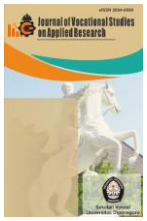
Contents lists available at JVSAR website

Journal of Vocational Studies on Applied Research

Journal homepage: https://ejournal2.undip.ac.id/index.php/jvsar



Research Article

Optimization of Virgin Coconut Oil (VCO) Production Using Papain Enzyme and Tempe Yeast to Enhance Medium Chain Triglycerides (MCT) Content

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Abstract

Virgin Coconut Oil (VCO) is a high-value derivative product of coconut that contains lauric acid as the major component of Medium-Chain Triglycerides (MCTs). This research aimed to optimize VCO production using a combination of papain enzyme and tempeh yeast to enhance MCT content. The study employed a Central Composite Design-Response Surface Method (CCD-RSM) with two factors: ratio of papain enzyme to tempeh yeast, and fermentation time. The results showed that the yield of VCO ranged from 5.00% to 7.96%, with the highest yield obtained at a fermentation time of 36 h and a papain/yeast ratio of 1/1.33. The water content ranged from 0.90% to 1.25%, free fatty acid (FFA) values ranged between 0.35% and 0.69%, and peroxide values between 1.06–2.41 meq/kg. GC-MS analysis indicated lauric acid (C12:0) as the dominant fatty acid, with a total Medium-Chain Fatty Acid (MCFA) content of 57.77%.

Article history:
Received: 28th August 2025
Revised: 4th December 2025
Accepted: 4th December 2025
Available online: 5th December 2025
Published regularly: 15th December 2025

Keywords:
Virgin Coconut Oil
Papain Enzyme
Tempe Yeast
Medium Chain Triglycerides

Permalink/DOI: http://doi.org/10.14710/jvsar.v7i1.29248

1. Introduction

Coconut (Cocos nucifera), often referred to as the "tree of life," has a wide range of applications in food, health, and industry. Indonesia is the world's largest coconut producer, with a total production of 18.3 million tons recorded in 2016. Virgin Coconut Oil (VCO) is one of the most valuable products derived from coconut, extensively used in the food, pharmaceutical, and cosmetic industries. VCO is particularly rich in lauric acid, a Medium-Chain Triglyceride (MCT) known for its antiviral, antibacterial, and antifungal properties. Despite its potential, VCO production in Indonesia remains underdeveloped. Therefore, optimization of VCO processing is necessary to increase MCT content (Mesu et al., 2018).

Papain enzyme, extracted from papaya latex, is a proteolytic enzyme widely utilized in various industries, including in the processing of Virgin Coconut Oil (VCO). Papain has the ability to hydrolyze proteins in coconut milk, which aids in breaking down the emulsion and facilitates the release of oil. As a protease enzyme, papain functions to cleave protein bonds associated with the oil present in the coconut milk emulsion (Diningsih, 2021).

However, enzyme papain does not produce that much VCO. Previous studies have highlighted the limitation of using single catalyst. Papain enzymes can produce VCO, but the result just showed low oil yield (10.9%) and increased oxidative activity because the enzyme increases the separation of oil rapidly (Erika et al, 2014). Conversely, using *tapai* yeast alone resulted in high Free Fatty Acid (FFA) content that failed to meet national standards and also produce rancid smell because of the effect of hydrolysis process (Razelita, 2024). Therefore, a gap exists in finding a method that balances yield and quality.

In the production of Virgin Coconut Oil (VCO), coconut cream is mixed with papain enzyme and tempeh yeast. Papain, a protease enzyme derived from papaya fruit, plays an important role in breaking down the proteins present in the coconut cream. Meanwhile, tempeh yeast contains various types of

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microorganisms, including fungi from the genus Rhizopus, which produce lipase enzymes. These lipases function to hydrolyze triglycerides in coconut oil into free fatty acids and glycerol. During fermentation, papain and lipase enzymes act synergistically. Papain facilitates the breakdown of proteins that encapsulate the oil droplets, thereby allowing lipase enzymes to more effectively access the triglycerides. The lipases subsequently hydrolyze triglycerides into free fatty acids and glycerol. The liberated free fatty acids then aggregate to form an oil layer on the surface of the mixture (Silaban et al., 2014).

The classification of fatty acids based on molecular size or the length of their carbon chain consists of Short-Chain Triglycerides (SCTs), Medium-Chain Triglycerides (MCTs), and Long-Chain Triglycerides (LCTs). MCTs are unique fatty acids characterized by saturated carbon chains ranging from C6 to C12 (caproic, caprylic, capric, and lauric acids). MCTs are produced through the esterification process of glycerol with fatty acids containing carbon chains from C6 to C12, which are predominantly derived from lauric acid–rich oils, particularly coconut oil (Safitri et al., 2022). One of the abundant sources of MCTs in Indonesia is coconut oil, which contains 92.1% saturated fat.

According to Damin et al. (2017), lauric acid and capric acid are the primary components of Virgin Coconut Oil (VCO). These fatty acids are converted in the human body into monolaurin and monocaprin, which exhibit antiviral, antibacterial, and antifungal properties. Lauric and capric acids are classified as medium-chain saturated fatty acids, possessing antimicrobial activity and being easily metabolized. In the body, lauric acid and caprylic acid are transformed into compounds that provide health benefits: lauric acid is converted into monolaurin, and caprylic acid into monocaprin. Monolaurin, a monoglyceride, demonstrates antiviral, antibacterial, and antiprotozoal activities. It supports the immune system of both humans and animals and is capable of inactivating lipid-coated viruses such as HIV, herpes, influenza, and various pathogenic bacteria. This study seeks to optimize VCO production by integrating papain enzyme and tempeh yeast, focusing on enhancing MCT content.

2. Materials and Methods

2.1 Materials and Equipment

The equipment used in this study include, digital balance, knife, beaker glass, measuring cylinder, filter cloth, blender, dropper pipette, glass funnel, burette, clamp and stand, stirrer, basin, rubber band, spoon, plastic cup, watch glass, centrifuge, Erlenmeyer flask, oven, desiccator, porcelain crucible, and cold-resistant container. While the materials used include, papain enzyme, mature coconuts, tempeh yeast (*Rhizopus* sp.), distilled water (aquadest), alcohol (95%), phenolphthalein (PP) indicator, 0.1 N NaOH solution, and $Na_2S_2O_3$ solution.

2.2 Analysis of the resulting VCO

The quality parameters observed in the Virgin Coconut Oil (VCO) produced in this study (based on Table 1) included yield, water content, free fatty acid (FFA) analysis, peroxide value analysis, and GC-MS analysis.

2.3 Yield

VCO yield was calculated by comparing the volume of VCO obtained with the volume of coconut milk used in the process. The yield of VCO was determined using equation (1).

$$Yield\ (\%) = \frac{VCO\ volume}{coconut\ milk\ volume} \times 100\% \quad (1)$$

2.4 Moisture Content

The moisture content of VCO was determined by first cleaning an empty cup in the oven and then cooling it in a desiccator. Approximately 2 g of the VCO sample were weighed together with the cup using a digital balance. The sample and cup were then placed in an oven at 105 °C for 3 h. After oven drying, the sample was cooled in a desiccator and reweighed until a constant weight was obtained. The moisture content of VCO was calculated using equation (2):

$$moisture\ content\ (\%) = \frac{a-b}{a} \times 100\% \quad (2)$$

Where a stands for the initial weight before oven (sample + cup) and b stands for weight after oven (sample + cup).

Table 1. Variation of independent variables with response surface method.

Sample	Papain Enzyme/Tempe Yeast Ratio (b/b)	Fermentation Time (h)
1	1/4.00	18
2	1/4.00	36
3	1/1.33	18
4	1/1.33	36
5	1/4.00	27
6	1/1.33	27
7	1/2.00	18
8	1/2.00	36
9(C)	1/2.00	27
10(C)	1/2.00	27
11(C)	1/2.00	27
12(C)	1/2.00	27

2.5 Free Fatty Acid (FFA)

The determination of free fatty acids (FFA) in VCO was carried out by weighing approximately 30 g of the sample into a 250 mL Erlenmeyer flask. A total of 50 mL of neutral 95% ethanol and 3–5 drops of phenolphthalein (PP) indicator were then added. The sample was titrated with 0.1 N NaOH standard solution until a stable pink color appeared (with no change for 15 s). The volume of NaOH used was recorded, and the FFA content was calculated using equation (3)

$$FFA\ (\%) = \frac{V_{NaOH} \times N \times BM}{1000 \times sample\ weight} \times 100\% \quad (3)$$

Where N stands for the normality of NaOH and BM stands for the molecular weight of NaOH (40 g/mol).

2.6 Peroxide Value

The peroxide value (PV) can be quantitatively measured using the iodometric titration method, in which the amount of iodine released is determined with potassium iodide. The liberated iodine is titrated with sodium thiosulfate, followed by the addition of starch indicator until a blue color appears. The titration is then continued with sodium thiosulfate until the blue color completely disappears. Peroxide value was calculated using equation (4).

$$Peroxide\ Value\left(meq/kg\right) = \frac{N \times V \times 56.1}{m} \quad (4)$$

Where N stands for the normality of $Na_2S_2O_3$, V stands for the volume of $Na_2S_2O_3$, 56.1 is the equivalent weight of oxygen, and m is the weight of oil sample (g).

2.7 GC-MS Analysis

GC-MS analysis was carried out to determine the fatty acid composition of Virgin Coconut Oil (VCO). The analysis was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. Gas chromatography (GC) produced a chromatogram showing the retention times and relative abundances of the components in VCO. The components separated by GC were subsequently analyzed using mass spectrometry (MS), resulting in a mass-to-charge (m/z) spectrum as a function of relative abundance. The identification of compounds in VCO was performed by matching the obtained spectra with reference spectra available in the instrument database (Novilla et al., 2017).

3. Results and Discussion

3.1 Yield Analysis Results

The yield analysis results show that the VCO yield ranges from 5.00% to 7.96% with an average of 6.30% (Table 2). The highest yield was obtained in run 4 under fermentation conditions of 36 h and an enzyme/yeast ratio of 1/1.33 (7.96%), while the lowest yield was in run 1 with a fermentation time of 18 h and an enzyme/yeast ratio of 1/4.00 (5.00%). This increase in yield is consistent with the research by Purba et al. (2020), which reported that the use of papain enzyme in VCO production can increase yield by up to 9.2% with optimized fermentation conditions, and the research by Nahak et al. (2023), which showed that fermentation with yeast can significantly increase VCO yield. These findings are also supported by the study by Jakfar et al. (2023), which stated that the combination of enzymes and microorganisms in coconut milk fermentation can optimize the oil extraction process.

3.2 Water Content Analysis Results

The moisture content of the VCO produced ranged from 0.90% to 1.25%, with an average of 1.03% (Table 3). The lowest moisture content was obtained in run 11 (0.90%) and the highest in run 7 (1.25%). Low moisture content is very important for VCO quality because it affects the shelf life and stability of the oil. According to Prasanna et al. (2024), the optimal moisture content

Table 2. Results of VCO yield analysis.

Sample	Coconut milk (mL)	Weight of coconut milk (g)	Weight of oil (g)	Yield (%)
1	87.50	86.63	4.33	5.00
2	82.50	81.68	5.31	6.50
3	87.50	86.63	5.20	6.00
4	82.50	81.68	6.50	7.96
5	85.00	84.15	5.05	6.00
6	85.00	84.15	5.72	6.80
7	87.50	86.63	4.81	5.50
8	82.50	81.68	6.35	7.80
9 (C)	85.00	84.15	5.47	6.50
10 (C)	85.00	84.15	5.41	6.43
11 (C)	85.00	84.15	5.55	6.60
12 (C)	85.00	84.15	5.39	6.41

Table 3. Results of VCO moisture content analysis.

Sample	Initial weight (g)	Final weight (g)	Water content (%)
1	4.33	4.29	0.92
2	5.31	5.25	1.13
3	5.20	5.15	0.96
4	6.50	6.43	1.08
5	5.05	5.00	0.99
6	5.72	5.65	1.22
7	4.81	4.75	1.25
8	6.35	6.29	0.94
9 (C)	5.47	5.42	0.91
10 (C)	5.41	5.35	1.11
11 (C)	5.55	5.50	0.90
12 (C)	5.39	5.34	0.93

of VCO ranges from 0.04-0.2% to maintain product quality and stability during storage. Research by Perera et al. (2020) shows that high VCO moisture content can accelerate the oxidation process and reduce the oil's organoleptic quality. A recent study by Mulyadi et al. (2019) confirms that moisture content control is a critical factor in maintaining VCO quality during production and storage. High moisture content is likely caused by incomplete phase separation or suboptimal drying conditions.

3.3 Free Fatty Acid Analysis Results

The free fatty acid (FFA) content of VCO ranges from 0.35% to 0.69%, with an average of 0.50% (Table 4). The lowest FFA value was obtained in run 8 (0.35%) under conditions of 27 h of fermentation time and a high enzyme-to-yeast ratio, while the highest value was in run 7 (0.69%) with a low enzyme-to-yeast ratio. According to Purba et al. (2020), the FFA content of VCO produced through fermentation with bromelain enzyme can reach 0.21%, indicating very good VCO quality. The study by Natalia et al. (2019) reported that FFA in VCO produced by fermentation ranged from 0.11–1.62%, with lower values indicating better quality. The study by Jakfar et al. (2023) showed that proper control of fermentation conditions can suppress FFA formation and improve VCO quality.

3.4 Peroxide Number Analysis Results

The peroxide value of VCO ranged from 1.06 to 2.41 meq/kg with an average of 1.51 meq/kg (Table 5). The lowest value was obtained in run 8 (1.06 meq/kg) and the highest in run 3 (2.41 meq/kg). The peroxide value is a primary oxidation indicator that shows the level of oil damage due to oxidation. Research by Perera et al. (2020) shows that the peroxide value of pure VCO ranges from 3.989 meq/kg, while VCO enriched with bioactive compounds shows lower values (3.626 meq/kg). The study by Negi et al. (2024) reported that thermal and non-thermal intensification processes can affect the peroxide value of VCO, where optimal conditions can produce low peroxide values. According to Flores & Camacho (2023), monitoring the peroxide value during thermal degradation shows that increases in temperature and heating time cause an increase in peroxide values.

3.5 Response Surface Methodology (RSM) Analysis

Table 6 shows the goal and constraint settings for the optimization process. Three responses—peroxide number, free fatty acid, and moisture content—are set to be minimized (Minimum). Conversely, the yield response is set to be maximized (Maximum). All parameters are assigned the same weight and importance, which is 1. This means that in the search for an optimal solution, the software will consider the achievement of the target for each response to be equally important.

The numerical summary of the best single solution produced by the optimization process, it confirms that the best treatment combination is to use a ratio of 0.506165 and a time of 35.2821 h. Under these conditions, the predicted values for peroxide number (1.40986 meq/kg), free fatty acids (0.395255%), moisture content (0.969294%), and yield (7.30067%) were obtained. The Composite Desirability value of 0.795445 serves as the final justification that this operating point is the most ideal for achieving the best balance among all the established objectives.

The yield analysis reveals positive value by showing a linear relationship between independent variable (enzyme papain and tempeh yeast ratio). This indicates the quantity of oil is directly driven by the intensity of the process as illustrated in the yield plot, the surface rises steadily as both the extraction time and the solvent-to-feed ratio increase. The slope appears steeper along the ratio axis, suggesting that the volume of solvent is the dominant factor; increasing the solvent-to-feed ratio significantly boosts the concentration gradient, thereby extracting more oil.

However, the Free Fatty Acids (FFA) and Peroxide Value shown non-linear behavior that make a contradictory strategy of simply maximizing input.

Table 4. Results of free fatty acid (FFA) analysis.

Sample	Weight Oil (g)	Volume of 0.1 N NaOH Required (mL)	FFA (%)
1	4.29	1.32	0.62
2	5.25	1.54	0.59
3	5.15	1.26	0.49
4	6.43	1.48	0.46
5	5.00	1.09	0.44
6	5.65	1.62	0.57
7	4.75	1.65	0.69
8	6.29	1.10	0.35
9 (C)	5.42	1.05	0.39
10 (C)	5.35	1.42	0.53
11 (C)	5.50	1.01	0.37
12 (C)	5.34	1.09	0.41

Table 5. Results of peroxide number analysis (meq/kg).

Sample	Weight Oil (g)	Volume of 0.1 N $Na_2S_2O_3$ Required (mL)	Peroxide Value (meq/kg)
1	4.29	1.13	1.48
2	5.25	1.15	1.23
3	5.15	2.21	2.41
4	6.43	2.23	1.95
5	5.00	1.15	1.29
6	5.65	2.24	2.22
7	4.75	1.15	1.36
8	6.29	1.19	1.06
9 (C)	5.42	1.14	1.18
10 (C)	5.35	1.13	1.18
11 (C)	5.50	1.14	1.16
12 (C)	5.34	1.13	1.19

Both quality plots display distinct "valley" or "bowl" shapes (Figure 1), indicating that there is an optimal region in the center of the experimental design where these undesirable values are minimized. For the Free Fatty Acids, the convex surface suggests a quadratic relationship where deviating from the middle range (either too low or too high) leads to increased hydrolysis and oil degradation. Similarly, the Peroxide Value plot indicates that while moderate conditions keep oxidation low, pushing the process parameters to their upper limits likely introduces excess stress or thermal exposure, causing the peroxide levels to rise and the oil to become rancid.

The overall interpretation of these three plots highlights a critical trade-off between quantity and quality. While pushing the time and ratio to their maximums ensures the highest yield, it simultaneously drives the process out of the optimal "valley" for chemical stability, resulting in oil with higher acidity and oxidation levels. The most effective processing condition is not at the extreme edges, but rather a "sweet spot" in the moderate-to-high range. This specific point represents a compromise where the yield is economically viable, yet the processing conditions

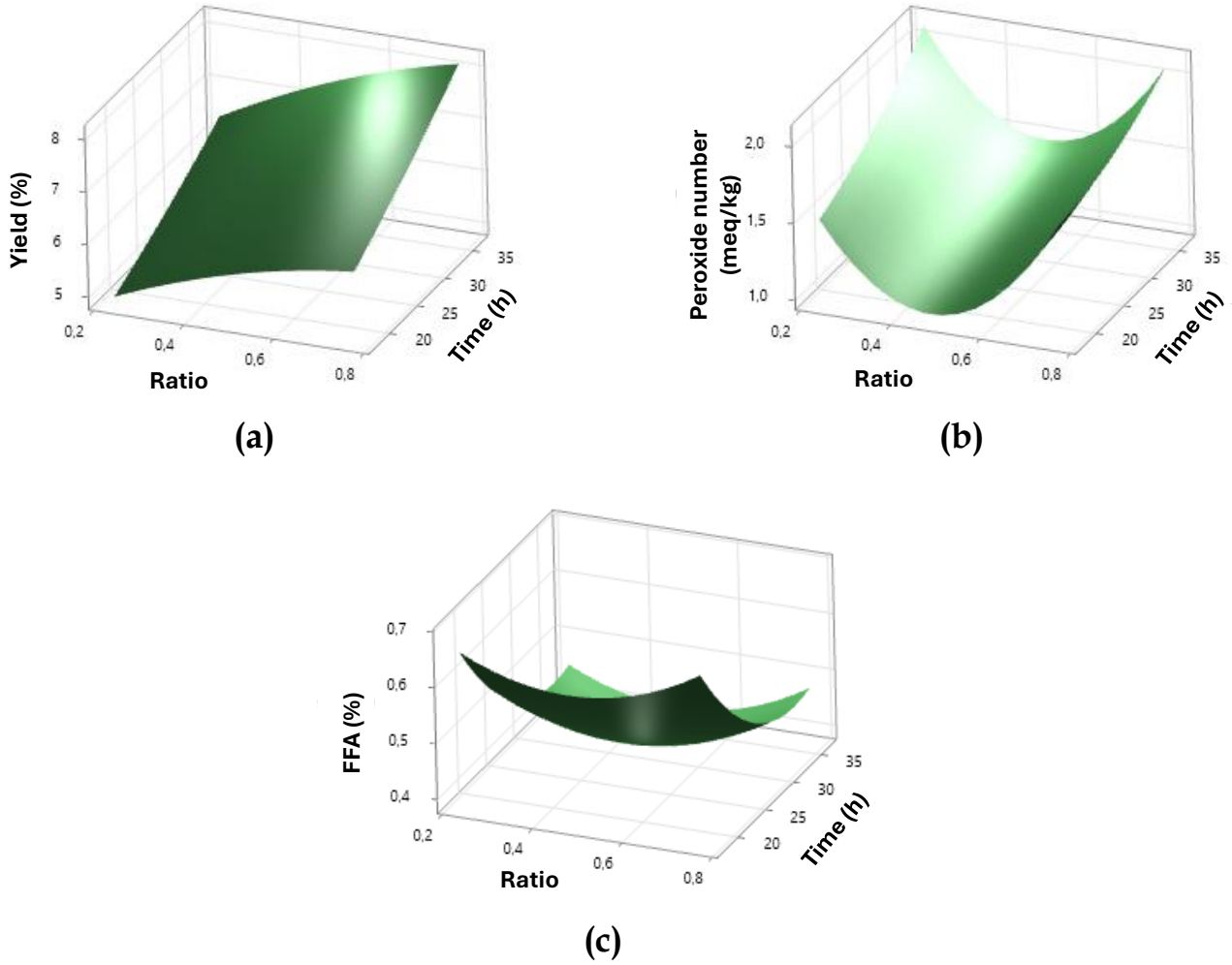

Figure 1. 3D plot surface analysis of (a) %Yield Value, (b) Peroxide Number, and (c) Free Fatty Acid.

Table 6. RSM-CCD optimization analysis parameters.

Response	Goal	Lower	Target	Upper	Weight	Importance
Peroxide Value (meq/kg)	Minimum		1.06	2.41	1	1
FFA (%)	Minimum		0.35	0.69	1	1
Moisture Content (%)	Minimum		0.90	1.25	1	1
Yield (%)	Maximum	5	7.96		1	1

Table 7. Results of response optimization data analysis validation.

Run	Time (h)	Enzyme (g)	Yeast (g)	Coconut milk (ml)	Yield (%)	Water content (%)	FFA (%)	Peroxide number (meq/kg)
1	35.28	5.00	9.9	85.10	6.28	0.74	0.64	1.24
Error %					13.98	23.63	62.02	14.18

remain gentle enough to maintain the Free Fatty Acids and Peroxide Value at their minimum, safe levels.

3.6 Data Validation Response Optimization Method

The validation results were obtained by conducting experiments under the optimal conditions recommended by the software, namely at 35.28 h with an enzyme/yeast ratio of 1/1.98 (w/w) (Table 7). The validation data were then compared with the predicted values from the RSM model to test the reliability and accuracy of the model. From the comparison, it was observed that there were differences or errors between the predicted values and the actual laboratory results. The error values for yield and moisture content were at an acceptable level, indicating that the model was sufficiently good at predicting these two responses. However, for the free fatty acid response, a very significant error was found, indicating that the model's prediction for this parameter was less accurate compared to the actual conditions.

3.7 GC-MS Analysis

GC-MS analysis of the selected samples, namely the samples from the data optimization validation, showed a profile consistent with the characteristics of virgin coconut oil (VCO). The dominant peak was lauric acid methyl ester (C12:0) with an area percentage of 46.13%. In addition to lauric acid, other MCFA compounds detected include caproic acid (C6) with an area percentage of 0.37%, caprylic acid (C8) with an area percentage of 6.03%, and capric acid (C10) with an area percentage of 5.24%. The total MCFA content in the sample based on GC-MS results is 57.77% of the total detected MCFA compounds.

The ideal VCO fatty acid always shows a predominance of medium-chain fatty acids (MCFA). Ghani et al. (2018) reported that VCO obtained through various extraction processes contains lauric acid (C12:0) as the main component, ranging from 48.40% to 52.84% of total fatty acids, followed by caprylic acid (C8:0) and capric acid (C10:0) at approximately 8–10% and 6–7%, respectively. Another study by Mena & Marfu'ah et al. (2020) also noted lauric acid between 48%–51% in commercial VCO, with total MCFA (C6:0–C12:0) reaching 55–62% of the overall fatty acid profile.

Table 8. GC-MS analysis of optimal VCO samples.

Fatty Acid Components	Structural Formal	Content (%)
Saturated Fatty Acid		
Caproic acid	$C_6H_{12}O_2$	0.37
Caprylic acid	$C_8H_{16}O_2$	6.03
Capric acid	$C_{10}H_{20}O_2$	5.24
Lauric acid	$C_{12}H_{24}O_2$	46.13
Myristic acid	$C_{14}H_{28}O_2$	19.51
Palmitic acid	$C_{16}H_{32}O_2$	9.81
Stearic acid	$C_{18}H_{36}O_2$	4.73
Unsaturated Fatty Acid		
Oleic acid	$C_{18}H_{34}O_2$	4.00
Linoleic acid	$C_{18}H_{32}O_2$	8.97

According to the specifications of the Asian and Pacific Coconut Community (APCC), the normal range of MCFA in VCO is: caproic (C6:0) 0.10–0.95%, caprylic (C8:0) 4–10%, capric (C10:0) 4–8%, and lauric (C12:0) 45–56%. Therefore, it can be concluded that this sample contains MCT as defined by the C6–C12 range and is therefore likely not pure VCO without any indication of contamination or issues with the FAME derivatization method.

4. Conclusion

Based on the research results, the combination of papain enzyme and tempeh yeast can increase the level of Medium Chain Triglycerides (MCT) in Virgin Coconut Oil (VCO) under optimal conditions at a ratio of papain enzyme to tempeh yeast of 1:1.98 and a fermentation time of 36 h. These conditions yield a yield of 7.30%, moisture content of 0.97%, free fatty acid content of 0.40%, and a peroxide value of 1.31 meq/kg, which meets the quality standards of SNI 7381:2008 and the specifications of the Asian and Pacific Coconut Community (APPC) for high-quality VCO, with a total MCFA content of 57.77%, dominated by lauric acid at 46.13%. The winterization method can increase the content of Medium Chain Triglycerides (MCT) because the winterization method can separate the Long Chain Triglycerides (LCT) fraction from the Medium Chain Triglycerides (MCT) fraction.

Notation

Recommendations for future research include further study of the ratio of papain enzyme and tempe yeast, winterization temperature and time, and conducting more analysis.

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