OPTIMISATION METHOD FOR COCOA FAT EXTRACTION USING OIL EXPRESSER

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ABSTRACT – *Cocoa beans are the main ingredients in chocolate and cocoa product, and are rich in polyphenols. The content of polyphenols can vary depending on the source of beans (growing conditions and cocoa variety), and processing conditions during fermentation and drying methods. This study investigated the total polyphenol content (TPC) of unfermented Malaysian cocoa beans and determined the suitable parameter for mechanical fat extraction using the oil expresser Model X8S compared to the previous method (fat expeller Model Komet). Initially, the polyphenol content of unfermented Cocoa Research and Development Centre (CRDC), Jengka cocoa beans was determined from cocoa nibs which undergone fat extraction at 60, 70, 80, 90 and 100°C. Results show that there was increasing TPC in cocoa nibs from CRDC, Jengka by increasing temperature used which are 68.20, 75.10, 85.80, 103.77 and 110.38 mg GAE/g respectively. Thus, a temperature of 100°C was chosen to extract cocoa butter from selected cocoa beans such as CRDC Bagan Datuk (Perak), CRDC Jengka (Pahang), Ong Chong Lim Plantation and Mr Tan Sow Phin (farmers). Cocoa beans from Ong Chong Lim Plantation contained the highest TPC but no significant difference ($p>0.05$) followed by CRDC Jengka, CRDC Bagan Datuk and Mr Tan Sow Phin which are 77.39, 73.53, 65.81 and 64.01 mg GAE/g respectively. However, this results might be different in the future depending on the primary and secondary processing carried out, weather and soil changes. In conclusion, the mechanical method is the safest way to remove cocoa butter in unfermented cocoa beans furthermore to gain high cocoa polyphenol extract which has some potential phytochemicals to be used in healthy food, beverages, cosmetics and skincare industries.*

Key words: Polyphenol, Cocoa Bean, Oil Expresser X8S, Total Phenolic Content, Cocoa Butter

INTRODUCTION

Cocoa was first developed as a crop in many ancient South American cultures (History of Cocoa: World Cocoa Foundation, 2018), and had been acknowledged by European Food Safety Authority (EFSA) as one of the most significant sources of polyphenols (Urbaska & Kowalska, 2019). Cocoa beans have a high phenolic content of about 12–18% (dry weight) in unfermented beans (Kim & Keeney, 1984. 60% of the total phenolics in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer) (Dreosti, 2000). These compounds were reported to be a potential candidate to combat free radicals, which cause degenerative human diseases such as cancer, heart disease, and cerebrovascular disease through multiple mechanisms (Lee *et.al.*, 2003).

The high amounts of polyphenols in fresh cocoa seeds depend on soil and climatic conditions (La Mantia *et al*., 2023). Besides, temperature and extraction time also affected polyphenol extract profiles both quantitatively and qualitatively. High temperatures and high exposure times reduced the yield of phenolic compounds detected (Antony & Farid, 2022). Therefore, growing cocoa plants in different places and extracting polyphenols that involved high temperatures may affect the antioxidant levels in cocoa beans.

Gallic acid (GAE) is an element of the phenolic compound made from hydroxybenzoic acid, which is commonly referred to as simple phenolic acid. Gallic acid was used in this analysis as a reference solution for calculating the quantities of total phenol components in samples. Because it is one of the stable, natural phenols and inexpensive compared to other solutions, gallic acid was chosen as the standard. During the preparation of the analysis TPC, each sample or standard gallic acid solution changed from being colourless to yellow when the FC reagent was introduced. This reveals when gallic acid or sample was combined with the FC reagent, the mixtures turn to yellow colour, indicating the presence of phenol (Hilma, 2018). On the contrary, as an antioxidant, phenolic elements can use oxidation-reduction reactions (Tyagi *et al*., 2022). The yellow solutions became blue when the sodium carbonate was added to the sample solution or gallic acid. This occur due to the FC reagent being reduced by phenolic elements, resulting in the formation of a blue colour (Hilma, 2018).

Thus, this study aimed to investigate the total polyphenol content (TPC) of unfermented

Malaysian cocoa beans and to determine the suitable parameter for mechanical fat extraction using oil expresser Model X8S.

MATERIALS AND METHODS

Raw Materials

Cocoa fruits for this study were obtained from *Pusat Cocoa Research and Development Centre (CRDC) Bagan Datuk (Perak) (A), CRDC Jengka (Pahang) (B), Mr Tan Sow Phin (farmers) (C) and Ong Chong Lim Plantation (D).*

Figure 2.1: Cocoa fruits

Chemicals

Analytical-grade chemicals from Sigma-Aldrich such as 2,2-diphenyl-2-picylhydrazyl hydrate (DPPH), ascorbic acid powder, Tris-HCl buffer, Folin-Ciocalteu′s phenol reagent, gallic acid and acetone were utilised in these tests. Other than that, ethanol from R&M Chemical and Fisher Scientific were also used. The chemicals from Fisher Chemical such as petroleum ether and sodium carbonate were also used in this experiment. Distilled water was also utilised.

Sample Preparation

Fresh cocoa beans had been separated from cocoa pod husk and then boiled for 20 minutes. It was then subsequently dried under the sun for 5 days. The cocoa shells were manually peeled from cocoa nibs. Nibs from the cocoa bean were used for further processing (Serena, 2021).

Figure 2.2: Fresh cocoa beans

Figure 2.3: Boiled cocoa beans

Figure 2.4: Drying cocoa beans

Figure 2.5: Dried cocoa beans

Figure 2.6: Cocoa shells

Figure 2.7: Cocoa nibs

Figure 2.8: Oil expresser X8S (mechanical defat machine).

Defatted Method

As polyphenols are a fat-soluble substance, the defatted process was employed to extract the fats from cocoa nibs. There are two kinds of defatting treatments: mechanical and chemical (Hua *et al*., 2020).

The Mechanical defat process was done by using X8S oil expresser. Dried cocoa beans were ground until became small sizes called nibs. 500g of nibs were weighed and placed inside the funnel of defat machine. The temperature was set to 60°C and the process started once the timer is done. Time spent on the defatting process as well as weights obtained from waste, crude, and by-products like oil/fat were recorded and percentages of the products were computed. The procedure was repeated for the next temperatures which were 70°C, 80°C, 90°C and 100°C.

Cocoa Oil/Fat

Cocoa nib

In chemical treatment, fats are primarily extracted during the chemical treatment via organic solvents. The lipid components in cocoa beans are extracted by organic solvent extraction, which involves using volatile organic solvents. Simple organic solvents or mixtures of organic solvents can be used in this method. Petroleum ether, dichloroethane, methanol, ethanol, chloroform, and acetone are examples of common solvents (Hua *et al*., 2020). The ether samples (low polarity) and alcohol substances (middle polarity) were obtained through sequential extraction methods at a 2:5 ratio. 6g of cocoa nibs (ground into a powder) and 15 mL of petroleum ether were used to develop the ether extract (supernatant). The mixtures were shaken and centrifuged for 5 minutes at 6°C, and then, the supernatant was drained. The following extracts were made using the same ratio, but began with the precipitate that formed during the first extraction. In a fume hood, the precipitate is allowed to dry overnight. The sequential extractions were carried out twice. Afterwards, the final pellet produced by this method was utilised to extract the polyphenol. This method was prepared according to Henrique *et al*. (2012) method with slight modifications.

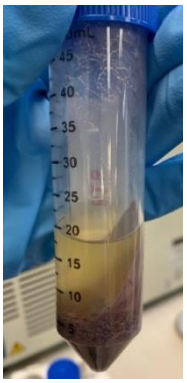

Figure 2.10: Ether extract (supernatant)

Figure 2.11: Alcohol extract (supernatant)

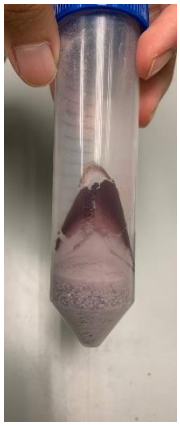

Figure 2.12: Final pellet

Polyphenol Extraction

0.25g of cocoa extract was placed into 25ml of 70% acetone in a 50ml centrifuge tube. The samples were then centrifuged at 12000 rpm, 4°C for 4 minutes. After that, the supernatant was filtered into a clean 50ml centrifuge tube using Whatman Qualitative Filter Paper (Sigma-Adrich) grade 42 to obtain polyphenol extract without contamination from the pellet. The polyphenol extracts were then stored in chiller at -20°C for further use.

Figure 2.13: Polyphenol extract

Determination of Total Polyphenol Content

Sample

Two types of cocoa beans with different defatted treatments were used; (a) the chemical defatted method with various locations, (b) the physical defatted method with different temperature.

Preparation Stock Solution

The Gallic acid stock solution was prepared according to Samarasiri *et al*. (2019) method with slight modification. Folin-Ciocalteu colorimetry was used to calculate the total phenolic content of cocoa nibs. The calculation of the amount of TPC required the use of a gallic acid standard curve. The gallic acid was diluted in ethanol (95% purity) to produce a stock gallic acid solution concentration of 5000 ppm. Following that, distilled water was used to dilute the stock gallic acid solution to various concentrations (200, 400, 600, 800, and 1000 ppm).

The required amount of gallic acid to prepare 200–1000 ppm gallic acid standard solution used equation (1):

$$M_1V_1=M_2V_2 \qquad (1)$$

Where,

M_1: 5000ppm of gallic acid

M_2: gallic acid concentration (200–1000 ppm)

V_2: 10ml of distilled water

Total Polyphenol Content (TPC) Analysis

100 µL of each extract or standard gallic solution was put into a vial along with 7.9 mL of distilled water and 500 µL of Folin-Ciocalteu (FC) reagent. The mixture was well blended after that and left for 30 seconds to 8 minutes. Each solution was subsequently given 1.5mL of a 20% (w/v) sodium carbonate solution (Na_2CO_3). The series of samples were then stored in a dark room for two hours, and the UV-Vis Spectrophotometer (Agilent Technologies, California) was used to determine the absorbance of every sample at 765 nm. This method was prepared according to Samarasiri *et al.* (2019) method with slight modifications.

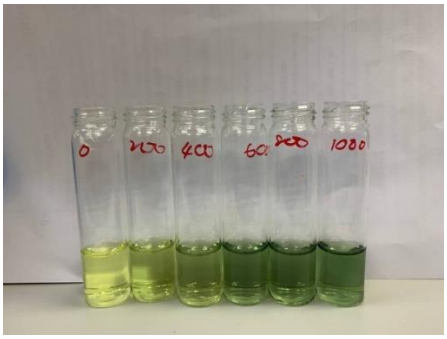

Figure 2.14: Standard gallic acid solution after adding 7.9 mL of distilled water and 500 µL of Folin-Ciocalteu reagent.

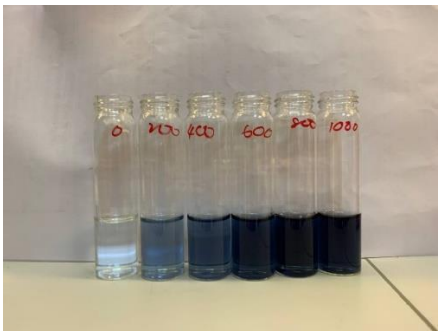

Figure 2.15: Standard gallic acid solution after adding 1.5mL of a 20% of Na_2CO_3.

The Gallic acid standard solution was evaluated at concentrations of 200, 400, 600, 800, and 1000 ppm, with analyses recorded at a maximum wavelength of 756 nm. The absorbing capacity of a standard gallic acid solution was determined at each concentration. It was discovered

a linear equation that could be used to calculate the total amount of phenolic compounds in samples of cocoa bean extract. The standard curve required for this TPC assay was created by plotting the absorbance data at 765 nm against gallic acid concentrations (Samarasiri *et al*., 2019).

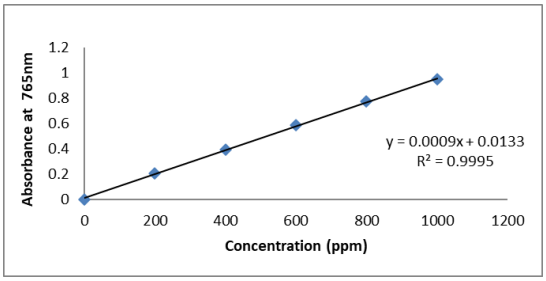

Figure 2.16: Gallic acid standard curve for TPC determination at 765 nm

The linear regression correlation (5) for the gallic acid standard curve is given in Figure. 2.16**.** TPC was determined and reported as mg of gallic acid equivalent per 1g of cocoa beans using the calibration curve.

y = 0.0009 [Gallic Acid] + 0.0133 (5)

where,

Gallic Acid was expressed as mg /L with R^2= 0.9995

TPC data from cocoa beans were plotted according to location. According to the findings of this study, phenolic molecules in cocoa beans from different regions had variable total phenolic concentrations,

RESULTS AND DISCUSSIONS

Total Polyphenol Content

The Total polyphenol content of selected cocoa beans was shown in Figure. 3.1 and Table 1. The highest TPC of cocoa beans obtained was 77.39 mg GAE/g which was obtained from farmer Mr Tan Sow Phin while the lowest TPC of cocoa beans obtained was 64.01 mg GAE/g which was obtained from Ong Chong Lim Plantation. The variation may be relied on by the soil type and the weather (La Mantia *et al*., 2023). Moreover, Heimler *et al*. (2017) concluded that a lower level of polyphenols is associated with a greater amount of nitrogen supply. The source of nitrogen may come via fertilising the soil with inorganic or organic materials.

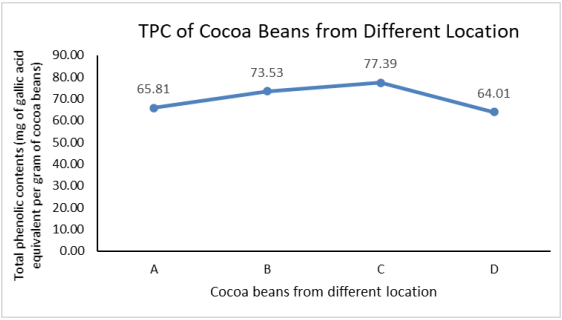

Figure 3.1: Total polyphenol content of cocoa beans from different locations

Table 1: Total phenolic content of cocoa beans from different location

Location	Total Phenolic Content (mg GAE/g)
A	65.81 ± 0.01
B	73.53 ± 0.05
C	77.39 ± 0.05
D	64.01 ± 0.05

The result of TPC obtained from the cocoa beans over defatted temperature was plotted. The results in Figure 3.2 and Table 2 study showed that when the temperature increase, the TPC of cocoa beans also increases. This result was supported by Vergara-Salinas *et al*. (2012) stated that total antioxidant levels extract increased when exposed to temperatures ranging from 50 to 200°C. The disparity in the proportion of fat found using the defatted machine may be the reason for this cause as polyphenols are soluble in fat. Cocoa beans have a fat content of between 40 and 50% (Rucker, 2009). When a higher temperature extraction was applied, more fat was produced. The proportion of fat produced when using 100°C temperature was about 35.8%, which was the highest compared to other temperatures as shown in Table 2.

Table 2: Total phenolic content of cocoa beans with different temperatures

Defat Temperature (°C)	Total Phenolic Content (mg GAE/g)
60	68.20 ± 0.03
70	75.10 ± 0.03
80	85.80 ± 0.02
90	103.77 ± 0.05
100	110.38 ± 0.03

This shows that most of the polyphenol content is no longer bound for the fat. As a result, the TPC value rises as the temperature extraction rises. Antony & Farid (2022) stated that it is very

difficult to gain a clear understanding of the impact of temperature on polyphenols due to the lack of sensitivity of the Folin-Ciocâlteu assay to assess TPC. To fully understand the phenolic composition of the extract, it is advised that TPC by Folin-Ciocâlteu assay be carried out together with HPLC analysis.

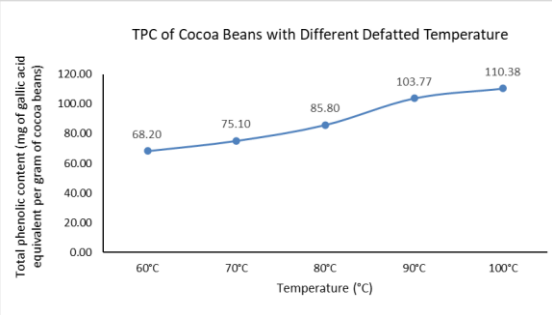

Figure 3.2: Total polyphenol content of cocoa beans at different temperature

CONCLUSIONS

In this study, the results conclude that the higher temperature applied to cocoa beans the more oil produced, and more polyphenol extract was obtained which indicated that it has some potential phytochemicals to be used in health industries, commercialize and further study.

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