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Effect of Pretreating Coconut Meat with Cellulase on Oil Extraction

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Abstract

In the production process of virgin coconut oil (VCO), by-products like coconut residue are underutilized. The residue is either used as an animal feed or roasted to give cooking coconut oil. However, the inactivated by the heat during the roasting. Therefore, this research aimed to study the effect of antioxidants of the oil were pretreating coconut meat with enzymes on the oil yield in order to maximize the yield. Cellulase enzymes (500, 1500, 35000, 44000, and 50000 U/ 20 g coconut meat) were used to pretreat coconut meat. Comparing oil yields obtained with the enzyme pretreatment and without one, it was found that enzyme pretreatment increased the oil yield but the amount of enzyme within the range studied had little effect on the oil yield. However, increasing the amount of enzyme decreased polysaccharides content in the water phase. It could be concluded that the enzyme pretreatment could improve VCO extraction and reduce oil remaining in coconut residue. Moreover, additional byproducts, e.g., polysaccharides, could be obtained.

Keywords
cellulase, coconut meat, enzymatic oil extraction, virgin coconut oil.

Introduction
Coconut residue is a by-product of the production of virgin coconut oil (VCO). It has been reported that oil remained in the coconut residue (Yalegama et al. 2013). In present, the coconut residue is roasted (heating process) to obtain oil but this oil has no antioxidant activity. Enzymes has been reported to assist oil extraction to produce VCO (Che Man et al. 1996). Hence, this work aimed to study the effects of treating coconut meat with cellulase on oil yield.

1.1 Objective
To investigate the effects of treating coconut meat with cellulase on oil yields without modifying the VCO process.

2. Literature Review
VCO production process is an oil extraction from fresh coconut meat that does not require high temperature or involve chemical extraction; VCO has been reported to have higher antioxidant activity than the Refined, Bleached and Deodorized Coconut Oil (RBD oil) (Negi et al. 2024). VCO is clear and colorless and has a coconut aroma and taste (Wickramasinghe et al. 2023). There are several wet processes for extracting oil from coconut meat to produce VCO, such as the traditional hand-pressed, fermentation, centrifugation, and enzyme process. (Figure 1.)

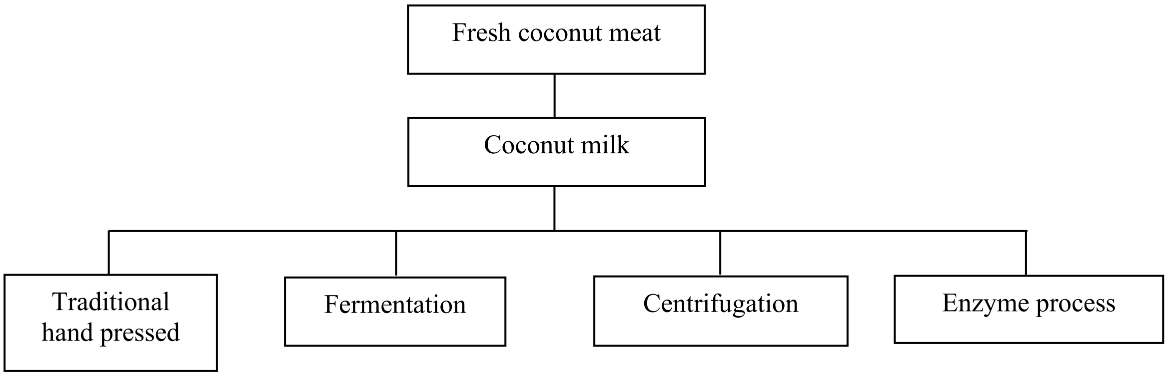

Figure 1. The VCO production processes

Traditional hand-pressed and fermentation processes are of low cost and simple. However, the oil quality may be poor due to high moisture content (Bosco 2013). Recently, VCO extraction by centrifugation has become more popular. With this process, coconut milk is centrifuged so that oil is separated from the water phase. An advantage of this method is that coconut oil is not heated during the extraction, so the quality of the oil is better (Nour et al. 2011). In addition to the above methods, several enzymes were also found to assist in the extraction of coconut oil. According to the research of Che Man et al. (1996), coconut meat was hydrolyzed by cellulase, polygalacturonase, protease, and α -amylase to improve the extraction efficiency of oil. The work showed that the extraction efficiency improved, and the quality of the oil obtained was maintained. However, the selection of concentrations of the enzymes used was not discussed in the work and some of the enzymes used could be costly.

With enzymatic extraction, coconut residue showed less oil content compared to the coconut residue obtained after coconut milk extraction (9.2 vs. 42.6%) (Che Man et al. 1996). This suggests that pre-treatment of coconut meat with enzymes could help improve VCO yield and reduce the waste used. Moreover, the oil obtained did not involve any heat and could maintain antioxidant capacity.

3. Methods

3.1 Enzymatic extraction

20 g grated coconut meat was placed in a conical flask (250mL) and mixed with 50 mL distilled water and cellulase (Iknowzyme, Thailand) at the following amounts: 0, 500, 1500, 35000, 44000, and 50000 U. The mixture was then incubated in a shaking water bath at 100 rpm for 5 hours. 100 rpm was chosen to ensure that the enzyme was not denatured by the shaking and 5 hours were chosen because a preliminary study indicated that these were the optimal extraction time. After that, the mixture was fed into a squeezing screwed juicer (Hurom HP Slow Juicer, Model, Thailand) and the coconut milk obtained was placed in a centrifuge tube (50 mL). The coconut milk was refrigerated at 4°C overnight and then centrifuged at 5000 rpm for 20 min. After the centrifugation, the milk was separated into 3 layers: (i) coconut oil at the top; (ii) white solid layer in the middle, and (iii) water layer at the bottom. The oil was used for further analysis.

3.2 Determination of polysaccharide content

To visualize the **effects of cellulase on the oil extraction**, the water phase obtained in 3.1 was analyzed for **coconut polysaccharide content** (CPC). CPC concentration was determined by the phenol-sulfuric acid method (Nielsen 2009) using D-glucose as a standard. After the centrifugation, the water phase (100 µL) was pipetted into a microcentrifuge tube and mixed with 5% phenol solution and 96% sulfuric acid (100 and 1200 µL, respectively). An absorbance of the mixture was measured at a wavelength of 484 nm using a microplate reader (SPECTROstar^{Nano}, BMG LABTECH Ltd., Germany)

4. Results and Discussion

After the enzymatic extracting step, all coconut milk samples were separated into 3 phases: the upper layer was oil, the middle layer was a thin white solid layer, and the lower layer was water, as shown in Figure 2.

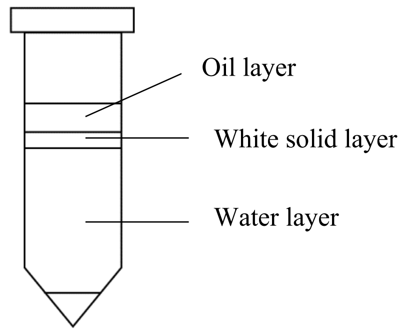

Figure 2. The coconut milk separation after extracting step diagram

Weights of the oil obtained from various samples are slightly different and shown in Table 1. According to the table, it was found that when smaller amounts of enzyme was used (< 44000U), increasing amount of enzyme increased the VCO yield. However, when the amount of enzyme was increased beyond 44000 U, the oil yields did not differ significantly. The enzyme pretreatment used could increase the oil yield because coconut meat was digested by cellulase; the oil then could be extracted from the meat more easily (Goulao et al. 2010). When a substrate is used up, increasing an amount of enzyme do not increase a product (Robinson 2015). This suggests that all the substrate was used up when the cellulase enzyme amount was larger than 44000 U.

Table 1. Effect of enzymatic pre-treatment on the amount of VCO extract

	No enzyme	Cellulase				
		500 U	1500 U	35000 U	44000 U	50000 U
Weight of VCO extract (g)	1.80±0.01	2.06±0.03	2.25±0.04	3.01±0.02	3.35±0.03	3.61±0.04

However, when increasing the amount of enzyme, polysaccharides could also be digested into sugars (Malhotra and Alghuthaymi 2022). According to Table 2, when the enzyme content is larger than 35000 U, the polysaccharide content begins to drop.

Table 2. Effect of enzymatic pre-treatment on the concentration of polysaccharides

	No enzyme	Cellulase				
		500 U	1500 U	35000 U	44000 U	50000 U
Concentration of polysaccharides (μg/mL)	278.21±4.20	295.26±21.93	320.38±9.23	293.72±31.49	283.14±13.04	278.97±4.89

According to the results, if considering the amount of oil extracted and the content of polysaccharide that may be further utilized, the amount of enzyme should be used in the range of 35000 - 44000 U. Under this condition, the enzyme pretreatment could promote the oil yield by 1.67-1.86 times compared to that obtained without the pretreatment.

5. Conclusion

The present study indicated that pretreating coconut meat with cellulase could improve VCO extraction by increasing the oil yield. We found that increasing cellulase enhanced oil extraction but decreased polysaccharides content. Hence, to utilize the polysaccharides without sacrificing the oil yield, around 35000-44000 U of cellulase would be appropriate for the enzyme pretreatment.

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