ENZYMATIC COCOA POD WAX EXTRACTION MONITORING DEVICE FOR COCOA FARMS

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Abstract

This research aims to explore and develop methods for extracting wax from cocoa beans, their components, and potential applications. The study focused on extracting wax from cocoa pods from Lees farm in Malaysia,by using response surface methodology (RSM) to optimize wax yield and validate its active ingredient. The highest wax yield was recorded at 20.4%, with the density of cocoa pod wax ranging from 0.94 to 0.98 grams per milliliter.

Keywords: *Cocoa, pods, extracction, enzyme, wax.*

INTRODUCTION

Malaysia is close to the equator and has a variety of terrain types, making it home to numerous plant species. Wax is a general term for a variety of pliable materials that may be produced from both natural and synthetic ingredients and is made up mostly of high molecular weight components. Cocoa beans are the seeds of the cacao tree that have been fermented to remove the sugars and oils and are then used to make chocolate. Malaysia is one of the main centers for grinding and processing cocoa beans, and the extraction of wax can be done by several methods, such as chemical-based extraction and enzyme-based extraction.

Malaysia is a rich country with abundant plant species, but there are few studies of extracting wax from cocoa beans due to the market's focus on producing chocolate. This research aims to learn more about and develop methods to extract wax from cocoa beans, their components, and potential applications.

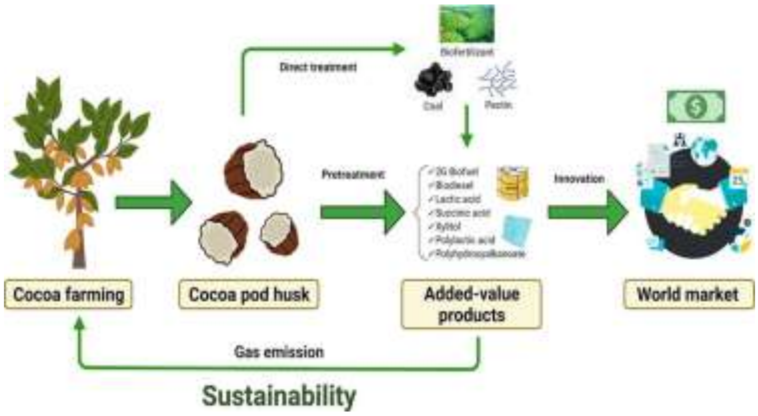
Investigating and exploring cocoa beans can help us discover their social and economic advantages, which could become a major global commercial interest.

The objective of this study is to study and develop the extraction methods of wax, optimize the extracted wax, and validate its active ingredient.

This study focused on extracting wax from cocoa pods from Lees farm in Malaysia, with potential applications and uses described in the report.

Raw Materials for Wax Extraction Process

Cocoa pods are the fruit of the cocoa tree and are harvested and processed to make cocoa goods such as chocolate and cocoa butter. Cocoa butter is a naturally occurring lipid derived from cocoa beans and is used in a range of products, including cosmetics, medicines, and food. Cocoa pod husks (CPH) make up 80% of the waste produced in the first stages of processing cocoa beans, and their disposal takes up huge territory. Recent research has shown that CPH can be utilized to create chemicals with moderate to high added value, with potential uses in food and agricultural, bioenergy, and other industries.



Source: (Porto de Souza Vandenberghe et al., 2022)

Figure 1: An example of the Cocoa Pod Products Process.

Cocoa Industry in Malaysia

According to the Ministry of plantation industries and commodities (MPIC) in 2021, there are 5955 hectares of cocoa plantations, producing 361 tons. Malaysia, which is ranked sixth in the world, is one of the main centers for grinding and processing cocoa beans. The extraction of wax can be done by several methods such as chemical-based extraction which involves using chemical solvents to complete the process. And another method can be used for extraction of wax which is enzyme-based extraction and in this method, enzymes involved in the process to get a higher purity product at the end of the extraction process.

Figure 2: Analysis of the cocoa market from MPIC for 2021.

Methods of Wax Extraction

There are several methods to extract wax and that depends on many factors such as the raw material plant that contain the wax, physical and chemical properties of the extraction process, and in this study comparison between two types of extraction were mentioned: Chemical base extraction and enzyme base extraction. And the Enzyme -based wax extraction from cocoa pods is used since it is a promising technology with advantages over existing solvent-based procedures. It eliminates the need for hazardous solvents, making it more environmentally friendly and sustainable. It is also more efficient and effective, resulting in higher yields and higher quality wax.

Chemical Based Extraction of Wax from Cocoa Pods

To extract wax from cocoa pods, the pods are harvested, and the seeds removed. To dissolve the wax, the husks are crushed and immersed in a solvent such as hexane. The mixture is filtered to eliminate any solid particles before the solvent is evaporated away, leaving only the pure wax. Non-chemical methods such as mechanical pressing or supercritical CO_2 extraction are also available. One chemical-based approach for obtaining wax from cocoa pods is as follows:

1) Remove the cocoa beans from the cocoa pods.

2) Using a grinder or mixer, ground the cocoa pods into small bits.

3) Place the pulverized cocoa pod pieces in a container and fill it with a solvent, such as hexane. The wax in the cocoa pods will be dissolved by the solvent.

4) Stir the mixture for many hours to dissolve the wax in the solvent.

5) Separate the solvent and dissolved wax from the crushed cocoa pod pieces by filtering the mixture.

6) Allow the solvent to evaporate, leaving the extracted wax behind.

Enzymatic Based Extraction of Wax from Cocoa Pods

Enzymatic extraction of wax from cocoa pods is a procedure that uses enzymes to break down the wax in the pods. The mixture is filtered to eliminate any solid particles before being allowed to stand for a period to allow the wax to separate from the liquid. The wax can then be purified and processed to make candles, soaps, and cosmetics. Enzyme extraction has been found to be more efficient and cost-effective than standard approaches such as solvent extraction. Enzyme-assisted wax extraction from cocoa butter has received a lot of attention in recent years since it has been proved to be an efficient and environmentally friendly process.

Lipases, esterases, and cutinases are enzymes that have been utilized in the extraction of wax from cocoa butter. Lipases are enzymes that catalyze the hydrolysis of ester bonds, and they have been proven to be particularly effective at hydrolyzing wax esters. Esterases are enzymes that have been found to be effective in the hydrolysis of wax esters as well as the hydrolysis of ester linkages. Cutinases are enzymes that catalyze the hydrolysis of ester bonds in cutin, a component of plant cuticles that has been found in cocoa butter. The extraction method normally involves an enzyme treatment of the pressed cocoa butter at certain temperature and pH levels, followed by centrifuged or filtered to separate it from the cocoa butter

METHODOLOGY

Preparation of the Cocoa pods

In this step there will be 5 main stages to prepare the pods for the extraction process which include:

- **Fermentation:** The beans need to be opened before starting the fermentation stage, usually by hitting them with a piece of wood. The fermentation process is done by putting the beans in boxes, with every two days being stirred and moved to another box. This process will take 6-10 days to ensure the best results.
- **Drying:** After fermentation, the beans are spread out to dry in the sun for 5 to 10 days.
- **Grinding:** The roasted beans are then ground to release cocoa butter, which is a source of wax.

Preparation of Enzyme

The most important details are to prepare a 2.5% solution of lipase by adding 2.5g of lipase powder to 100mL of distilled water, a 0.01M sodium carbonate solution by dissolving 1.06g of sodium carbonate powder in 100mL of distilled water, a 5%

solution of bile salts by adding 5g of bile salts powder to 100mL of distilled water, and a hydrogen carbonate indicator by adding 10mL of the concentrate to 90mL of distilled water. The extracted cocoa butter then goes through enzyme treatment for 48 hours at a temperature of 45 C° with 5.5 PH and is mixed with enzymes to break down waxes and other unwanted components.

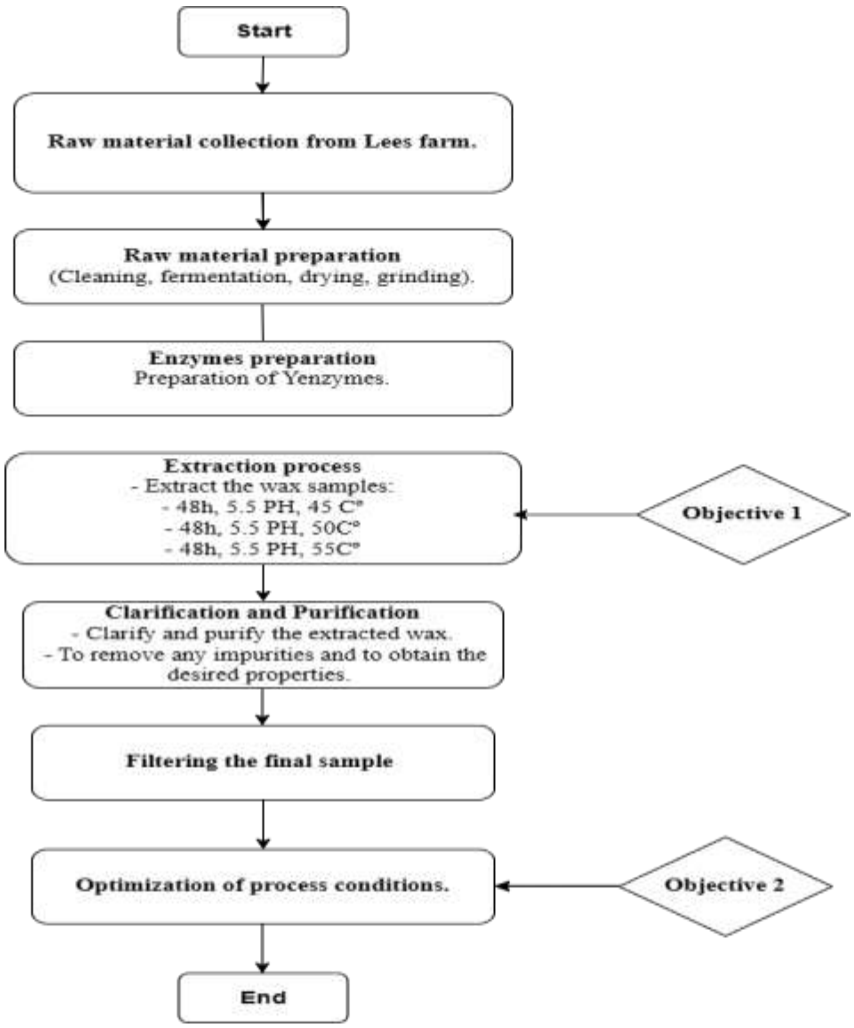


Figure 3: Overall methods performed in the study.

RESULTS AND DISCUSSION

Response surface methodology (RSM) was used to evaluate the effect of the different factors and the interaction between them. The optimization of wax yield was performed based on the 2 parameters: temperature and enzymatic loading. The range of enzymatic loading was between 2.5% to 7.5%, and the range of temperature was between 45°C to 55°C. The physical observation made throughout the experiment proved several theoretical knowledge based on the result and the variation of wax formation.

Figure 4 illustrates the physical variation of the wax formation. It was found that the higher number of enzymes added into the mixture resulted in more visible quantity of wax production, and the higher amount of solid (cocoa fruits) added into the mixture also showed a similar higher production of wax as compared to lower addition of enzymes and solids (cocoa pods). The highest enzymatic loading was observed at 7.5%, while the highest temperature was 55°C. The highest wax yield obtained in this study was 20.4%. The addition of distilled water facilitated not only.

Figure 4: Image showing the physical variation of wax formation.

Overall, based on the 13 runs performed in the optimization step, the highest Wax yield is recorded at 20.4 %, where it was calculated as shown below:

$$Wax\ yield = \frac{Mass\ of\ extracted\ wax\ (g)}{Mass\ of\ sample\ (g)} \times 100\%$$

$$Wax\ yield = \frac{3.57}{17.5} \times 100\%$$

$$Wax\ yield = 20.4\ \%$$

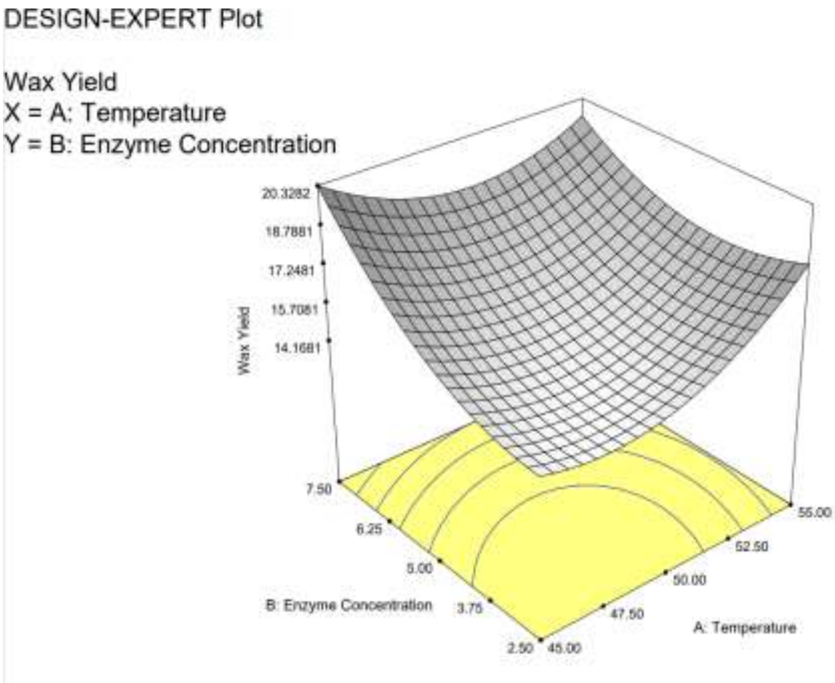


Figure 5: Screening of temperature versus enzymatic loading graph.

Figure 5 illustrates the effect of the variation of the temperature versus enzymatic loading to give a maximum yield of 20.4 % of wax. This graph shows and confirms the progressive effect on the wax yield by the constant increase of the enzymatic loading.

Table 1: Data of Percentage error between theoretical and experimental yield.

Run	Theoretical Yield (%)	Experimental Yield (%)	Error (%)
9	20.40	20.33	0.34
10	16.40	15.08	8.04
8	15.40	14.59	5.25

The method of calculation for the % error using the formula is shown below.

$$\%error = \frac{theoretical\ value\ -\ experimental\ value}{theoretical\ value} \times 100\%$$

$$\%error = \frac{20.40 - 20.33}{20.40} \times 100$$

$\%error = 0.34\%$

The percentage error for the 3 runs of the optimization experiment showed the lowest error at 0.34 % and the highest error at 8.04%.

The density of cocoa pod wax can range from 0.94 to 0.98 grams per milliliter (g/ml). However, it's important to note that actual values may vary depending on factors such as cocoa variety, pod maturity, and extraction methods.

In fulfilment of Objective 2, the density was calculated using the formula as shown below. The physical observation of the wax floating on top of the solvent is further justified through the findings of the actual density value of the wax. Since, water has a density, ρ = 1.00g/ml., the lower density of wax (ρ < 1.00) is in harmony with the physical observation.

$$Density = \frac{Mass}{Volume} = \frac{95.5g}{100ml} = 0.955g/ml$$

CONCLUSION

Enzyme-based wax extraction from cocoa pods is a promising technology with various advantages over existing solvent-based procedures. It eliminates the need for hazardous solvents and is more efficient and effective in extracting wax from cocoa pods, resulting in higher yields and higher quality wax. However, more research is needed to optimize the enzyme extraction process and assess the economic feasibility of this technology on a larger scale. Enzymes are more successful than solvents at breaking down the cell walls of the cocoa bean, resulting in larger wax yields. However, optimizing the enzyme extraction technique to maximize yields and quality is one of the most difficult challenges. This entails selecting the appropriate enzymes, optimizing the extraction conditions, and determining the best enzyme-to-substrate ratio.

REFERENCE

Chun Lin, Y., & Meng Choong, Y. (2022). Characterization and Yield of Crude Cocoa Butter Extracted from Taiwanese Cocoa Beans under Different Fermentation Degree and Roasting Conditions. *Journal of Food and Nutrition Research*, *10*(2), 151–157. https://doi.org/10.12691/jfnr-10-2-8.

Gutiérrez-Macías, P., Mirón-Mérida, V. A., Rodríguez-Nava, C. O., & Barragán-Huerta, B. E. (2021). Cocoa: Beyond chocolate, a promising material for potential value-added products. *Valorization of Agri-Food Wastes and By-Products*, 267–288. https://doi.org/10.1016/b978-0-12-824044-1.00038-6

Hennessey-Ramos, L., Murillo-Arango, W., Vasco-Correa, J., & Paz Astudillo, I. C. (2021). Enzymatic Extraction and Characterization of Pectin from Cocoa Pod Husks (Theobroma cacao L.) Using Celluclast® 1.5 L. *Molecules*, *26*(5), 1473. https://doi.org/10.3390/molecules26051473

Lan, Y. (2019). Waxes. *Encyclopedia of Food Chemistry*, 312–316. https://doi.org/10.1016/b978-0-08-100596-5.22344-5

Tinto, W., Elufioye, T., & Roach, J. (2017). Waxes. *Pharmacognosy*, 443–455. https://doi.org/10.1016/b978-0-12-802104-0.00022-6

Yusof, F., Khanahmadi, S., Amid, A., & Mahmod, S. S. (2016). Cocoa pod husk, a new source of hydrolase enzymes for preparation of cross-linked enzyme aggregate. *SpringerPlus*, *5*(1). https://doi.org/10.1186/s40064-015-1621-3