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Effect of Papain Enzyme Concentration on Virgin Coconut Oil Yield

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ABSTRACT

The papain enzyme is now increasingly used in the production of virgin coconut oil (VCO) due to its ability to efficiently break down coconut milk emulsions. Papain enzyme is also widely available in the market under various brands, each with different enzymatic activities. In this study, we investigated the effect of different papain enzyme brands (A, B, C) and concentrations (1–3.5 g) on the quality of VCO, specifically focusing on free fatty acid (FFA) content, moisture content, and yield. In the enzymatic method, coconut cream is fermented with papain for 8 hours to separate the oil components, resulting in a distinct oil layer that separates. The results showed that the smallest FFA content (0.12%), the largest yield (31.32%), and the lowest moisture content (0.14%) were achieved using brand B papain enzyme at a concentration of 3.5 g. This study confirms that the choice of enzyme brand and concentration significantly impacts VCO quality, with brand B demonstrating superior performance in meeting SNI 7381:2008 standards.

Keywords: *Free Fatty Acids, Moisture Content, Virgin Coconut Oil, Papain Enzyme, Yield*

INTRODUCTION

In general, there are 3 methods for making coconut oil or virgin coconut oil (VCO) such as the fishing method, the gradual heating method and the enzymatic method, for the enzymatic method as it is done depends on the concentration of the enzyme and the ratio of the weight of the enzyme added to the weight of the coconut cream (Mari'i et al., 2022; Prayitno, 2019; Rismiati, et al., 2016). Some researchers also use the proteolytic enzyme papain to make virgin coconut oil from coconuts. As in the research by Rasmito et al. (2024); Rasmito et al. (2025), Virgin Coconut Oil is made using papain proteolytic enzyme, with the best results at a fermentation time of 28 hours and the addition of papain enzyme by 9.5%. Researcher Iskandar et al. (2015) also make virgin coconut oil using the proteolytic enzyme papain, which produces the highest yield of 31.53%. Likewise, in research, Irawan et al. (2023) conducted research to make virgin coconut oil products by utilizing proteolytic enzymes from papaya peels. Researcher Diningsih et al. (2021) made a conclusion from their research that the yield of virgin coconut oil produced was getting larger when the papain enzyme used was large, but the level of free fatty acids from virgin coconut oil produced did not meet the quality standard 7381: SNI 2008. Nuraisyah et al. (2023) also researched empowering coconut farmers to make coconut oil using the enzymatic method and sell it instead of just selling coconuts. Muna (2017) also reveals, based on research from existing journals, that the production of pure coconut oil or virgin coconut oil can be achieved using the best method with ultrasonic methods. The quality of the oil, based on free fatty acid values and iodine content values using ultrasonic methods, meets the quality standards of SNI 7381:2008. According to Idris and Armi's (2022) research, virgin coconut oil can be extracted from coconut milk through various techniques, including gradual heating, oil fishing, and enzymatic fermentation. The gradual heating technique remains the most prevalent approach for VCO production. Kusuma and Putri (2020) conducted a systematic literature review revealing that virgin coconut oil, derived from fresh coconut flesh (non-copra), maintains its clarity and distinctive aroma through chemical-free processing without excessive heat application. Mesu et al. (2018) also researched to find out various methods of utilizing coconut pulp in virgin

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coconut oil and the right stirring time in making virgin coconut oil with the addition of papain enzymes and acidification. The conclusion of his research was to use acidification and stirring for a time of 40 minutes to produce virgin coconut oil according to standards, also with a clear color and distinctive aroma of coconut. Research was conducted by Setyorini et al. (2022) on the manufacture of virgin coconut oil from coconut flesh with a fermentation process for ≥ 24 hours using baker's yeast with a yeast nutrient concentration of 6% b/v. The results of this study were that the highest yield of virgin coconut oil was obtained at a fermentation time of 24 hours, which was 18.1%. The yield decreased with increasing fermentation time. The resulting virgin coconut oil product has physical properties that comply with the quality requirements of the Indonesian National Standard (SNI) 7381:2008, namely colorless and clear, a distinctive aroma of fresh coconut, and a distinctive taste of coconut oil. The pH price analysis of the virgin coconut oil product produced at a fermentation time of 24 to 36 hours has the same pH value, namely 5. Haedar et al. (2024) demonstrated through community service research that cold-processed virgin coconut oil (VCO), known for preserving its natural antioxidants, contains significant lauric acid content. This medium-chain fatty acid metabolizes into monolaurin, a bioactive compound that enhances immune function and promotes tissue regeneration. The production of virgin coconut oil (VCO) typically employs a fermentation process using baker's yeast. Rifdah et al. (2021) investigated an alternative enzymatic approach utilizing bromelain extracted from pineapple stems (at concentrations of 5–20%). This protease enzyme facilitates protein hydrolysis, enabling the separation of oil and water in coconut milk emulsions. Their optimal parameters (24-hour fermentation with 5% enzyme concentration) produced VCO that met the standards of SNI 7381:2008. This study aims to find the type of proteolytic enzyme product brand that produces the largest yield of the 3 three brands on the market that we use.

METHODS

The experimental method for making virgin coconut oil is based on research conducted by Perdani et al. (2019); Yuniati et al. (2021), who designed their research on making virgin coconut oil using the proteolytic enzyme papain. In its manufacture, it is divided into 2 stages, namely the stage of making coconut cream and the stage of making virgin coconut oil. In the stage of making coconut cream, the coconut is grated to reduce its size because it will undergo a coconut milk extraction process. Grated coconut is mixed with water with a ratio of water and grated coconut of 1:2 (100 grams of grated coconut and 200 grams of warm water). Water is added 3 times from the total water used, or as much as possible, until all the coconut milk is perfectly extracted. Then filtered to separate the coconut dregs from the coconut milk. The coconut milk is collected in a transparent container. The coconut milk in a transparent container is left for approximately 1 hour until it separates into skim and cream. The cream part will be used as a raw material for making virgin coconut oil. The stage of making virgin coconut oil begins by putting the coconut cream into a container and adding the proteolytic enzyme papain. Next, the mixture is fermented for 6 hours until three layers are formed, namely: blondo on the top layer, then oil on the middle layer, and the bottom layer is water.

The stage of making coconut milk cream, making grated coconut, and mixing with water with a ratio between grated coconut and water is 1:3 (250 grams of grated coconut and 750 grams of warm water). The addition of water can be done as much as 1 (one) or 2 (two) times of the total water used. In this case, water serves to dissolve the coconut milk in the

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grated coconut. Then the mixture is filtered, and the coconut milk obtained is accommodated in a transparent container. The coconut milk was left in a transparent container for 2 hours until two layers formed: skim (bottom) and cream (top) due to differences in density. The cream that will be used as raw materials for making virgin coconut oil we take.

The process of making virgin coconut oil begins by putting 200 ml of coconut milk cream into a glass beaker. After that, papain enzymes from brand A, brand B, and brand C are added as variables, as much as 1gr, 1.5gr, 2gr, 2.5gr, 3gr, and 3.5gr. Stir the mixture of coconut milk, cream, and papain enzyme until homogeneous. Next, close the beaker glass containing coconut milk cream and papain enzymes. Then, let it sit for ± 8 hours at room temperature until three layers are formed, namely, blondo on the top layer, oil on the middle layer, and water on the bottom layer. Take the oil that is in the second layer. Furthermore, the oil that has been taken is analyzed for free fatty acid content, moisture content, and the percentage of the yield.

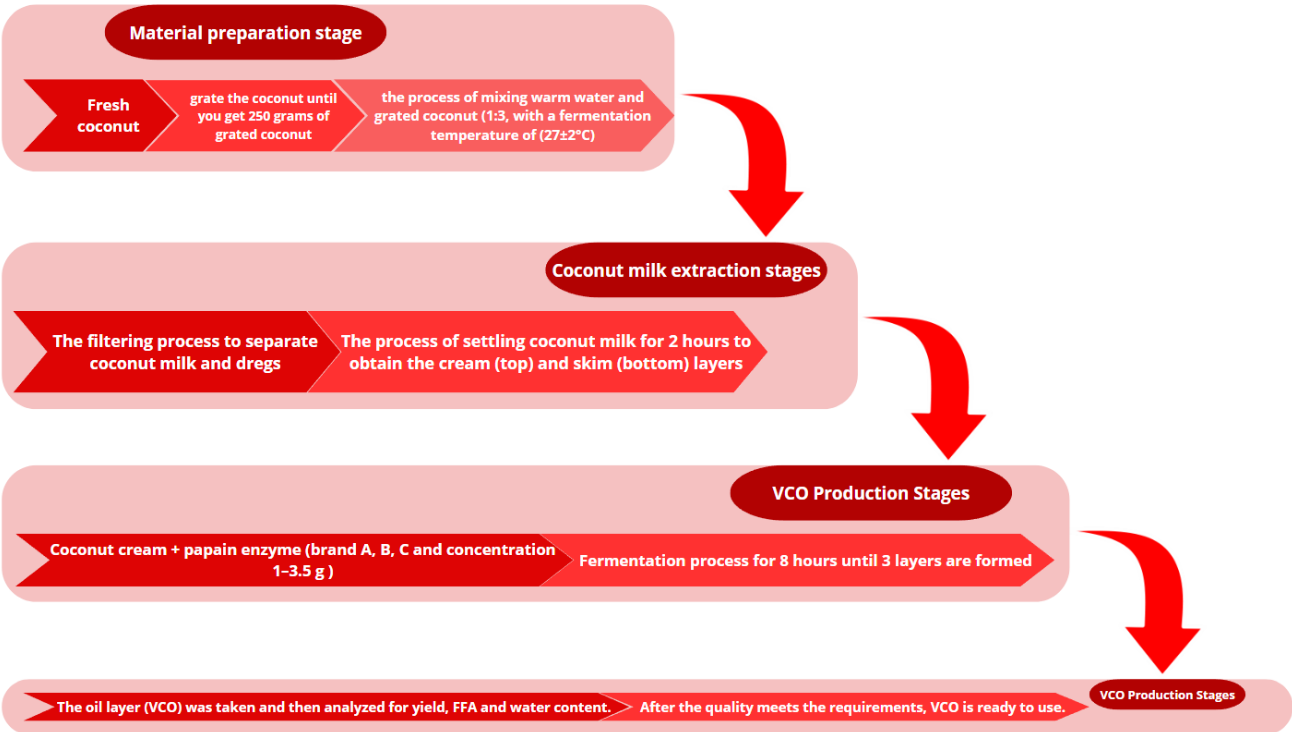

Figure 1. Flowchart of Virgin Coconut Oil Production

In the coconut cream preparation stage, grated coconut is mixed with warm water at a ratio of 1:3 (250 grams of grated coconut to 750 grams of water) to achieve optimal coconut milk extraction. Water can be added again 1 (one) or 2 (two) times from the total water used. In this case, water functions to dissolve the coconut milk in the grated coconut. Then the mixture is filtered, and the coconut milk obtained is collected in a transparent container. The coconut milk in the transparent container is left for ± 2 hours until 2 layers are visible, namely the skim layer and the coconut cream. This part of the coconut cream is what we will take and use as raw material for making virgin coconut oil.

The stages of producing premium coconut oil are shown in Figure 2, which illustrates the production process of Premium Coconut Oil (VCO) using the enzymatic fermentation method with papain enzyme. The process begins with grating the coconut and mixing it with warm water in a 1:3 ratio, then filtering it to obtain coconut milk. The coconut milk is allowed to

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settle until a cream layer forms, after which 200 ml is taken as the main ingredient. This coconut cream is treated with papain enzyme from three brands (A, B, C) with varying concentrations (1–3.5 g), then mixed until homogeneous and left for 8 hours at room temperature. During sedimentation, the mixture will separate into three layers: blonde (upper layer), VCO (middle layer), and water (bottom layer). Pure coconut oil is taken from the middle layer as the final product of this process.

Figure 2. Flowchart of Virgin Coconut Oil Production

Research Design

The study used a factorial experimental design with two independent variables: papain enzyme brands (A, B, and C) and enzyme concentrations (1 g, 1.5 g, 2 g, 2.5 g, 3 g, and 3.5 g), resulting in 18 treatment combinations. Each treatment was carried out in triplicate (n = 3) to ensure data validity. The response variables observed were yield (%), free fatty acid content (%), and water content (%). Data were analyzed descriptively and presented in tabular form. In addition, to determine whether there were significant differences between treatments, especially between enzyme brands on each VCO quality parameter, statistical analysis was carried out using the one-way ANOVA test. The use of the ANOVA test aims to test treatments statistically and to ensure whether the variation in results obtained was caused by differences in treatment or simply by random variation in the data.

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RESULTS AND DISCUSSIONS

Research Design

From the experiments that we have conducted, the results are in the form of data: %Yield, %Moisture Content, and %Free Fatty Acid Content that we have tabulated in Table 1.

Table 1. Results of Yield Analysis, Free Fatty Acids, and Moisture Content

enzyme (gr)	Analysis Results (%)								
	Yield			Free Fatty Acids			Moisture Content		
	Enzyme Brand			Enzyme Brand			Enzyme Brand		
	A	B	C	A	B	C	A	B	C
1	23.19	24.22	23.24	0.16	0.14	0.17	0.18	0.16	0.19
1.5	25.44	26.57	25.48	0.15	0.14	0.16	0.17	0.16	0.18
2	27.32	28.41	27.63	0.17	0.13	0.15	0.19	0.15	0.17
2.5	29.21	30.83	29.37	0.16	0.13	0.17	0.18	0.15	0.19
3	30.19	31.25	30.45	0.17	0.12	0.16	0.19	0.14	0.18
3.5	30.21	31.32	30.51	0.16	0.12	0.16	0.19	0.14	0.18

The ANOVA results show that there is no significant difference in yield among enzyme brands ($p = 0.757$), indicating that enzyme concentration is the dominant factor. However, Brand B consistently produced the highest yield (31.32%), presumably due to its more stable proteolytic activity. This indicates that the increase in VCO yield is more influenced by the enzyme concentration than by the brand.

On the other hand, in the free fatty acids and moisture content parameters, the ANOVA test results showed a very significant difference between the three enzyme brands ($p < 0.001$). Papain enzyme brand B consistently produced VCO with lower FFA and water content than brands A and C. This indicates that brand B is not only able to break down emulsions efficiently, but is also able to maintain the stability of chemical components in the oil. Thus, in terms of chemical quality, brand B shows the most superior performance and is by VCO quality standards based on SNI 7381:2008.

Unlike the research by Rasmito et al. (2024) which required 28 hours of fermentation, this study achieved an optimal yield of 31.32% in just 8 hours using Brand B papain (3.5 g). This time efficiency is supported by high proteolytic activity that accelerates the breakdown of coconut milk emulsion (Figure 3).. However, above 3 g, the rate of yield increase slows down (only 0.07%), indicating enzyme-substrate saturation. The three enzyme brands (A, B, and C) show a similar trend, namely an increase in yield as the enzyme concentration increases, with the peak yield achieved at a concentration of 3.5 grams. However, enzyme brand B consistently produces the highest yield at each concentration level, with a maximum value reaching 31.32% at a concentration of 3.5 grams. This indicates that the papain enzyme brand B has a more effective proteolytic activity in breaking down coconut milk emulsions and facilitating oil separation. However, the increase in yield began to decline after a concentration of 3 grams, indicating a possible saturation point between the enzyme and the substrate. This means that the addition of enzymes that exceed the substrate capacity no longer significantly increases yields and can even be inefficient in terms of production costs. Clearer results can be seen in Figure 3:

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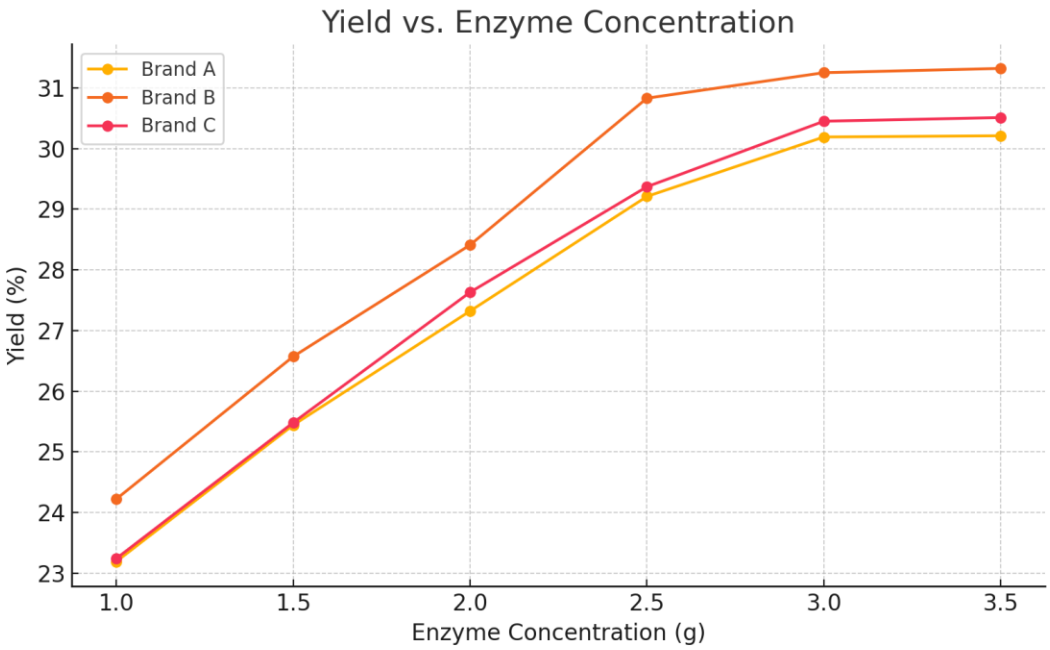

Figure 3. Yield vs Enzyme Concentration

The Free Fatty Acids (FFA) vs. Enzyme Concentration graph shows that the free fatty acid content in VCO generally decreases with increasing papain enzyme concentration, especially for enzyme brand B. The most significant decrease in FFA occurred at concentrations of 3 grams and 3.5 grams, where enzyme brand B produced the lowest FFA content of 0.12%. In contrast, enzyme brands A and C showed relatively higher and fluctuating FFA content, with the highest value of 0.17%. These results indicate that enzyme brand B is not only effective in breaking down emulsions but is also able to maintain the stability of the fatty acid structure, thereby preventing an increase in FFA due to triglyceride degradation. Low FFA content is very important because it indicates high oil quality and resistance to oxidation. According to the SNI 7381:2008 standard, the maximum FFA content for VCO is 0.2%, and all treatments in this study were still within this safe limit, but only enzyme brand B achieved optimal performance in terms of chemical quality. Clearer results can be seen in Figure 4:

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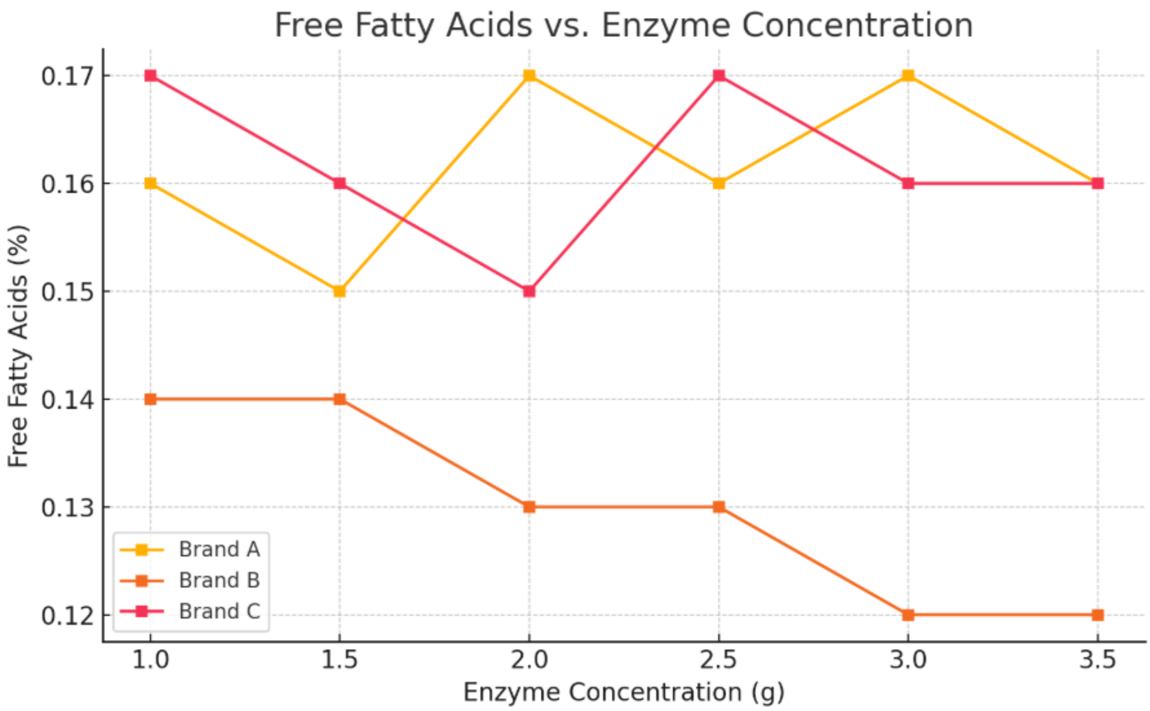

Figure 4. Free Fatty Acids vs Enzyme Concentration

The FFA content in VCO decreases with increased concentration of papain, especially for brand B (0.17% → 0.12%). This decline is believed to be due to the more stable hydrolysis activity of brand B, thereby preventing triglyceride degradation (Febriandini et al., 2023). At enzyme concentrations of 1 gram to 3.5 grams, it can be seen that brand B consistently produces the lowest moisture content compared to brands A and C. The lowest moisture content value was recorded at 0.14% at concentrations of 3 and 3.5 grams with the use of enzyme brand B. Meanwhile, brands A and C showed moisture content that tended to be higher and relatively stable in the range of 0.17% to 0.19%, even experiencing a slight increase at higher enzyme concentrations. These results indicate that the papain enzyme brand B is more effective in helping to separate water from oil during the fermentation process, resulting in better quality VCO in accordance with the SNI 7381:2008 standard, which requires a maximum moisture content of 0.2%. Low moisture content is very important because it can extend the shelf life of VCO and prevent degradation processes such as hydrolysis and oxidation that can reduce oil quality. Clearer results can be seen in Figure 5:

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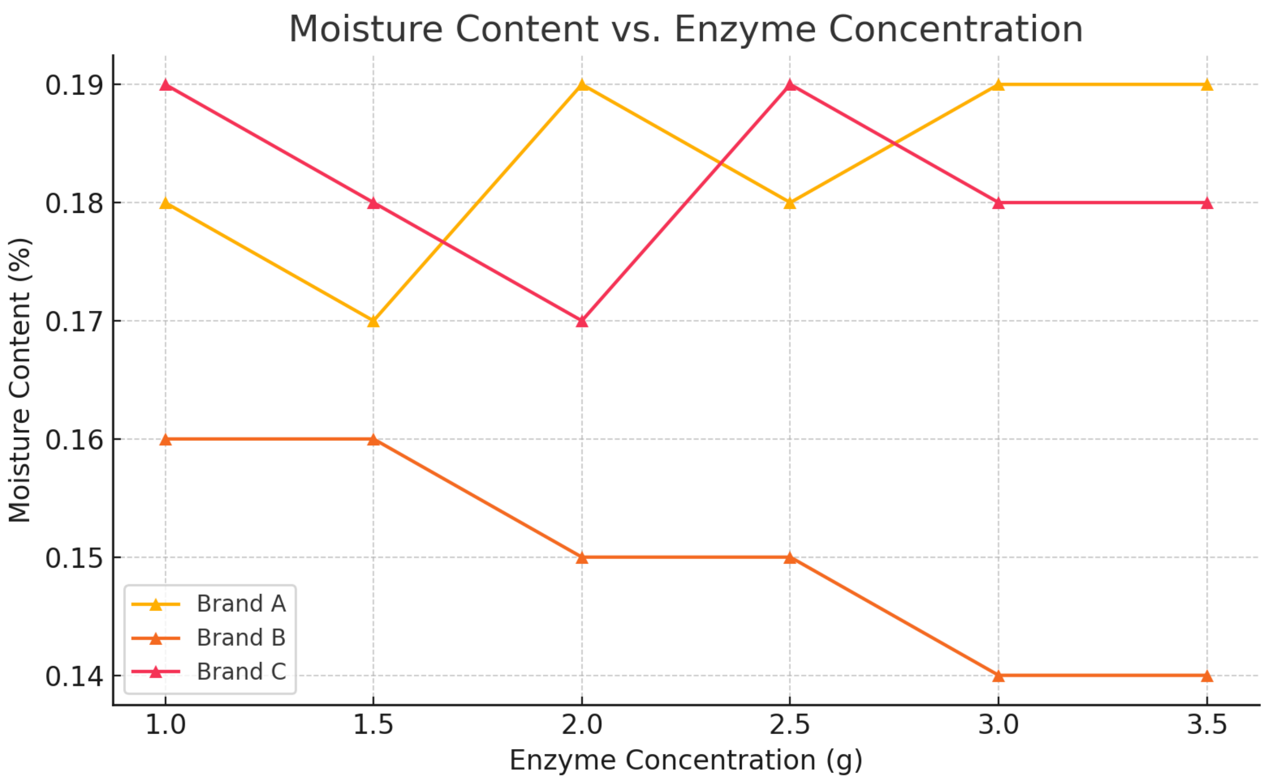

Figure 5. Moisture Content vs. Enzyme Concentration

Percentage of Yield

The research results show that the yield range of VCO is between 23.19% (lowest) to 30.51% (highest), depending on the brand and the concentration of the papain enzyme used. Each brand of proteolytic enzyme contains a different number of enzymes, even though they weigh the same. Following what was done by Andaka & Fitri (2017), His research aims to study the effect of papain enzyme concentration and incubation time on the amount (volume) of coconut oil taken. Yield stabilization at 3-3.5 g concentrations across brands suggests enzymatic saturation, where further additions don't proportionally increase output. This aligns with Michaelis-Menten kinetics, where reaction velocity plateaus once enzymes are fully engaged with substrate molecules.

The increase in the number of enzymes must be proportional to the increase in the number of substrates; if the enzymes added is more than the number of substrates, then the enzymes cannot react to produce products, so the process becomes inefficient. In the research (Suirta & Astitiasih, 2020) Makes virgin coconut oil is made from coconut with papaya leaf extract as a source of papain enzyme. Research by Rasyadan et al. (2023) also used the papain enzyme to separate the oil and protein contained in coconut flesh. His research aims to produce the greatest possible coconut oil yield. The variables in this study were the fermentation time between 20 hours to 28 hours, and the addition of the papain proteolytic enzyme between 5.5% to 9.5%. The conclusion of his research was obtained when the fermentation time was 28 hours, and the addition of 9.5% papain enzyme resulted in the largest coconut oil yield was 36%.

(Emilia et al., 2021) Conducted community service by providing material on coconut processing in Gunung Megang village, Gunung Megang sub-district, Muaraenim. The community service material aims to produce pure oil or virgin coconut oil, which is the result of processing fresh coconuts through fermentation without going through a heating process, without the addition of chemicals, dyes, and preservatives so that it can last as vegetable oil. Her research used an experimental method. The results of her research showed that making virgin coconut oil using 2 coconuts produces 100 ml of virgin coconut oil. Virgin coconut oil, using 3 coconuts, produces 300 ml of pure oil.

Moisture Content

The water content of virgin coconut oil in our study ranged from 0.14% at the lowest to 0.19% at the highest. The water content of virgin coconut oil is shown in Table 1, where the highest water content is found in enzyme brand A and enzyme brand C, which is 0.19%. The lowest water content of 0.11% is found in enzyme brand B. Water content is one of the important determining factors of oil quality. Decreased aqueous phase concentration in lipid systems prevents rancidity by simultaneously limiting hydrolysis and oxidation reactions that compromise oil quality. VCO's characteristically low water content stems from efficient emulsion breakdown, promoting complete phase separation between the oil, protein aggregates (blondo), and aqueous fractions during processing. The shelf life of oil is partly influenced by water content. Rancidity will easily occur when the oil-water content is relatively high. High oil water content can cause bacteria to grow in virgin coconut oil and hydrolyze fat molecules. This was stated by Anggraini & Elfidiah (2019), who, in their research, utilized the proteolytic enzyme papain from papaya leaves to produce coconut oil enzymatically. Fathurahmi and Siswanto (2020) systematically investigated how baker's yeast concentration and fermentation duration impact VCO quality parameters, including moisture content, yield efficiency, free fatty acid levels, and organoleptic properties (clarity, aroma, and color).

Free Fatty Acid Levels

From the data in Table 1, the free fatty acid content of virgin coconut oil in our study was between 0.17% at the highest and 0.12% at the lowest. The highest free fatty acid content was found in enzyme brands A and C, which was 0.17%. The lowest free fatty acid content of 0.12% was found in enzyme brand B. The free fatty acid content is one of the determining factors for the quality of the virgin coconut oil produced, which is a maximum of 0.12% according to the SNI 2008 standard.

An inverse relationship exists between papain concentration and free fatty acid (FFA) levels in VCO, as the proteolytic enzyme catalyzes triglyceride hydrolysis, cleaving ester bonds to yield fatty acids and glycerol molecules (Fathurahmi & Siswanto, 2020). Unlike Fitri's (2017) research which required a fermentation time of 19 hours to achieve optimal yield (30 ml), this study achieved a higher yield (31.32%) in a shorter time (8 hours). This difference may be due to the use of a higher enzyme concentration (3.5 g vs. 2 g) and the effectiveness comparison between different brands of commercial enzymes. and a papain enzyme weight of 2 grams, with the amount of oil taken as much as 30 ml.

Also conducted by Rindawati (2020) said that the traditional virgin coconut oil-making technique is considered not good for the characteristics of the oil produced because it can cause the oil to quickly smell rancid, and the color of the oil changes due to the oxidation process during heating. The use of enzymes and bait systems is different from the traditional system because both are carried out without heating. Her research aims to determine the effect of making VCO bait and enzymatic systems on the characteristics of the resulting pure coconut oil (VCO). In her research, a comparison was made between making VCO by adding 10% to 20% papaya pieces to the cream for the enzymatic system and adding 10% to 20%

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VCO bait oil to the cream. After 24 hours of fermentation, the results were obtained in the form of VCO, which was then analyzed for fatty acid content, water content, and yield. As a result, this study shows that the best enzymatic method of making VCO is at a concentration of 10% with a free fatty acid content of 0.04%. While VCO with a good bait method at all concentrations, with the same free fatty acid content of 0.03%. However, the water content values of these two methods still do not meet the quality requirements according to SNI 7381: 2008 because the values of these two methods exceed the standard of 0.2%. As for the yield, the highest enzymatic method is the addition of 20% papaya pieces, with a yield of 17%. The baiting method that obtained the highest yield was the 10% baiting method of 24.9%. This is almost the same, but still better than that done by 3. Agung Rasmito (2023), who in his research used enzymes to separate oil and protein contained in coconut seed endosperm cells. The purpose of his research was to produce the greatest possible coconut oil yield (Febriandini et. al., 2023). The variables in this study were fermentation time: 20 hours to 28 hours, and the addition of papain proteolytic enzyme: 5.5% to 9.5%. This study concludes that the largest coconut oil yield of 36% was obtained when the fermentation time was 28 hours, and the addition of 9.5% papain enzyme was made.

CONCLUSION

This finding not only meets SNI 7381:2008 but also offers a The low water content (0.14%) in Brand B meets the SNI 7381:2008 standard (<0.2%), thus potentially extending the shelf life of VCO up to 12 months at room temperature storage., due to shorter fermentation time (8 hours vs. 24-28 hours) and optimal enzyme concentration (3.5 g). Recommendation: Pilot scale testing for industrial application validation.

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