

The results show a very significant influence on individuals and interactions of the parameters. The effect of adding enzymes (X_1, PEM) F-value (F-v) = 41.38 and *p*-value = 0.0004, fermentation time (X_2, FT), F-value = 27.93 and *p*-value = 0.0011 is very significant in increasing yield of BAVCO. The incubation time only affects the growth or exponential phase by preliminary research and proven by ANOVA. For PJVCO, the effect of fermentation time (F-value = 37.54), *p*-value = 0.005 significantly increase VCO yields. The effect of enzymes and fermentation time for BVCO yield was very significant on an F-value = 28.40 and *p*-value = 0.001. Incubation time was affected during the manufacture of enzymes testing the adaptation phase, exponential, and death phases. The exponential phase for enzyme growth is optimal so that the results are immediately used for processing actual data, as in the experimental design for ANOVA testing. While the incubation time is not included in the test and it is only needed during the preliminary test.

Tables 2 and 3 explain the effect of each independent variable (X_1, X_2) and its interaction (X_1X_2) quadratic on the response of the variables (Y_1, Y_2, Y_3, Y_4, Y_5, Y_6) in the form of a mathematical model according to Table 3 for various *p*-values and significance levels can be formulated in the form of mathematical Equations (1)–(6).

$$\text{BAVCO Yield } (Y_1) = 22.6 + 2.7\,X_1 + 1.9\,X_2 + 1.6\,X_1X_2 - 1.9\,{X_1}^2 + 1.5\,{X_2}^2 \quad (1)$$

$$\text{PJVCO Yield } (Y_2) = 22.3 + 2.1\,X_1 + 1.6\,X_2 + 0.884\,X_1X_2 - 1.7\,{X_1}^2 + 0.354\,{X_2}^2 \quad (2)$$

$$\text{BVCO Yield } (Y_3) = 22.3 + 2.1\,X_1 + X_2 + 0.996\,X_1X_2 - 3.2\,{X_1}^2 + 1.5\,{X_2}^2 \quad (3)$$

$$\text{WC } (Y_4) = 0.146 - 0.108\,X_1 - 0.034\,X_2 + 0.056\,X_1X_2 - 0.042\,{X_1}^2 + 0.028\,{X_2}^2 \quad (4)$$

$$\text{FFA } (Y_5) = 0.014 + 0.0001\,X_1 - 0.0001\,X_2 - 0.001\,X_1X_2 - 0.003\,{X_1}^2 + 0.0003\,{X_2}^2 \quad (5)$$

$$\text{PN } (Y_6) = 0.014 + 0.002\,X_1 + 0.006\,X_2 + 0.003\,X_1X_2 - 0.004\,{X_1}^2 + 0.003\,{X_2}^2 \quad (6)$$

Table 2. ANOVA for the effect of the mass of crude papain enzyme and fermentation time on the quantity and quality of Aceh virgin coconut oil.

R_1	Source	SS	df	MS	F-v	*p*-v	R_2	SS	df	MS	F-v	*p*-v	R_3	SS	df	MS	F-v	*p*-v
BAVCO Yield (Y_1, %)	Model	69.5	5	13.9	16.9	0.0009	PJVCO Yield (Y_2, %)	44.5	5	8.9	16.2	0.001	BVCO Yield (Y_3, %)	44.5	5	8.9	16.2	0.001
	X_1-PEM	33.9	1	33.9	41.4	0.0004		20.6	1	20.6	37.5	0.0005		20.6	1	20.6	37.5	0.0005
	X_2-FT	22.9	1	22.9	27.9	0.001		15.6	1	15.6	28.4	0.001		15.6	1	15.6	28.4	0.001
	X_1X_2	10.8	1	10.8	13.2	0.008		3.1	1	3.1	5.7	0.048		3.1	1	3.1	5.7	0.048
	X_1^2	7.9	1	7.9	9.7	0.017		5.6	1	5.6	10.3	0.015		5.6	1	5.6	10.3	0.015
	X_2^2	4.8	1	4.8	5.8	0.047		0.3	1	0.3	0.5	0.506		0.3	1	0.3	0.5	0.506
	Residual	5.7	7	0.82				3.9	7	0.5				3.9	7	0.5		
R_4	Source	SS	df	MS	F-v	*p*-v	R_5	SS	df	MS	F-v	*p*-v	R_6	SS	df	MS	F-v	*p*-v
WC (Y_4, %)	Model	0.084	5	0.017	12.1	0.002	FFA (Y_5, %)	0	5	7×10^{-6}	8.6	0.006	PN (Y_6, mg O_2/g VCO)	0.0003	5	0.0001	18.3	0.0007
	X_1-PEM	0.053	1	0.053	38	0.0005		7×10^{-8}	1	7×10^{-8}	0.084	0.78		0	1	0	7.7	0.028
	X_2-FT	0.007	1	0.007	5	0.05		7.7×10^{-8}	1	7.7×10^{-8}	0.091	0.772		0.0002	1	0.0002	65.2	<0.0001
	X_1X_2	0.013	1	0.013	9.1	0.02		5×10^{-6}	1	5×10^{-6}	6.3	0.04		0	1	0	9.9	0.016
	X_1^2	0.004	1	0.004	2.5	0.156		0	1	0	27.4	0.001		0	1	0	11.3	0.012
	X_2^2	0.002	1	0.002	1.2	0.309		2×10^{-7}	1	2×10^{-7}	0.211	0.66		0	1	0	4.6	0.068
	Residual	0.01	7	0.001				6×10^{-6}	7	8.5×10^{-6}				0	7	3×10^{-6}		

Sum of squares (SS); Degree of freedom (df); Mean square (MS); F-value (F-v); *p*-value (*p*-v).

Table 3. Thematic sales model of the effect of each variable and its interaction on yield, WC, FFA, and PN and the effect quadratic for *p*-value shading.

Name	Intercept	X_1	X_2	X_1X_2	X_1^2	X_2^2	R^2
BAVCO Yield (Y_1, %)	22.6	2.7	1.9	1.6	−1.9	1.5	0.92
p-values		0.0004	0.001	0.008	0.017	0.047	
PJVCO Yield (Y_2, %)	22.3	2.1	1.6	0.884	−1.7	0.354	0.92
p-values		0.0005	0.001	0.048	0.015	0.506	
BVCO Yield (Y_3, %)	22.3	2.1	1	0.996	−3.2	1.5	0.89
p-values		0.002	0.024	0.059	0.001	0.044	
WC (Y_4, %)	0.146	−0.108	−0.034	0.056	−0.042	0.028	0.90
p-values		0.0005	0.06	0.019	0.156	0.309	
FFA (Y_5, %)	0.014	0.0001	−0.0001	−0.001	−0.003	0.0003	0.86
p-values		0.78	0.772	0.04	0.001	0.659	
PN (Y_6, mg O_2/g s)	0.014	0.002	0.006	0.003	−0.004	0.003	0.93
p-values		0.028	< 0.0001	0.016	0.012	0.068	

The results of the analysis of variance listed in Table 2 and Equations (1)–(3) show that adding the papain enzyme and the stopping time increased the coconut oil yields of BAVCO, PJVCO, BVCO very significantly. The interaction of the two variables also dramatically influences the increase in oil yield. Oil yields: BAVCO > PJVCO > BVCO. The influence of the mass of the papain enzyme and time has an effect on the water content because there is still a little residual water which is not wholly separated during separation, but the quality of VCO is excellent because WC, FFA, and PN is still much smaller than the threshold determined by the VCO.

The oil consists of triglycerides or long-chain aliphatic acid esters with high molecular weights, both saturated and unsaturated. The formation of triglycerides is generally the result of the reaction between glycerol and fatty acids to produce triglycerides and water, as can be seen in Figure 2.

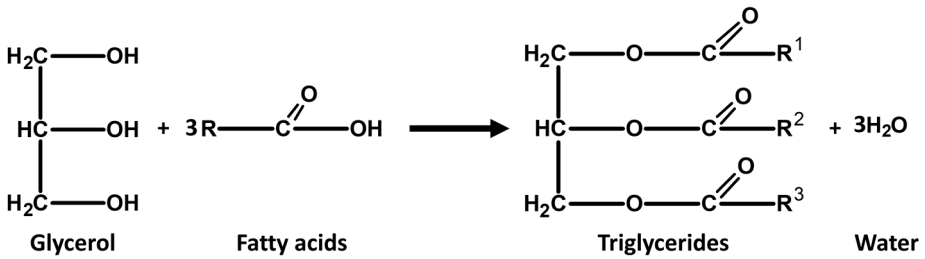

Figure 2. Formation reaction of triglycerides.

Coconut oil is in the form of granules in the form of an emulsion with water, and each grain is covered by protein. Basically, the process of taking the oil is to break down the protein layer so that the oil can diffuse out. The papain enzyme is able to break down protein layers because it has proteolytic properties that target peptide bonds, namely bonds between amino acids. Hydrolyzed protein peptide chains so that the bond between CO and NH is broken, which causes the protein to break so that the oil emulsified in water can flow out [30,31]. The reaction of peptide decomposition by enzymes is shown in Figure 3.

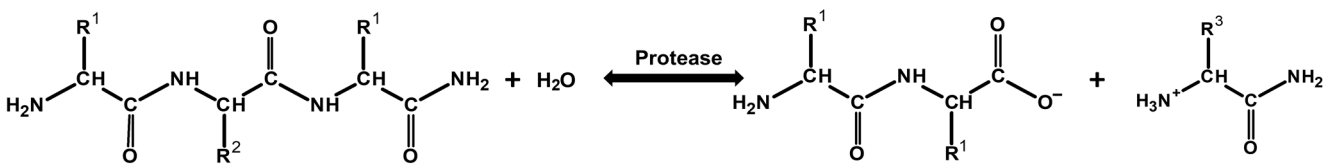

Figure 3. Decomposition reaction of the peptide.

Based on its fatty acid content, coconut oil is classified as lauric acid because its content is higher than other fatty acids. Fermentation, namely a microbial activity that causes reactions on organic substrates, in this case, carried out anaerobically to prevent outside air from entering, and proteolytic microbes, which can break down proteins and nitrogen components, causing a foul odor, lipolytic microbes will hydrolyze fats, phospholipids, and their derivatives to produce odors rancid [32–34]. This research was carried out by means of anaerobic fermentation, modifying it with papain enzymes so that maximum enzymatic combustion is expected. The effect of the mass of papain enzymes and fermentation time can increase the yield of coconut oil very significantly. The interaction effect of the two variables also has a significant effect. This can be seen in Table 2. ANOVA, the effect of X_1, X_2, and X_1X_2 on Y_1, Y_2, and Y_3 is very significant for all p-values < 0.05. The effect of individual raw papain enzyme masses, fermentation time, and the interaction of the two variables is very significant. The quality of coconut oil is affected by the mass of the enzyme and fermentation time because p-values < 0.05. This is due to the presence of water in the coconut oil emulsion, which does not separate completely, and also contains little water in the crude papain enzyme. Regarding quantity, the water content is still << standard threshold of VCO. Optimized the effect of X_1 and X_2 on Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6. The optimization $Y = f(X_1, X_2)$ is explained in Figure 4a–f.

Figure 4a–f shows the relationship between the influence of the mass of the papain enzyme (g), which is a numerical and categorical variable because it is the locality of the papain enzyme from papaya saree Aceh province and the curing time is a numerical and categorical control variable because it uses Acehnese coconut from Banda Aceh, coconuts from Pidie Jaya and coconuts from Bireuen to the yield of VCO from each local coconut, the results show a different response than when the coconuts are in different locations or areas where they grow, namely a: yield of BAVCO (Y_1), b: yield of PJVCO (Y_2), and c: yield of BVCO (Y_3), obtained optimum results respectively: Y_1 = 29.1%, Y_2 = 25.6%, and Y_3 = 24.6%. The results show a significant difference, namely: BAVCO > PJVCO > BVCO. Interestingly, there is a different quantitative increase in yield in terms of using the same process and treatment, and this means that there are small components that make up the composition in the three local coconuts that affect the fermentation process and enzyme activation, which affect the quantity of yield of coconut oil produced. The reaction in the ripening process does not produce water. It reduces the quality of the coconut oil, as well as the enzymatic reaction, which can improve the quality of the coconut oil from the three Acehnese coconuts in different locations. Figure 4d–f explains the values of WC, FFA, and PN, respectively: WC = 0.05%, FFA = 0.01%, and PN = 0.024%. From the values listed, the quality of coconut oil is still well controlled and meets the VCO quality standards, even the WC, FFA and PN are much lower than the VCO quality standard values, meaning that the quality of the VCO produced is better than which is expected because the smaller the WC, FFA and PN the better the quality of the VCO [35–38].

To achieve optimum conditions with maximum desirability, an objective limit is designed: X_1, X_2, Y_1, Y_2, Y_3 maximum, Y_4, minimum, Y_5, and Y_6 within the range. Desire graphs are shown in Figure 5a–c. Figure 5a RSM contour plot of the effect of papain enzyme mass (PEM) and fermentation time (FT) on desirability showed very satisfactory results. The effect of both PEM and FT independent variables was very significant, as the results are also shown in Tables 2 and 3. The correlation of the two variables to the response variable is also very high, this is indicated by the desirability value reaching 0.98, meaning that the predicted value is very close to or almost the same. Figure 5b RSM Pareto plot effect and each independent variable and variable response to desire: PEM, FT, Y_1, Y_2, Y_3, Y_4, Y_5, Y_6 and Y combined values range from 0.99 to 1 (combined 0.99). Y = f(X), desire 0.998. The predicted value can be explained by the actual data of 99% (≈100%). Figure 5b 3D RSM plot of PEM and FT on desirability reveals very good accuracy results, this can be seen from the desirability value close to 1 (desirability 0.983). This indicates that the predicted value and the actual value obtained are very close or the deviation is minimal. The optimal limit and the maximum possible yield are listed in Table 4.

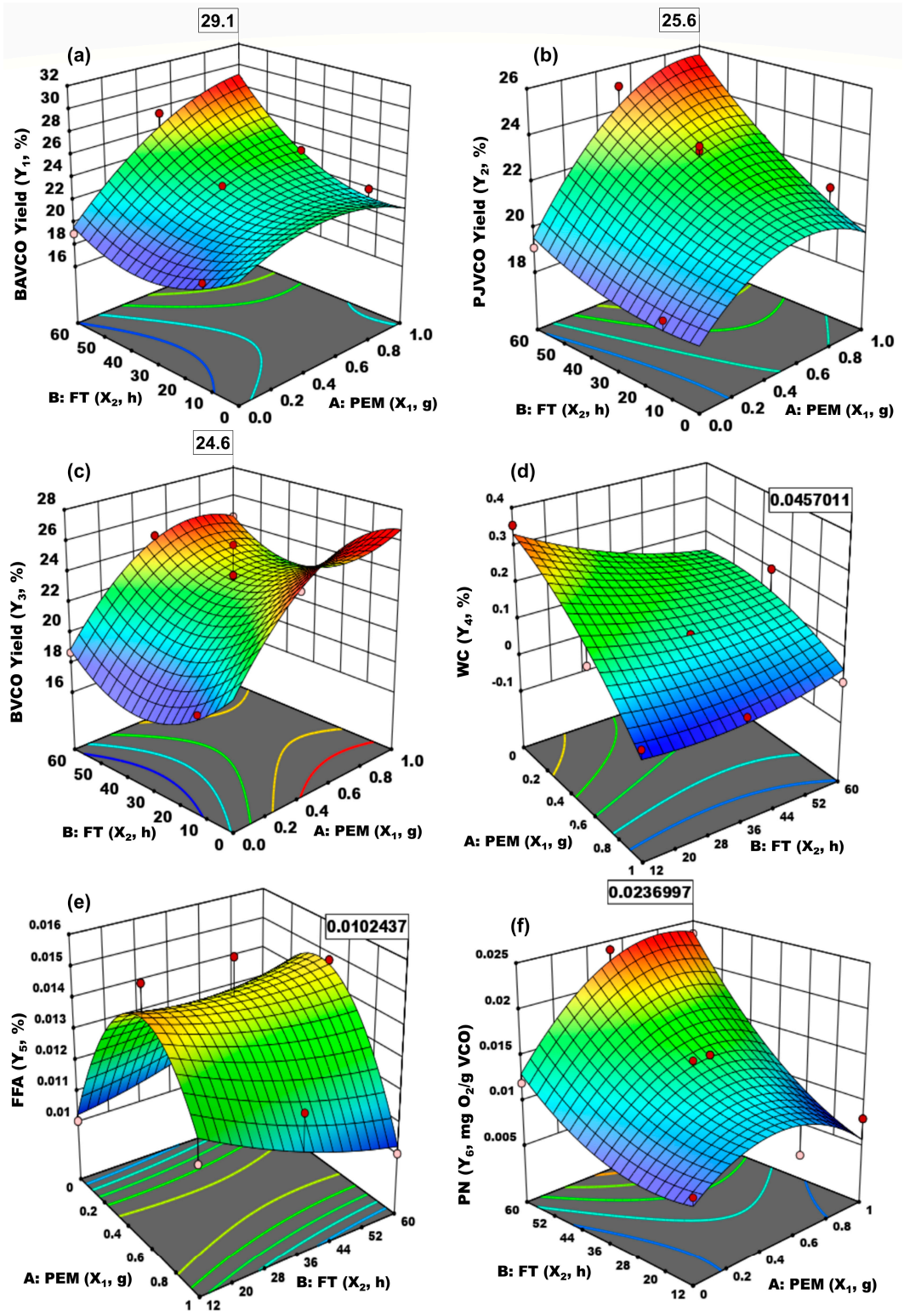

Figure 4. (**a**) 3D RSM plots of PEM and FT on BAVCO yield; (**b**) PJVCO yield; (**c**) BVCO yield; (**d**) WC; (**e**) FFA; (**f**) PN.

Based on the constraints designed in Table 4, the optimum numerical ramp condition is obtained, as shown in Figure 6 (Design Expert 13.0.11.0, RSM CCD).

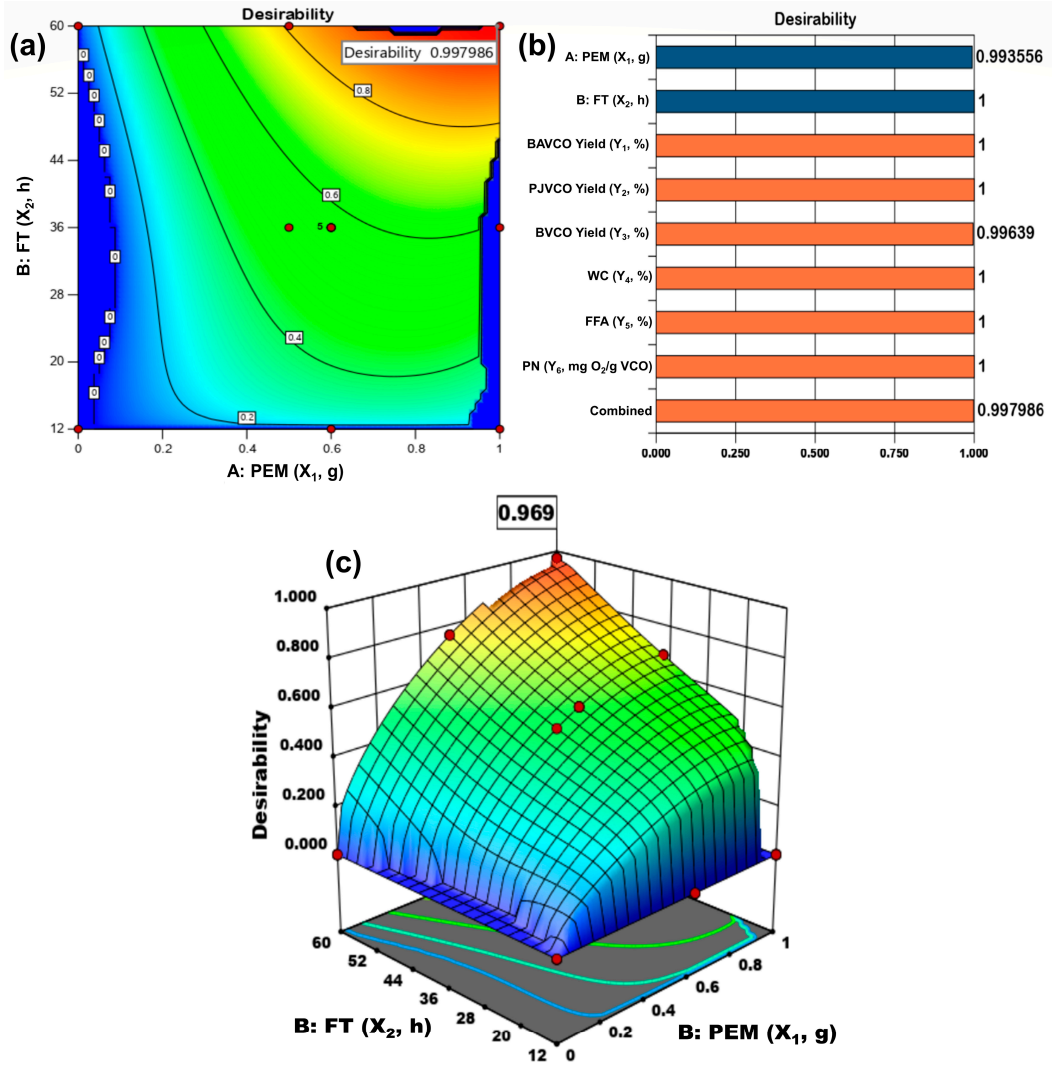

Figure 5. (**a**) Contour RSM plots of PEM and FT on desirability; (**b**) Pareto plots on each variable response desirability and the desirability combined; (**c**) 3D RSM plots on desirability.

Table 4. The design of independent variable constraints and response variables.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: PEM (X_1, g)	maximize	0	1	1	1	3
B: FT (X_2, h)	maximize	12	60	1	1	3
BAVCO Yield (Y_1, %)	maximize	19	27.7	1	1	3
PJVCO Yield (Y_2, %)	maximize	18.5	25.18	1	1	3
BVCO Yield (Y_3, %)	maximize	18.32	24.75	1	1	3
WC (Y_4, %)	minimize	0.111	0.354	1	1	3
FFA (Y_5, %)	is in range	0.92	1.48	1	1	3
PN (Y_6, mg O_2/g VCO)	is in range	0.07	0.24	1	1	3

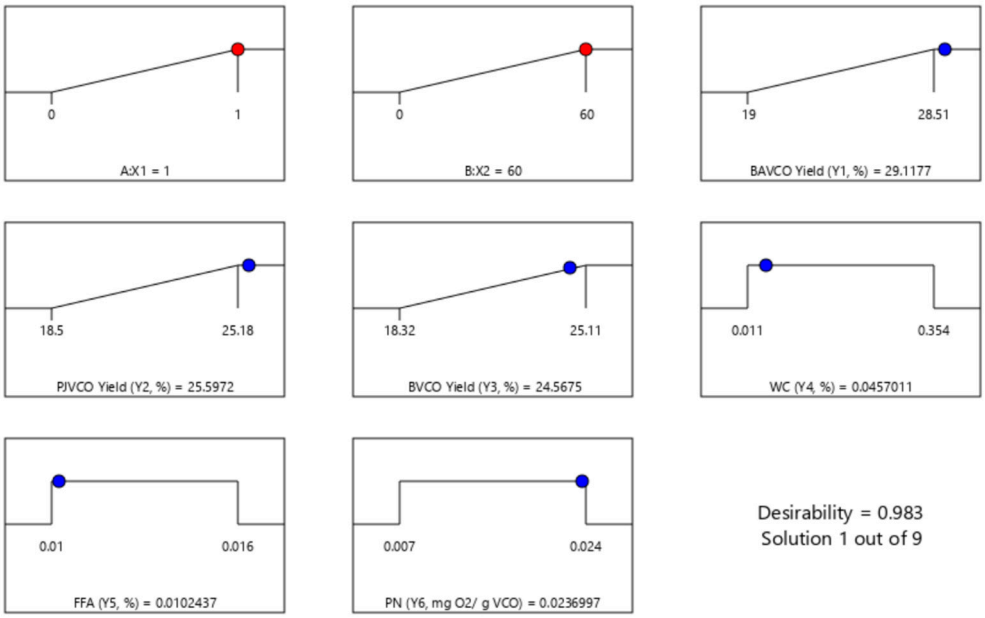

Figure 6. Desirability ramp for numerical optimization of goals for PEM, FT, BAVCO, PJVCO, BVCO, WC, FFA and PN.

Table 4 shows the selected constraints based on criteria that match the objectives to be achieved. The desirability of all variables and specific response variables is close to 1. Its value describes the correlation of all independent and response variables under optimum conditions. This design is needed to predict optimal conditions for numerical optimization. Figure 6 shows the desirability of optimizing the influence of PEM, and FT on response variables, namely: BAVCO yield (Y_1, %), PJVCO yield (Y_2, %), BVCO yield (Y_3, %), WC (Y_4, %), FFA (Y_5, %) and PN (Y_6, mg O_2/g VCO). The independent variables involved in this study are actually, in addition to the independent numerical variables, namely FFA and FT, there are categorical variables, namely Banda Aceh coconuts, Pidie Jaya coconuts, and Bireuen coconuts, but using CCD instead of a mixture. Design is not shown as a variable in the experimental design because it can be read directly as a numerical or categorical variable. In contrast, the response variables are quantitative and qualitative all appear, namely (Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6. X_2 = 60 h), BAVCO (Y_1 = 29.118%), PJVCO (Y_2 = 25.6%), BVCO (Y_3 = 24.6%), WC (Y_4 = 0.046%), FFA (Y_5 = 0.01%), PN (Y_6 = 0.024 mg O_2/g VCO) and desirability 0.983. From the optimal conditions obtained from the influence of PEM, FT from coconut sources, namely Banda Aceh, Pidie Jaya, and Bireuen coconuts, the yield of BAVCO > yield of PJVCO > yield of BVCO. In terms of quality, in terms of WC, FFA, odor, and desired aroma is 0.983, meaning that this research has better prospects to make VCO from coconut raw materials in Aceh Province.

For comparison, the existing coconut oil extraction methods include the cold extraction process, which produces lower yields [9,18,19]. In the hot extraction procedure, the anti-oxidant ability decreases at a temperature exceeding 40 °C [4,6]. The route also needs a more significant capital cost [20]. Cooling, freezing, and thawing have considerable operating costs because the temperature must be lowered below 0 °C [18,21]. In addition, conventional fermentation requires an extended extraction period [22]. The advantages of induction fermentation include ease of processing and high yields. At the same time, the disadvantages of the method could decrease the quality of coconut oil, a long extraction time, and the product's smell during the fermentation process [18,23]. Also, the centrifugation method makes lower yields.

The advantages of the enzymatic fermentation-modified coconut oil extraction method include higher yield and quality of VCO above the grade of VCO conventional methods. In optimum conditions, mass papain enzyme, fermentation time, maximum VCO yield (BAVCO > PJVCO > BVCO), minimum water content (WC min), free fatty acids in range

(FFA), and peroxide number in range (PN). Optimization using the RSM Box-Behnken design resulted in a maximum yield, water content (WC), minimum free fatty acid content (FFA), minimum peroxide number, and maximum desirability of 0.983. The results show that actuality values are very good with optimization predictions, and the quality of the VCO produced is above the grade of commercial VCO [9,18,19,24]

4. Conclusions

Without the addition of the crude enzyme papain, the yield of virgin coconut oil during natural fermentation is 19%, and it reaches a maximum of 27.7% with the addition of 1 g of the enzyme. The best results were obtained with a mass of 993.5 mg of the crude papain enzyme, a fermentation time of 60 h, and yields of 28.4%, 25.6%, and 24.7% for BAVCO, PJVCO, and BVCO, respectively. The quality of VCO was WC = 0.04745%, FFA = 0.0103%, and PN = 0.02374%. VCO modified by fermentation with the addition of CPE elevated the yield. VCO satisfies the required level of quality in terms of WC, FFA and PN. It has clear color with a distinct scent that is not rotten and the desirability optimization of 0.998.

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Abbreviations

ANOVA	Analysis of variance
BAVCO	Banda Aceh virgin coconut oil
BVCO	Bireun virgin coconut oil
CCD	Central composite design
CPE	Crude papain enzyme
FFA	Free fatty acid
FT	Fermentation time
PEM	Papain enzyme mass
PJVCO	Pidie Jaya virgin coconut oil
RSM	Response surface methodology
VCO	Virgin coconut oil
WC	Water content
PN	Peroxide number

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