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Utilization of pineapple stems as biocatalysts in the production of coconut oil using the enzymatic method

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ABSTRACT

The utilization of pineapple fruit stems as a by-product of the agricultural process has not been optimally utilized. Meanwhile, pineapple fruit stems have promising economic potential in the field of biotechnology and food processing due to a high content of bromelain enzyme compared to ripe fruit flesh. This bromelain enzyme functions as a biocatalyst in breaking down protein and fat through the hydrolysis reaction of coconut oil. In this work, the catalytic activity of bromelain enzyme in pineapple fruit stems is observed to produce coconut oil through an enzymatic process with variables of the coconut milk weight (20, 30, 40, and 50%) and enzymatic time (6, 12, 18, and 24 h). The best variable was obtained at 30% biocatalyst dose and 24 h enzymatic process with the highest yield of 67.9%; the product characteristics have met CODEX STAN 210-1999. This shows that pineapple fruit stems have the potential material as biocatalyst in producing coconut oil as well as providing added value to pineapple fruit stems in an effort to verify agricultural waste.

1. Introduction

Indonesia is known as the largest producer of tropical fruit in the world. One of the most famous tropical fruit commodities in Indonesia is pineapple. Based on data released by Satudata Pertanian, the level of pineapple consumption has always increased [1]. Where in 2022, pineapple consumption in Indonesia reached 2.27 million tons and is projected to continue to increase to 2.55 million tons in 2027. This shows that the fairly high level of pineapple consumption in Indonesia is not only driven by the popularity of the fruit, but also due to the health benefits offered by pineapple, such as being anti-inflammatory, containing quite high levels of vitamin C and antioxidants, and can improve bone health and improve the body's digestive system [2]. This incident certainly resulted in an increase in the amount of pineapple stem waste as a by-product of the production process. This results in an increase in the amount of organic waste. This is inseparable from the use of pineapple stems which is still not optimal because it is still thrown away. In fact, pineapple stem waste has quite a large potential as a biocatalyst in making coconut oil because the bromelain enzyme content is higher than ripe pineapple [3].

On the other hand, coconut oil has long been an important commodity in Indonesia, both for local consumption and export activities. This is because coconut oil has various applications, ranging from food ingredients, the pharmaceutical industry, the chemical industry, to the cosmetics industry. Where so far, the production of coconut oil is still carried out by the heating method through a stirring process at high temperatures. As a result, this process has various shortcomings, such as the degradation of nutrients due to the fairly high temperatures in the processing process and requires quite large production costs due to the use of quite high energy [4].

Therefore, to overcome the various shortcomings in the production of coconut oil using the heating method, various developments in production methods have been carried out, one of which is the enzymatic method. In this enzymatic method, enzymes are needed as biocatalysts in the process of obtaining oil from coconut milk [5]. On the other hand, this method also offers various advantages, such as relatively low production costs because it does not require a large amount of energy, does not damage the nutritional content in coconut oil, and is environmentally friendly because it does not cause pollution from production activities. So this enzymatic method is considered more efficient and economical [6].

To further increase the level of efficiency and economic value in the coconut oil production process, a study was conducted that examined the potential of pineapple stems as a biocatalyst in the production of coconut oil using the enzymatic method as an effort to diversify to provide innovative solutions to the problems of agricultural waste and coconut oil production in Indonesia. In this study, the variables used were the dose of biocatalyst addition and the duration of the enzymatic reaction to the amount of coconut oil produced. Where the biocatalyst dose variable used was 20-50% with an

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interval of 10% for each variable. While the enzymatic reaction time variable used was 6-24 hours with an interval of 6 hours for each variable. Furthermore, the coconut oil produced will undergo a purification process using activated carbon and product characterization based on SNI 7381: 2008. On the other hand, to determine the catalytic level of the bromelain enzyme in pineapple stems, product yield calculations were carried out on all variables used.

2. Materials and Methods

2.1. Tools

Plastic basin, coconut grater, knife, chopping board, beaker glass, measuring cup, separating funnel, centrifuge, storage bottle, analytical balance, oven, weighing bottle, desiccator, crucible, Erlenmeyer flask, pipette, burette, glass funnel, glass spatula, and measuring flask.

2.2. Materials

Pineapple stem, grated coconut, distilled water, sodium hydroxide, acetic acid, cyclohexane, potassium iodide, sodium thiosulfate, starch indicator, Wijs solution, 96% alcohol, phenolphthalein indicator, and chloroform

2.3. Methods

2.3.1. Biocatalyst synthesis

The pineapple stem is cut into several smaller pieces, washed in running water while rubbing the surface of the stem to remove any dirt that is still attached, then the pineapple stem is grated using a coconut grater, and stored in a beaker glass, then the beaker glass is tightly closed using plastic wrapping to avoid contamination.

2.3.2. Coconut *milk synthesis*

Weigh the prepared old grated coconut, then add distilled water with a ratio of 1:1 to the weight of the grated coconut. Knead the two mixtures of ingredients until they feel even, then squeeze the coconut milk using a strainer cloth. Store the coconut milk in a storage bottle to wait for the next process.

2.3.3. Coconut oil synthesis using enzymatic methods

100 mL of coconut milk is put into a separating funnel that has been labeled according to the variables being worked on. Add biocatalyst according to the dose added, which is 20-50% with an interval of 10% for each variable. Shake the separating funnel for 10-15 minutes until the mixture of coconut milk and biocatalyst is felt to be homogeneous. Let the two mixtures stand according to the time variable of the enzymatic process being worked on, which is 6-24 hours with an interval of 6 hours for each variable. After the enzymatic process time is complete, separate the three layers in different beaker glasses. For beakers containing blondo, carry out the separation process again using a centrifuge to ensure that no oil content is included in the blondo. Next, store the coconut oil that has been produced in a storage bottle according to the processing variables.

2.4. Coconut oil purification

The purification process is used to remove any remaining unpleasant odors or unwanted content in coconut oil. The purification process is carried out using activated carbon weighing 5% of the amount of oil produced. Where in this process a glass funnel that has been equipped with Whattman filter paper size 42 and activated carbon is designed on a stand and clamp that has been provided. Furthermore, coconut oil is flowed from the top of the funnel, so that it passes through the activated carbon and filter paper that has been provided. Then the coconut oil will come out of the bottom of the funnel and be directly collected in a measuring cup that has been labeled for processing. The coconut oil produced will then be calculated for yield and product characterization based on SNI Pure Coconut Oil number 7381: 2008.

3. Results and Discussion

3.1. Research result

From the gas analysis using the Multi Gas Detector Analyzer, three gas components were identified in the gas collection we conducted.These include carbon dioxide (CO_2) and hydrogen (H_2), which were produced due to the failure of these two gases to react and form methane (CH_4). The percentages of the gas components can be seen in the following table.

3.2 Discussion

3.2.1. Effect of addition of dosage and enzymatic reaction time on mL of coconut oil product produced

Based on Figure 1, it can be seen that as the enzymatic time increases with the addition of a 20% biocatalyst dose, the amount of coconut oil produced also increases. This is inseparable from the kinetics of the enzymatic reaction which tends to run slowly due to the addition of a fairly low dose. So it takes longer for the enzyme to break down the substrate into oil [7]. In addition, the addition of a fairly low dose of biocatalyst allows the enzyme not to easily reach saturation. Therefore, the enzyme can still operate efficiently for a longer period of time without being hampered by excess substrate or product produced. So that the oil conversion will increase continuously as the enzymatic time increases without a drastic decrease in the efficiency of the reaction that occurs in it [8].

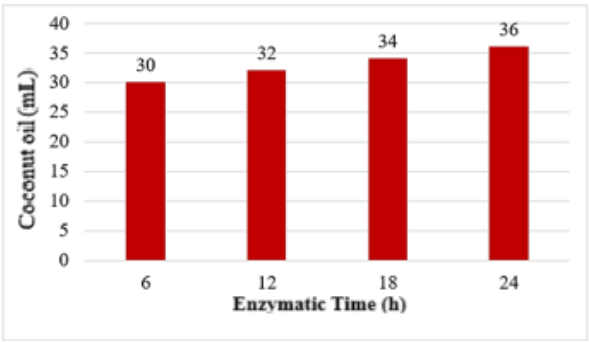

Fig. 1. oil produced with the addition of a 20% biocatalyst dose

Based on Figure 2, it can be seen that the amount of coconut oil produced from the 6-18 hour enzymatic time variable continues to increase. This is due to the abundant availability of substrates at the beginning of the enzymatic process, so that the enzyme can work with high efficiency to break down the substrate into oil [9]. Meanwhile, at the 24 hour enzymatic time variable, the amount of oil produced decreased compared to the 18 hour enzymatic time variable. This event is caused by the enzyme saturation condition. Where after a certain point, the available enzyme is already bound to the substrate, so that it has reached its maximum activity limit. When the enzyme is working at full capacity, the addition of time can no longer increase the amount of oil conversion because there are no more active sites available to catalyze new reactions [9].

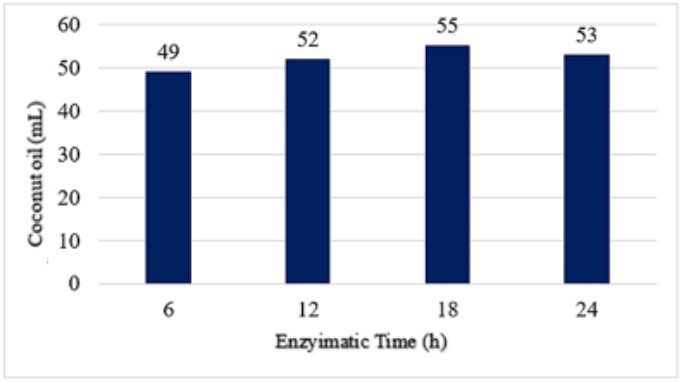

Fig. 2. oil produced with the addition of a 30% biocatalyst dose

The graphic pattern presented in Figure 3 is almost the same as the graphic pattern presented in Figure 2. Where oil conversion increases in the 3 initial enzymatic time variables (6, 12, 18 hours) and decreases in the final enzymatic time variable (24 hours). This is because over time the amount of substrate decreases, especially after the oil conversion reaches its peak. Where when most of the substrate has been broken down into oil, the reaction rate will decrease because there is no more substrate available to be broken down by the enzyme [10]. In addition, it is also caused by enzyme denaturation and inactivation factors, considering that enzymes are a group of proteins that are sensitive to environmental conditions such as temperature, pH, and time. Over time, enzymes can denature. As a result, the enzyme structure will change and the enzyme loses its ability to catalyze the reaction. This will certainly reduce the level of enzymatic reaction efficiency and decrease oil conversion [11]. In addition, the decrease in the amount of oil produced is also due to the effect of the reaction balance that occurs. Where after reaching a certain point the reaction can reach equilibrium, so that the rate of product formation will be the same as the rate of reverse reaction. In this condition, even though time increases, oil production no longer increases [12].

Based on Figure 4 regarding the graph of coconut oil yield from the variable of adding 50% biocatalyst dose and 6-24 hours of enzymatic time, it is known that the conversion of coconut milk to coconut oil tends to increase faster at the initial enzymatic time compared to longer enzymatic time. This is caused by several factors related to enzyme kinetics, enzyme saturation, and the effect of the reaction environment. Where at enzymatic times of 6 and 12 hours, the interaction between the enzyme and the substrate takes place efficiently because the enzyme in a large enough amount can directly bind the substrate without any saturation constraints. This allows the reaction rate to run quickly in producing significant amounts of coconut oil in a shorter time [13]. Meanwhile, after 12 hours of enzymatic time, oil conversion actually decreased due to product inhibition. Product inhibition can occur when the oil produced interferes with the interaction between the enzyme and the substrate or changes the reaction environment and reduces reaction efficiency at longer times. In addition, the use of doses that are too high under long reaction conditions actually causes the enzyme to lose structural stability, especially when the enzyme is affected by environmental factors, such as temperature and pH [14].

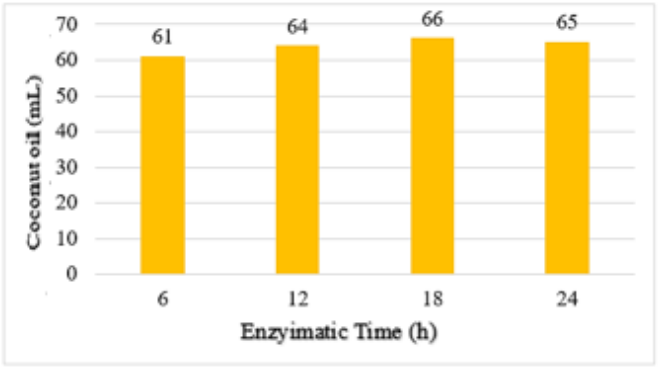

Fig. 3. oil produced with the addition of a 40% biocatalyst dose

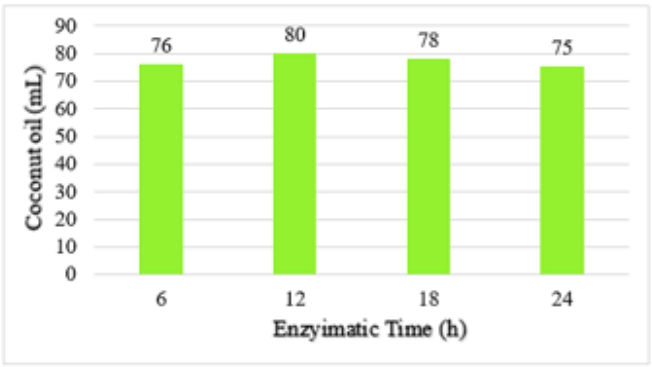

Fig. 4. oil produced with the addition of a 50% biocatalyst dose

3.2.2. Effect of addition of dosage and enzymatic reaction time on the yield of coconut oil produced

The addition of biocatalyst dosage and the length of enzymatic reaction time are two important factors in influencing the yield of coconut oil produced. Theoretically, the more biocatalyst dosage added and the longer the enzymatic reaction takes place, the more the conversion of coconut milk into coconut oil increases. This is because the increased biocatalyst dosage causes the reaction rate to increase, due to the enzyme being more effective in breaking down the complex components in coconut milk into coconut oil so that it can increase the yield of coconut oil produced [15]. On the other

hand, the addition of enzymatic reaction time can extend the reaction process that occurs, so that the enzyme has more time to break down the protein in coconut milk into coconut oil [16]. In this study, the catalytic activity of the biocatalyst can be measured from the percentage of yield calculated using equation 1 above. Figure 5 shows the results of the yield calculation obtained.

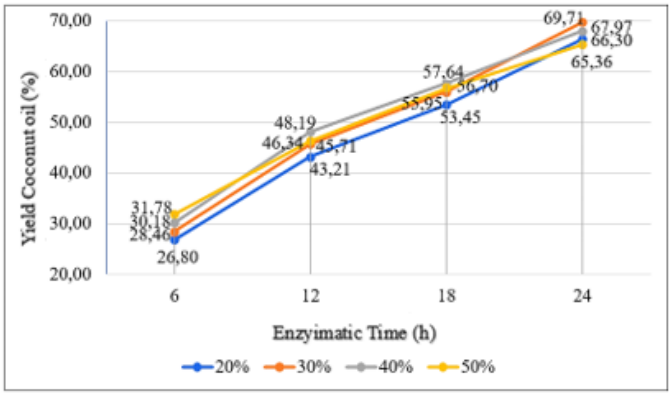

Fig. 5. Coconut oil yield

From the graph above, it can be seen that in general, the yield of coconut oil increases with increasing enzymatic time for all biocatalyst doses. For example, at a dose of 20%, the yield increased from 26.80% at an enzymatic reaction time of 6 hours to 66.30% at an enzymatic reaction time of 24 hours. This shows that longer times provide more opportunities for enzymes to break down the complex components contained in coconut milk, so that the percentage of reaction conversion produced in the enzymatic process is higher [17]. On the other hand, for the same enzymatic time, increasing the dose of biocatalyst can generally also increase the percentage of coconut oil yield, although the resulting graph is not always linear [18]. For example, at an enzymatic time of 6 hours, the yield increased from 26.80% with a biocatalyst dose of 20% to 31.78% with a biocatalyst dose of 50%. However, at 24 hours of enzymatic time, the highest yield was obtained from a biocatalyst dose of 30% with a value of 69.71% and experienced a decrease in conversion to 65.36% with an additional biocatalyst dose of 50%. Where this incident shows that the biocatalyst has an optimal limit for a certain enzymatic time. Where the addition of the dose does not always result in a higher increase in yield [19].

3.2.3. Characteristics of coconut oil enzymatic method

To determine the quality of the coconut oil produced, product characterization is carried out based on applicable quality standards. Where in this study the quality standard used is SNI Pure Coconut Oil Number 7381 of 2008. Where the test parameters carried out include the condition of coconut oil (odor, color, taste), water content and volatile compounds (moisture content), iodine value, free fatty acids, and peroxide value. Below are the results of the characterization of the best coconut oil product from the variables of adding a 30% biocatalyst dose and a 24-hour enzymatic process time.

Table 1. Characterization results of coconut oil

Test Parameter	Unit	Requirement	Result	Description
Odor	-	Characteristic of coconut, fresh, not rancid	Characteristic of coconut, fresh, not rancid	Meets CODEX STAN 210-1999
Taste	-	Normal (characteristic coconut taste)	Normal	Meets CODEX STAN 210-1999
Color	-	Colorless to pale yellow	Pale Yellow	Meets CODEX STAN 210-1999
Moisture and Volatile Compounds	%	0.1 (max)	0.09	Meets CODEX STAN 210-1999
Iodine Number	g Iod/100 g	4,1 - 11,0	6,38	Meets CODEX STAN 210-1999
Free Fatty Acids (calculated as Lauric Acid)	%	0.5 (max)	0,095	Meets CODEX STAN 210-1999
Peroxide Number	mg ek/kg	5.0 (max)	1.66	Meets CODEX STAN 210-1999

From table 1 above, it can be seen that the coconut oil product produced has met all the test parameters contained in SNI 7381:2008. Where the odor that is not rancid indicates that the product does not undergo oxidation and hydrolysis reactions [20]. The oxidation reaction is caused because the unsaturated fatty acids in the oil react with oxygen in the air to produce other compounds, such as peroxides, aldehydes, and ketones which cause an unpleasant aroma to appear [21]. While the hydrolysis reaction occurs when the oil is contaminated with water or moisture. So that the fat will be broken down into free fatty acids and glycerol. On the other hand, the oxidation process can also reduce the nutritional content of coconut oil, such as vitamin E [22].

Abnormal taste as the taste of coconut oil in general occurs because the product experiences a change in quality due to contamination from a poor production process [23]. Meanwhile, in the food industry, taste is one of the most important factors in determining product quality. Coconut oil with an abnormal taste can cause negative perceptions of the product as well as being a sign that the oil has been contaminated with microbes or other hazardous chemicals. On the other hand, an abnormal taste also poses a health hazard because it is carcinogenic [24].

4. Conclusion

From the results of the research that has been done, the highest yield percentage was obtained at 69.71% on the variable of adding a biocatalyst dose of 30% and an enzymatic reaction time of 24 hours. Where the amount of coconut oil produced reached 80 mL. In addition, the coconut oil product produced has also met the requirements contained in SNI 7381:

2008. On the other hand, the addition of 5% activated carbon in the coconut oil purification process is optimal for removing a little of the distinctive aroma of pineapple contained in the coconut oil product.

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