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Optimization of Oil Extraction from Cocoa Bean Shells Using Three Solvents with Solvent Reusability

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ABSTRACT: Cocoa bean shells from chocolate processing are byproducts of the winnowing process used to remove the shells from the cocoa nibs. The cocoa bean shells have a residual oil content of 11.30 wt % and good nutritional value for animal feed. This study aims to determine the optimal conditions for oil extraction from dried cocoa bean shells using three solvents (hexane, anhydrous ethanol, and hydrous ethanol) while reusing the solvent. The mass ratio of solvent-to-dried cocoa bean shell (2.5−29.5 g/g), stirrer speed (50−550 rpm), and extraction time (0.3−13.7 min) parameters are varied to optimize the oil yield from dried cocoa bean shell using the response surface method. The optimal conditions for hexane were 26.0:1 g/g hexane-to-dried cocoa bean shell ratio, 550 rpm stirrer speed, and 5.2 min extraction time; for anhydrous ethanol, it was 29.5:1 g/g anhydrous ethanol-to-dried cocoa bean shell ratio, 330 rpm stirrer speed, and 7.1 min extraction time; and for hydrous ethanol, it was 27.4 g/g hydrous ethanol-to-dried cocoa bean shell ratio, 550 rpm stirrer speed, and 12.1 min extraction time. The results of oil yields showed that 10.80, 10.50, and 8.90 wt % cocoa bean shell oil yields from the extraction process under optimal conditions using hexane, anhydrous ethanol, and hydrous ethanol, respectively. The high yields of cocoa bean shell oil from hexane and anhydrous ethanol conditions were selected to test the extraction efficiency using reused miscella. Therefore, the extraction efficiency of dried cocoa bean shells with reused miscella using hexane and anhydrous ethanol was examined under optimal conditions to save the amount of solvent and energy. It was found that six cycles of hexane and two cycles of anhydrous ethanol were required to extract oil from cocoa bean shells, with an efficiency of over 80%. The compositions in cocoa bean shell oil from extraction consisted of 99.53% triglycerides, 0.01% free fatty acids, 0.43% diglycerides, and 0.05% monoglycerides for the hexane condition, while the compositions in cocoa bean shell oil were found to be 98.37% triglycerides, 0.53% free fatty acids, 1.02% diglycerides, and 0.08% monoglycerides after being extracted with ethanol. In addition, cocoa bean shell was oil extracted with reused miscella of hexane and anhydrous ethanol solvents to save energy and chemicals during solvent evaporation. This study recommends ethanol over hexane because it is safer for the environment. Both dried and defatted cocoa bean shells could successfully produce feed pellets by using pelletization. The process was achieved by adding 25 wt % water to defatted cocoa bean shells before forming them with a pellet machine.

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.), also known as cacao, is a natural product obtained from the seeds of the cocoa tree. Once harvested and processed, cocoa serves as a fundamental ingredient in chocolate production and various other food and beverage applications. Cocoa's distinct flavor profile and adaptability have made it a globally recognized ingredient. In recent years, following the global COVID-19 pandemic, the cocoa market has shown progressive growth owing to industry expansion in premium and high-quality chocolate, organic and locally sourced cocoa, and innovative cocoa-based products. In addition, consumers are increasingly interested in the origins and production methods of cocoa used in desserts.[1,2] According to predictions, the overall volume of cocoa imports is anticipated to reach 4.2 billion kg by 2026, reflecting an average annual increase of 1.2% since 2016.[2] Cocoa is primarily grown in tropical countries near the equator, with global output reaching 4.9 million tonnes per year, with 81, 15, and 4% produced in Africa, the Americas, and Asia and Oceania, respectively, during the 2021−2022 harvest season.[3] Ivory Coast, Ghana, Indonesia, Nigeria, and Cameroon are the leading global cocoa producers. Thailand's average cocoa market growth is projected to be 5.15% yearly between 2023 and 2028, and cocoa volumes are projected to reach 50.4

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million kg by 2028. In addition, it is predicted that volumes will grow by 3.0% by 2024.[4] However, the Horticultural Research Institute reports that Thai cocoa production is still small compared to the world's leading cocoa producers. Approximately 43% of Thailand's cocoa is grown in the North, 21% in the Northeast, 15% in the East, and 6% in the South.[5]

The cocoa bean process consists of several steps: harvesting, fermentation, drying, roasting, winnowing, and nib grinding. Cocoa beans are harvested over several months, with the pods carefully removed to avoid damage. After harvesting, the cocoa beans are cleaned to remove foreign contaminants and then stored for fermentation.[6] Fermentation affects chocolate production enormously by improving its taste and flavor. The cocoa beans are fermented on banana and cocoyam leaves in baskets, wooden boxes, or fermentation barrels for 4−7 days. The beans are turned over often during this process, and the humidity is monitored. After fermentation, natural drying by sunlight takes 8−15 days.[7] After being dried, the cocoa beans are sorted and stored in a dry, well-ventilated location to prevent mold growth. The roasting of cocoa beans is a unique procedure that varies based on the taste of the cocoa market. After the roasting process, the cocoa beans are winnowed to remove the cocoa bean shells (CBSs) from the beans before grinding. Cocoa beans are ground and processed into cocoa powder, cocoa butter, chocolate, and other products.[8−10] CBSs are a byproduct of the winnowing process that contains 10−20 wt % of dried cocoa bean from the transformation of cocoa beans, depending on the fermenting procedure.[8−10] CBSs are frequently dumped as waste, which may lead to environmental deterioration and contamination.[8−10] However, several nutrients in CBSs after extraction have high amounts that can be utilized in the food, pharmaceutical, cosmetic, and agricultural industries.[9,10] Therefore, the compositions in CBSs are interesting in terms of dietary fiber, protein, and various bioactive compounds.[9,10] In addition, CBS oils may be suitable for use in food because their lipid profiles are quite similar to cocoa butter from cocoa bean.[9,10]

Several studies recommended using CBSs as an animal feed due to its high protein content. CBSs has also been considered an additive for animal feeding functions.[8,11,12] For instance, Soares and Oliveira[11] found that the protein content of CBSs was 16−18 wt %, making them a high-protein source for animal feed. Recently, CBSs were used in consumer products to manufacture dietary supplements, animal feed, cakes, tea, medicines, cosmetics, natural additives, and adsorbents.[8,12−15] Furthermore, there have been reports related to residual oil in CBSs. Rojo-Poveda et al.[8] found that CBSs still contain 8.5% of the oil residue from cocoa beans. The oil from CBSs has received attention from researchers because of the beneficial fatty acids that it contains.[8,11,12]

There are three main techniques for oil extraction from seeds or kernels: mechanical, chemical, and enzymatic. Solvent extraction is the most common technique for extracting oil because of its higher yield, particularly the accumulation of 5−15% residual oil in the raw material.[16−18] During the solvent extraction process, the solvent permeates the raw material to dissolve the oil. Once this is accomplished, evaporation removes the solvent from the oil and solvent solutions, known as miscella. The recovered solvent is then put back into use in the solvent extraction procedure.[18] Several factors have been identified as influential in determining the effectiveness of solvent extraction, including the raw material particle size, solvent type, ratio of raw material to solvent, temperature, extraction time, and mixing intensity.[16,17] The oil extraction method with a solvent depends on the solute−solvent affinity. Nonpolar solvents disrupt the hydrophobic interactions between nonpolar lipids, while polar solvents break down hydrogen bonds between polar lipids. Solvents used for extraction must consider low boiling points, low cost, and solvent reusability. Therefore, hexane and ethanol are commonly recommended solvents for oil extraction.[19] Wang et al.[20] used response surface methodology (RSM) to optimize oil extraction yields from rice bran with different solvents. Nonpolar solvents have been shown to be more effective in oil extraction than polar solvents. Conversely, Mabona et al.[21] reported that polar solvents extract more oil than nonpolar solvents. The oil content of the extraction was between 10.50 and 21.40% for polar solvents (acetone and ethanol), while the oil content was between 9.08 and 10.13% for nonpolar solvents (toluene and hexane). The results showed that the oil content increased with increasing extraction time, but not more than 60 min. Yang et al.[22] studied oil extraction from rubber seed meal using a solvent. The hexane solvent was found to remove the most oil (95.12%), and the protein content in the rubber seeds reached 90% after oil extraction. They also found that the raw material's moisture content significantly affected the oil solvent's extraction efficiency. Therefore, the moisture content in the raw materials must be minimized by air-drying before oil extraction. Efthymiopoulos et al.[23] obtained similar results by studying the influence of factors on oil extraction using the Soxhlet process. They discovered that having more than 2 wt % moisture content in spent coffee grounds affected the oil production following extraction. The oil concentration varied from 13.4−30.4 wt %, with an optimal spent coffee grounds-to-solvent ratio of 1:9 w/v and an extraction duration of 8 h. To investigate the oil extraction from cashew and pistachio shells, which are related to the raw materials of CBSs, Haque[24] reported that oil yields ranging from 0.484−0.581% were achieved using a Soxhlet extractor with hexane solvent at 70 °C for 10 h. Darkunde et al.[25] studied the extraction method and utilization of cashew nut shell liquid oil (CNSL) from cashew nut shells. The results showed that oil extraction with a Soxhlet extractor gave the CNSL yields of 32, 35, and 37% under conditions of 1:3, 1:4, and 1:5 g/mL ratio of shell-to-ethanol, respectively. However, considering the contaminants that may remain in animal feed, ethanol is a greener solvent than hexane. Ethanol is less toxic than many other organic solvents, making it safer to handle. Moreover, it is renewable and biodegradable.[26] In Thailand, ethanol is a renewable resource primarily derived from the fermentation of sugars found in crops like corn, sugar cane, and wheat.[27] However, hexane is widely used in oil extraction from seeds and vegetables due to its good solubility, low boiling point, and easy oil recovery.[28] Hexane is generally considered less green than ethanol because it is derived from fossil fuels, and its production and use can have a more significant environmental impact. Moreover, oil extraction using hexane as a solvent is expensive and complex and can pose health and safety hazards.[29]

To the best of the authors' knowledge, the value of oil extraction from CBSs using various solvent types (hexane, anhydrous ethanol, and hydrous ethanol) to produce animal feed has not been previously reported. Therefore, this work aims to fill existing research gaps in oil extraction from CBSs with various solvent types. The design of experiments using RSM can be used for a variety of applications, including oil extraction,[20] biodiesel production,[30] emulsification,[31] pharma-

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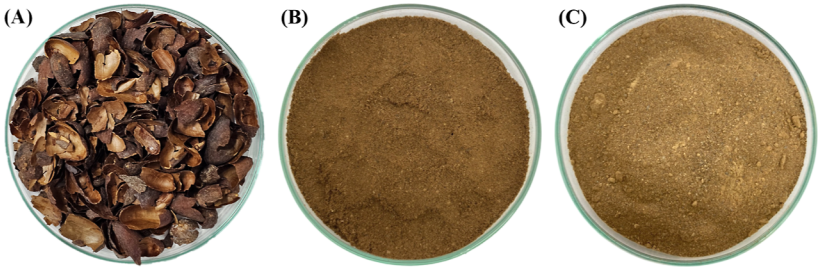

Figure 1. Raw material products from each processing step: (A) CBSs, (B) DCBSs, and (C) DFCBSs.

ceutical production,[32] and the synthesis of substances.[33,34] Thus, RSM is used to determine the most suitable oil yields with the solvent-to-CBS mass ratio, extraction time, and stirrer speed as parameters. The oil production from the recirculating extraction process is also described in terms of the number of extraction cycles, extraction efficiency, and the number of times the reused miscella (RM) is reused. Finally, a low-fat pellet feed is produced from extracted CBSs using a pellet machine.

2. MATERIALS AND METHODS

2.1. Materials. The CBSs are a waste material produced during the cocoa bean separation process, containing approximately 10−20 wt % dry cocoa bean.[8,10] In this study, CBSs were obtained from a cocoa plantation in southern Thailand. The CBSs of the cocoa bean were collected after the winnowing process. The CBSs (in Figure 1A) were ground using an electric grinder (model: 800Y; Yongkang Boou Hardware Products Co. Ltd.; China) to prepare the raw material for the oil extraction process. The small particle powder from CBSs was then dried in an oven at 105 °C for 24 h. This process aimed to decrease the moisture content in the raw material throughout all experiments and minimize the possible effect of moisture content on the oil extraction yield using solvents.[22] Figure 1A shows fresh CBSs, with a moisture content of approximately 1 wt %. The samples were carefully sealed in a ziplock bag to protect the dried cocoa bean shells (DCBSs). The oil was extracted using three commercial-grade solvents: hexane, anhydrous ethanol (99.9 vol %), and hydrous ethanol (95 vol %). Table 1 shows the physical and chemical compositions of DCBSs and defatted cocoa bean shells (DFCBSs).

2.2. Laboratory Scale Oil Extraction from CBSs with Three Solvents. The oil extraction was carried out using hexane, anhydrous ethanol, and hydrous ethanol as the solvents. In the first step, 10 g of CBSs was put into a 600 mL beaker and weighed with an accuracy of 0.0001 g on a digital analytical balance (AL204; Mettler-Toledo; Küsnacht, Switzerland). The required solvent-to-DCBS ratio (g/g) was poured into the beaker, which was then wrapped in aluminum foil to prevent solvent evaporation. The DCBSs and solvent were constantly mixed using a magnetic stirrer (model: RCT basic; IKA, Germany) with a set stirrer speed and extraction time at approximately 32 °C. The oil yield from the solvent extraction process was optimized by varying the solvent-to-DCBS ratio, stirrer speed, and extraction time. To separate the miscella from the DFCBSs after the oil extraction process, the DFCBSs were filtered using filter paper (Genuine Whatman no. 1; W. & R. Balston Ltd.; Kent, UK). A simple distillation separated the solvent from the cocoa bean shell oil (CBSO) in the miscella. After oil extraction, DFCBSs and CBSO were dried at 105 °C for 6 h to ensure that residual moisture and solvent were removed from DFCBSs and CBSO. Finally, the compositions of DCBSs, DFCBSs, and CBSO were analyzed for their composition after drying. The procedures for oil extraction from the DCBSs were performed three times for each of the three solvents.

Table 1. Physical Properties and Chemical Compositions of DCBSs and DFCBSs

		DFCBSs	
property	DCBSs	hexane	anhydrous ethanol
HHV (kJ/kg)	16,144.85		
LHV (kJ/kg)	14,926.46		
ash (wt %)	8.98		
protein (wt %)	13.39	20.87	19.97
crude fiber (wt %)	15.64	21.29	38.04
total sugar (wt %)	1.06	0.71	1.10
oil content[a] (wt %)	11.30	0.50	0.80
element			
carbon (wt %)	44.53	44.25	
hydrogen (wt %)	5.67	5.73	
nitrogen (wt %)	2.52	3.19	
sulfur (wt %)	0.12	0.18	
oxygen (wt %)	40.69	41.55	

[a]Note: The oil content was analyzed using the Soxhlet extraction method.

2.3. Analysis Methods. The DCBS compositions were analyzed for carbon (C), hydrogen (H), nitrogen (N), sulfur (S), oxygen (O), high heating value (HHV), and low heating value (LHV) using a CHNS/O analyzer (model: Flash 2000, ThermoScientific, Italy). The nutritional values of the DCBSs and DFCBSs were examined for protein content according to AOAC method 981.10 and for crude fiber content using a fiber analyzer (model: ANKOM200, ANKOM Technology, New York, NY, USA). The percentage of ash was tested according to the AOAC 942.05 official method, and the percentage of total sugar was tested based on the AOAC method (2019) 9215.35 (B). The surface characterizations of the CBSs before and after oil extraction were analyzed by scanning electron microscopy (SEM, model: SU3900, Hitachi, Japan). The residual oil in the DCBSs was analyzed using Soxhlet (method D5369-93(2008)e1; American Society for Testing and Materials, 2008). The fatty acid compositions of the CBSO were analyzed using a GC−FID (model: 7820A, Agilent Technologies, USA). Oil composition analysis for triglycerides (TG), diglycerides (DG), monoglycerides (MG), and free fatty acids (FFA) was performed using a thin layer chromatograph with flame ionization detection (TLC/FID, model: IATRO-SCAN MK-65; Mishubishi Kagahu Latron Inc., Tokyo, Japan). The CBSO yield was calculated from

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$$Y = (W_{CBSO}/W_{DCBS}) \times 100 \quad (1)$$

where Y is the CBSO yield (wt %), W_{CBSO} is the CBSO weight (g), and W_{DCBS} is the DCBS weight (g).

2.4. Experimental Design. The optimal yield of oil extracted from DCBSs was evaluated using the RSM by using a circumscribed central composite design (CCD) with 5 levels of three independent variables. In this design, each independent variable in the CCD is given a design rotatability encoded as $-\alpha$, -1, 0, $+1$, and $+\alpha$. The star point (α_x) is the extreme point for the low and high settings for all variables. The rotatable CCD's star points (α_x) are at some distance alpha from the center based on the design's number of variables (k). In this study, oil extraction from DCBSs has three independent variables. Thus, the number of variables (k) is equal to 3. Consequently, the experiments were designed with 5-levels for each independent variable, coded levels as -1.682, -1, 0, $+1$, and $+1.682$, as dictated by eq 2.[35]

$$\alpha_x = \sqrt[4]{2^k} \quad (2)$$

where α_x is the star point for rotatability, and k is the number of variables.

The solvent-to-DCBS ratio (2.5−29.5 g/g), stirrer speed (50−550 rpm), and extraction time (0.3−13.7 min) independent variables were studied in batch mode to obtain the maximum oil yield. The independent variables and coded variable levels are shown in Table 2. The coded levels were repeated for the three solvents (hexane, anhydrous ethanol, and hydrous ethanol). The oil yield from the extraction was modeled using a second-degree polynomial and multiple regression

Table 2. Independent Variables and Coded Variable Levels for Oil Extraction from DCBSs

		coded variable level				
independent variables	units	−1.682	−1	0	+1	+1.682
solvent-to-DCBS ratio (R)	g/g	2.5	8	16	24	29.5
extraction time (t)	min	0.3	3	7	11	13.7
stirrer speed (S)	rpm	50	150	300	450	550

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where Y is the response; x_i and x_j are the uncoded independent variables; β_0, β_i, β_{ii}, and β_{ij} denote a constant term and the linear, quadratic, and interaction coefficients, respectively; k is the number of variables; and ε is the error.

2.5. DCBS and DFCBS Pelletization Process. DFCBSs are a byproduct of the oil extraction process and can be formed into pellets for animal feed. Therefore, the pelletization technique was chosen to investigate the various parameters recommended for animal feed production. The advantages of using pellets are increased palatability, reduced eating waste, easy storage, and reduced transportation costs. In addition, the pellet diet helps reduce dust formation, which can lead to respiratory disease in animals.[36−38] The DCBS and DFCBS raw materials were first prepared for pelletization by adding water. As a result, they can be extruded without the need for a chemical binder. Moreover, adding an appropriate amount of water improves feed softness, resulting in easier palletization.[39] The DCBSs and DFCBSs were mixed with water in differing proportions of 15, 20, 25, and 30 wt %. After mixing, the feed mixture was extruded using the pellet machine (model: KL120B/C, Laizhou Chengda Machinery Co., Ltd., CHINA), compressing it into cylindrical pellets through a flat die with a 5 mm diameter. The pellets were then cut to length with a blade and dried at 105 °C to reduce their moisture content and prevent spoilage. Finally, the pellets were examined using a drop shatter test according to ASTM D3038-93 (2018) and force tested on a manual direct drive test stand with a basic force gauge (model: BFG 1000N, Mecmesin, UK).

3. RESULTS AND DISCUSSION

3.1. Experimental Results for CBSO Extraction. Figure 1 shows the products after oil extraction from DCBSs. After removing the CBSs from the roasted cocoa bean, these husks have a brown color and a crisp, fibrous piece, as shown in Figure 1A. After the grinding and drying processes, the physical characteristics of DCBSs were quite fine and brown, as shown in Figure 1B. After oil extraction, the color of the DFCBS powder changed to pale brown, as shown in Figure 1C.

Table 1 shows the physical properties and chemical compositions of the DCBSs and DFCBSs. The DCBS protein content was 13.39 wt %, while the DFCBS protein content was increased to 20.87 and 19.97 wt % after extraction using hexane and anhydrous ethanol, respectively. The crude fiber in the DCBSs was 15.64 wt %, while the crude fiber in the DFCBSs was 21.29 and 38.04 wt % after oil extraction using hexane and anhydrous ethanol, respectively. The protein and crude fiber content of DFCBSs increased after oil extraction because the residual oil in the DCBSs was removed. The total weight of DFCBSs decreased after the oil in DCBSs was removed by solvent extraction, resulting in higher protein and crude fiber contents in DFCBSs compared to DCBSs when considering the same weight of DCBSs. Therefore, DFCBSs have improved protein content and can be used as animal feed. The total sugar in the DCBSs was 1.06 wt %, while the total sugar in the DFCBSs was 0.71 and 1.10 wt % after extraction using hexane and anhydrous ethanol, respectively. After oil extraction with hexane and anhydrous ethanol, CBSO was analyzed for fatty acid compositions and the physical properties listed in Table 3. The HHV and LHV of the CBSO were 40,975.04 and 38,192.01 kJ/kg, respectively, after extraction with hexane. The experimental design matrix and laboratory-scale results using the three solvents (hexane, anhydrous ethanol, and hydrous ethanol) in this study are described in the next section.

3.2. RSM Predictive Model and Statistical Analysis. The correlation prediction equations were used to find the optimal CBSO yield after the extraction of oil from DCBSs by employing a regression analysis method. According to Table 4, these analysis results were used to fit the response models with experimental results using the multiple regression add-in tool with a 95% confidence level for the Excel software package (version 2013; Microsoft Corp.; Redmond, WA, USA). These results relate the dependent variable of CBSO yield (Y) to the three independent variables—solvent-to-DCBS ratio (R), extraction time (t), and stirrer speed (S)—using quadratic models for three solvents. The prediction equation for the relationship between CBSO yield and the three independent variables of hexane, anhydrous ethanol, and hydrous ethanol is shown in eqs 4, 5, and 6, respectively.

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Table 3. Fatty Acid Composition and Properties of CBSO with Hexane and Anhydrous Ethanol

fatty acids	symbol	content (wt %)	
		hexane	anhydrous ethanol
lauric acid	C12:0	0.02	
myristic acid	C14:0	0.04	0.12
pentadecanoic acid	C15:0	0.01	0.04
palmitic acid	C16:0	23.64	26.59
palmitoleic acid	C16:1	0.18	
heptadecanoic acid	C17:0	0.18	0.27
stearic acid	C18:0	35.15	30.26
oleic acid	C18:1	34.09	31.80
linoleic acid	C18:2	3.53	4.15
alpha linolenic acid	C18:3	0.16	
arachidic acid	C20:0	0.98	1.19
paullinic acid	C20:1	0.05	0.08
behenic acid	C22:0	0.19	0.33
lignoceric acid	C24:0	0.10	0.35
property	CBSO		
density at 32 °C (kg/L)	0.928		
viscosity at 40 °C (cSt)	43.56		
HHV (kJ/kg)	40,975.04		
LHV (kJ/kg)	38,192.01		

Table 4. Experimental Design Matrix and Results for Oil Extraction from DCBSs[a]

run	R (g/g)	t (min)	S (rpm)	Response, Y (wt %)		
				hexane (Y_1)	anhydrous ethanol (Y_2)	hydrous ethanol (Y_3)
1	2.5	7.0	300	7.00	5.50	3.55
2	8.0	3.0	150	7.50	5.70	4.55
3	8.0	3.0	450	8.30	6.30	5.15
4	8.0	11.0	150	8.50	7.25	5.30
5	8.0	11.0	450	8.65	7.85	5.75
6	16.0	0.3	300	8.95	7.60	6.55
7	16.0	7.0	50	9.40	8.10	6.85
8	16.0	7.0	300	9.60	8.95	7.20
9	16.0	7.0	300	9.65	8.95	7.25
10	16.0	7.0	300	9.65	8.90	7.20
11	16.0	7.0	300	9.70	8.90	7.25
12	16.0	7.0	550	9.80	8.95	7.60
13	16.0	13.7	300	9.70	9.30	7.60
14	24.0	3.0	150	9.80	9.40	7.90
15	24.0	3.0	450	10.10	9.60	8.15
16	24.0	11.0	150	10.10	9.80	8.30
17	24.0	11.0	450	10.20	10.00	8.45
18	29.5	7.0	300	10.30	10.15	8.50

[a]Note: R is the solvent-to-DCBS ratio; t is the extraction time; S is the stirrer speed; Y_1 is the CBSO yield from hexane; Y_2 is the CBSO yield from anhydrous ethanol; Y_3 is the CBSO yield from hydrous ethanol.

$$Y_1 = \beta_0 + \beta_1 R + \beta_2 t + \beta_3 S + \beta_4 Rt + \beta_5 RS + \beta_6 R^2 + \beta_7 t^2 + \beta_8 tS \quad (4)$$

$$Y_2 = \beta_0 + \beta_1 R + \beta_2 t + \beta_3 S + \beta_4 Rt + \beta_5 RS + \beta_6 R^2 + \beta_7 t^2 + \beta_8 S^2 \quad (5)$$

$$Y_3 = \beta_0 + \beta_1 R + \beta_2 t + \beta_3 S + \beta_4 Rt + \beta_5 RS + \beta_6 R^2 + \beta_7 t^2 \quad (6)$$

where Y_1 is the CBSO yield from hexane (wt %), Y_2 is the CBSO yield from anhydrous ethanol (wt %), Y_3 is the CBSO yield from hydrous ethanol (wt %), R is the solvent-to-DCBS ratio (g/g), t is the extraction time (min), S is the stirrer speed (rpm), and β_i are the coefficient values.

Table 5 shows each term's coefficient and probability of error (p-value) with statistical significance for the CBSO yield predictive model. The p-value was used to estimate the statistical significance of each coefficient (β) in the correlation prediction equation. A p-value of less than 0.05 for each coefficient was considered significant in the prediction model, where the smallest p-value indicates the most significant influence on the oil extraction process. In contrast, a p-value greater than 0.05 means that the term of the model is not significant enough in the equation and will be removed from the equation.[40] The terms $\beta_1 R$ and $\beta_6 R_2$ in eqs 4−6 derived the lowest p-value for the correlation prediction analysis. Therefore, increasing the amount of solvent significantly impacts the CBSO yield achieved after oil extraction. The influence of extraction time is shown with the $\beta_2 t$ condition in the third ranking. A high mass ratio of solvent-to-DCBS increases the oil yield due to an improved mass transfer rate when the solvent solution increases. Although the stirrer speed only slightly affects the extraction efficiency, it plays an essential role in increasing mass transfer during solvent extraction. The coefficients of multiple determination (R^2) were 0.996, 0.999, and 0.999, while the adjusted coefficients of multiple determination ($R_{adjusted}^2$) were 0.992, 0.998, and 0.998 for the prediction models of hexane, anhydrous ethanol, and hydrous ethanol, respectively. The analysis of variance (ANOVA) results were used to consider the quality of the prediction model, as shown in Table 6. To confirm the adequacy of the prediction model with three solvents in explaining the variation in the data, the lack of fit test is used to test the adequacy of the fitted model. In this study, the lack of fit test results are 5.80, 7.11, and 7.27 lower than the F-distribution values of 8.94 ($F_{0.5,6,3}$), 8.94 ($F_{0.5,6,3}$), and 8.89 ($F_{0.5,7,3}$) for the prediction models for hexane, anhydrous ethanol, and hydrous ethanol, respectively. It indicates that there is no significant lack of fit since the inherent error is small when compared to the experimental data, implying that the relationship in all predictive models is possible.[41,42] The F-test results in Table 6 show that the F_0 values of 265.89, 288.61, and 849.56 were higher than the F_{crit} values of 3.23 ($F_{0.5,8,9}$), 3.23 ($F_{0.5,8,9}$), and 3.14 ($F_{0.5,7,10}$) for the prediction models for the hexane, anhydrous ethanol, and hydrous ethanol solvents, respectively. Therefore, the prediction models for oil extraction with all types of solvents were found to be statistically significant.

3.3. Analysis of the Response Surface and Optimization. Figure 2 shows the response surface plots of oil extraction based on the correlation of the regression model's response variable (CBSO yield) and independent variables (solvent-to-DCBS ratio, stirrer speed, and extraction time) via a solvent extraction in batch process. According to the statistical analysis of the p-value, as described in the previous section, this study found that the solvent-to-DCBS ratio had the most impact on CBSO yield, followed by the extraction time and stirrer speed. Figure 2A−C shows the relationships between the solvent-to-DCBS ratio and the extraction time of three solvents. A higher solvent-to-DCBS mass ratio improves

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Table 5. Values of Coefficients for the Predictive Model

coefficient	hexaneeq 4		anhydrous ethanoleq 5		hydrous ethanoleq 6	
	value	p-value	value	p-value	value	p-value
β_0	4.249105	0.000000015860	0.740658	0.002294504550	0.8201398	0.000472825767
β_1	0.335715	0.000000004098	0.458148	0.000000000030	0.4397585	0.000000000007
β_2	0.269126	0.000008018313	0.413496	0.000000007610	0.1659728	0.000011091498
β_3	0.003148	0.000269426630	0.006585	0.000001501839	0.0024112	0.000032020093
β_4	−0.003711	0.003045217964	−0.008984	0.000000542585	−0.0025391	0.006451442664
β_5	−0.000057	0.045194413630	−0.000083	0.001853988175	−0.0000677	0.006451442664
β_6	−0.005486	0.000000092228	−0.005995	0.000000005617	−0.0067328	0.000000000437
β_7	−0.007244	0.000735702332	−0.010415	0.000008090103	−0.0039566	0.006637903189
β_8	−0.000177	0.005824924327	−0.000006	0.000032641444		
R^2	0.996		0.999		0.999	
$R^2_{\text{adjiusted}}$	0.992		0.998		0.998	

Table 6. ANOVA of the Predictive Mode

source	sum of squares	mean square	F_0	F_{signif}	degrees of freedom
Hexane, eq 4					
regression	14.8931	1.8616	265.89	3.23	8
residual	0.0630	0.0070			9
lack of fit error	0.0580	0.0097	5.80	8.94	6
pure error	0.0050	0.0017			3
total	14.9561				17
Anhydrous ethanol, eq 5					
regression	34.6669	4.3334	1024.33	3.23	8
residual	0.0381	0.0042			9
lack of fit error	0.0356	0.0059	7.11	8.94	6
pure error	0.0025	0.0008			3
total	34.7050				17
Hydrous ethanol, eq 6					
regression	34.5929	4.9418	1100.11	3.14	7
residual	0.0449	0.0045			10
lack of fit error	0.0424	0.0061	7.27	8.89	7
pure error	0.0025	0.0008			3
total	34.6378				17

mass transfer and increases the contact area between solvent and solid. A similar result was described by Ntalikwa .[43] They mentioned that a high solvent-to-solid ratio concentration in the extraction improves mass transfer due to increased solvent diffusion on the particle surface. In addition, longer extraction times significantly increased the chance of solute mass transfer from the solid to the solvent phase.[44] Figure 2D−F shows the relationship between the solvent-to-DCBS ratio and the stirrer speed of the three solvents. The relationship between these two variables is also highly significant for CBSO yield. A suitable blending speed enables the solvent's fairly uniform distribution on the solids, which increases the yield and efficiency of the extraction. Hussain et al.[45] reported that the stirrer improves mass transfer efficiency because the stirring intensity improves the dispersion between solvent and solids and reduces extraction time. The optimal conditions for the three solvents were determined by solving the predictive models, as shown in Table 7. The actual experimental yield was examined to verify the oil yield predictive model. The actual experimental yield was 10.80 wt % for hexane under the optimal conditions using a 26.0:1 g/g hexane-to-DCBS ratio, 550 rpm stirrer speed, and 5.2 min extraction time. The actual experimental yield for anhydrous ethanol was 10.50 wt % under the optimal conditions using a 29.5:1 g/g anhydrous ethanol-to-DCBS ratio, 330 rpm stirrer speed, and 7.1 min extraction time. The actual experimental yield for the hydrous ethanol solvent was 8.90 wt % under the optimal conditions using a 27.4:1 g/g hydrous ethanol-to-DCBS ratio, 550 rpm stirrer speed, and 12.1 min extraction time.

These results suggest the use of either the hexane or anhydrous ethanol solvent to achieve the highest yield from the oil extraction process. However, comparing these two solvents, anhydrous ethanol is a better choice because it is less environmentally harmful.[46] Ethanol is a renewable, nontoxic, and biodegradable solvent, making it a much more sustainable and eco-friendly option for oil extraction.[47] Moreover, Thailand can manufacture ethanol from sugar cane molasses, thus minimizing dependency on chemical imports.[48] The material balance of oil extraction using a magnetic stirrer was calculated under optimal conditions of anhydrous ethanol on a laboratory scale. The final product after extraction was 1.50 g of CBSO (10.50 wt %) and 8.50 g of DFCBS (85 wt %), compared to the initial 10 g of DCBS. The recoverable anhydrous ethanol solvent was 284.68 g (96.50 wt % compared to the initial 295 g of anhydrous ethanol), and the energy required to evaporate the solvent is 0.13 kW/h by the hot plate. The Reynolds number (Re) is a dimensionless number which can be used to scale up on a commercial scale. It is calculated to determine the flow pattern of the solvent in the beaker.

$$Re = \rho D^2 N/\mu \tag{7}$$

where ρ is the fluid density (kg/m^3), D is impeller diameter (m), N is stirrer (rev/s), and μ is fluid viscosity (kg/m·s). It is considered a laminar flow for a mixing tank when the Reynolds number is less than 10 and a turbulent flow when the Reynolds number is greater than 10^4.[49] For the optimal condition of anhydrous ethanol, the Re was calculated to be 7617 using eq 7,[49] where ρ = 779 kg/m^3 at 32 °C, D = 0.04 m, N = 5.5 rev/s, and μ = 0.0009 kg/m.s.

3.4. SEM Images. Figure 3 shows images from SEM examinations of DCBSs and DFCBSs. Figure 3A (at 500× magnification) and 3B (at 4000× magnification) show that the DCBS appears to have a thin oil layer before oil extraction. The technique of winnowing cocoa beans leads to the separation of the thin, crisp shell covering the cocoa bean from the bean. The grinders were used to minimize the size of the remaining oily CBS particles on the shell's surface. Once DCBS from the grinding process had dried to eliminate the

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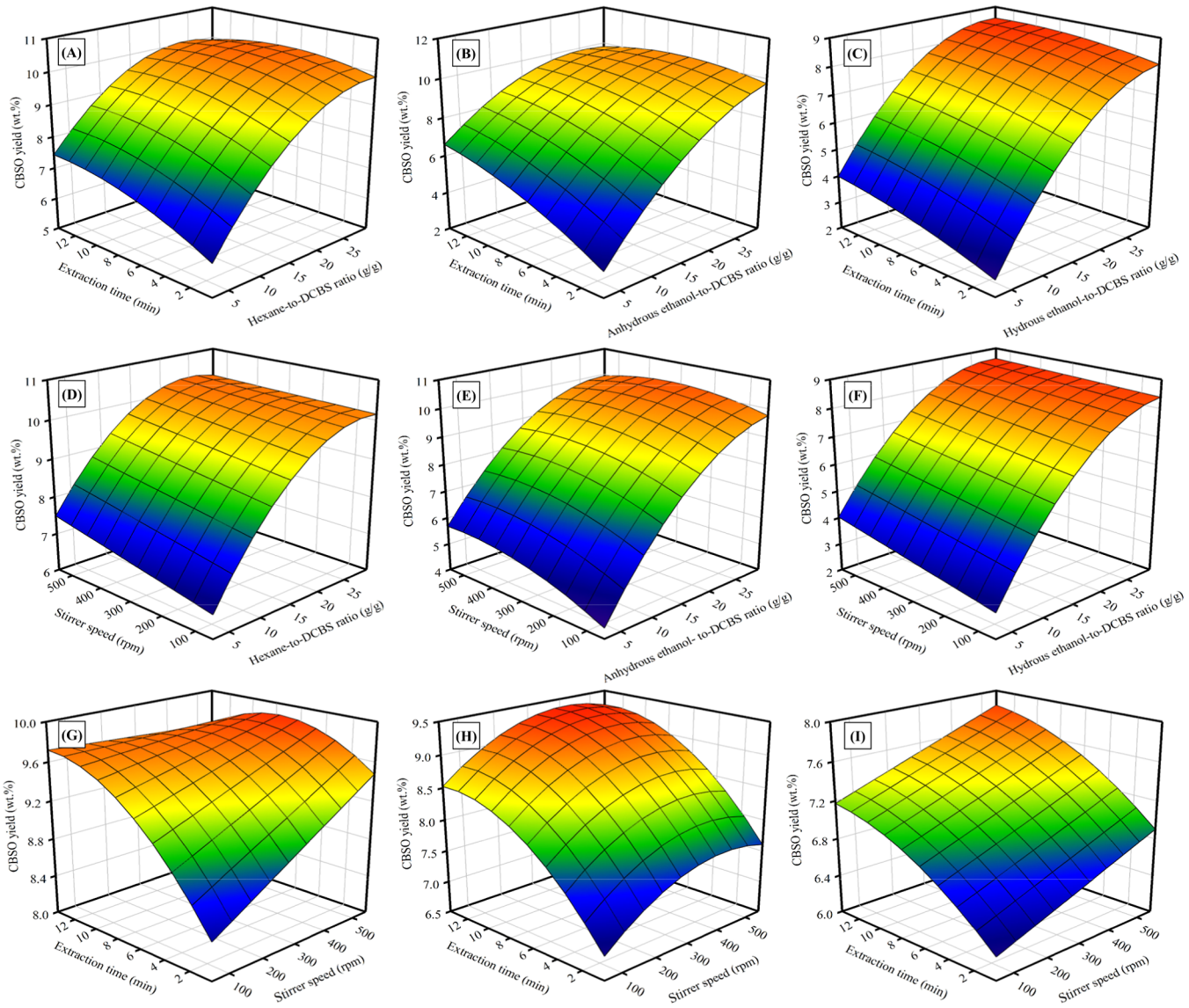

Figure 2. Three-dimensional response surface plots of the effects of the three parameters on the oil-extracted yield: the solvent-to-DCBS ratio and extraction time of (A) hexane, (B) anhydrous ethanol, and (C) hydrous ethanol; the solvent-to-DCBS ratio and stirrer speed of (D) hexane, (E) anhydrous ethanol, and (F) hydrous ethanol; and the stirrer speed and extraction time of (G) hexane, (H) anhydrous ethanol, and (I) hydrous ethanol.

Table 7. Optimal Conditions and CBSO Composition Were Determined Using Three Different Solvents

parameter	hexane	anhydrous ethanol	hydrous ethanol
Condition of extraction			
ratio of solvent to DCBS (g/g)	26.0	29.5	27.4
stirrer speed (rpm)	550	330	550
extraction time (min)	5.2	7.1	12.1
predicted oil yield (wt %)	10.38	10.20	8.72
verified oil yield (wt %)	10.80	10.50	8.90
Composition of CBSO			
triglyceride (wt %)	99.53	98.37	99.05
free fatty acid (wt %)	0.01	0.53	0.18
diglyceride (wt %)	0.43	1.02	0.70
monoglyceride (wt %)	0.05	0.08	0.07

remaining moisture, it was prepared for processing by solvent oil extraction. The DFCBS was obtained after the oil extraction, and its appearance in Figure 3C (at 500× magnification) and 3D (at 4000× magnification) indicate that its surface is smoother and dryer than that of the CBS.

3.5. Oil Extraction from CBSs with RM. The extraction efficiency from DCBSs with RM using hexane and anhydrous ethanol was studied by using their optimum conditions, as shown in Table 7. Heat energy must be added to evaporate the solvent in the miscella to obtain the oil. Energy was saved in each evaporation cycle by extracting the miscella with fresh raw material before evaporation to determine the extraction efficiency using RM. The results show that increased CBSO content accumulated in each cycle of RM for both solvents, as shown in Figure 4. According to the results presented in Figure 4, the CBSO content using hexane increases from 0.76 to 6.20 wt % after 1 to 9 cycles, while the oil yield increases from 0.27 to 0.96 wt % after 1 to 5 cycles for anhydrous ethanol. Subsequently, each cycle using RM was compared to the first

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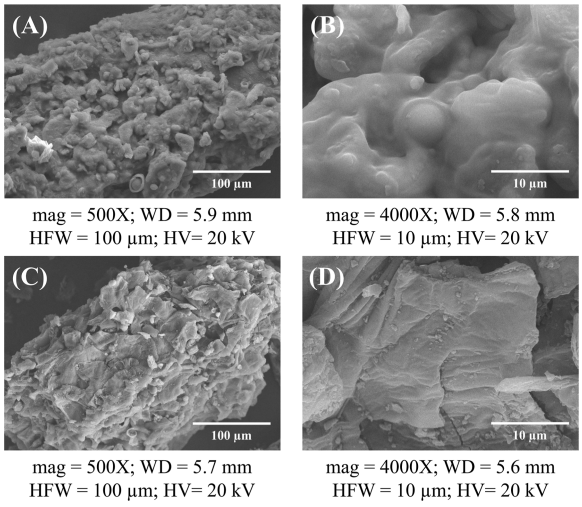

Figure 3. Scanning electron microscope images: DCBS at magnifications of (A) 500× and (B) 4000× and DFCBS at magnifications of (C) 500× and (D) 4000 ×.

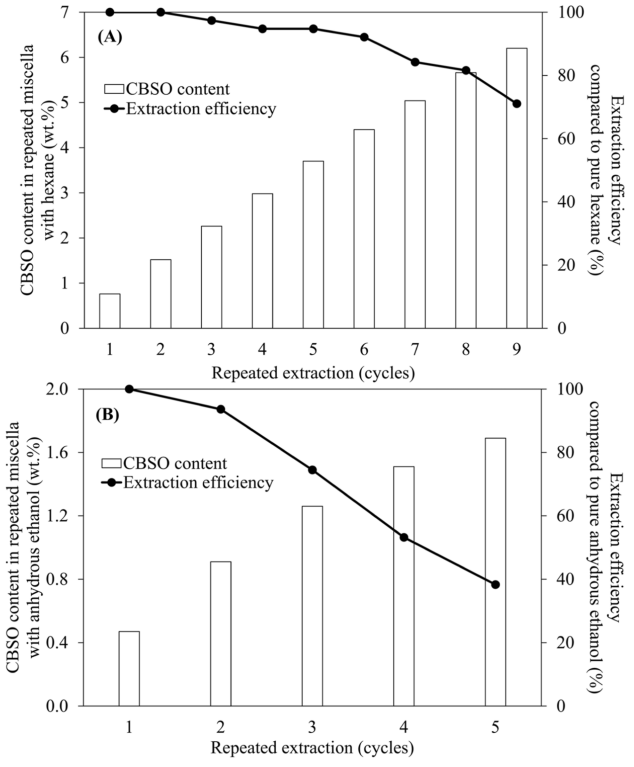

Figure 4. CBSO content in RM and extraction efficiency in each cycle with (A) hexane and (B) anhydrous ethanol solvents.

oil extraction cycle in terms of efficiency. The extraction efficiency of hexane with RM declines to 97.37% in the third cycle and 84.21% in the seventh cycle. For the anhydrous ethanol solvent, the oil extraction efficiency with RM drops to 93.62% in the second cycle and quickly falls to 74.47% in the third cycle. Figure 5 shows the color intensity of CBSO, which increases with each extraction cycle, indicating that the oil concentration in the miscella increases with each oil extraction cycle.

3.6. DCBS and DFCBS Pelletization. For pelletization without chemical binders, the DCBS and DFCBS were mixed with water in differing proportions (15, 20, 25, and 30 wt %) to

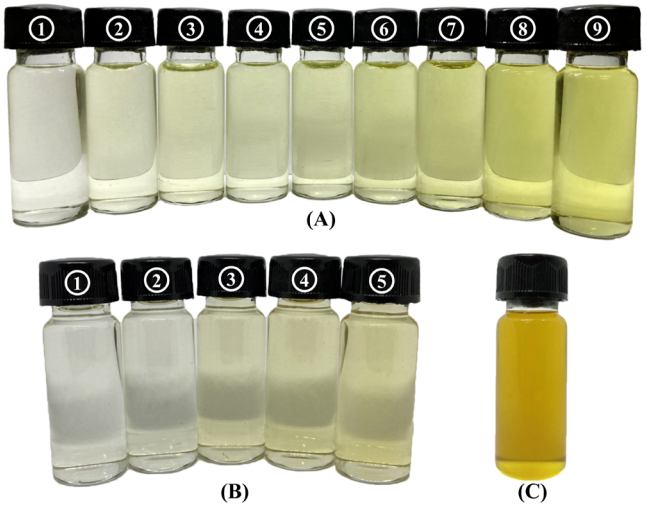

Figure 5. Color intensity of miscella after oil extraction with (A) hexane miscella, (B) anhydrous ethanol miscella, and (C) CBSO after solvent evaporation.

determine the optimum mixing ratio for pelletization. The various mixing ratios are evaluated against these parameters to identify the proportion of water blended into DCBS and DFCBS and study the pellet durability and stability. The results in Figure 6 demonstrate that pelletization can occur at a

Figure 6. Pellets from (A) DCBS and (B) DFCBS.

die temperature of 50 °C and a water ratio of 25% without pellet cracking. Thus, 25% water is recommended to produce the pellets, which have a diameter of 5 mm and a length of 20−30 mm. The densities of the DCBS and DFCBS are in the ranges of 1.44−1.54 and 1.47−1.68 g/cm^3, respectively. The resistance shattering of the pellets is analyzed using drop-shatter testing. The test results show that the DFCBS was better than the DCBS, with drop-shatter test values of 0.89 and 0.93 for DCBS and DFCBS, respectively. The compression test result values are 112.28 N for DCBS and 143.44 N for DFCBS on the vertical axis and 405.12 N for DCBS and 623.04 N for DFCBS on the horizontal axis.

4. CONCLUSIONS

The CCD of the RSM was used to evaluate the optimal conditions for oil extraction from CBSs using three different solvents. In a laboratory-scale oil extraction experiment using hexane, a 10.80 wt % yield of oil was achieved under optimum conditions of 26.0:1 g/g hexane-to-DCBS ratio, 550 rpm stirrer speed, and 5.2 min extraction time. Anhydrous ethanol yielded 10.50 wt % of oil under the optimum conditions of the 29.5:1

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g/g anhydrous ethanol-to-DCBS ratio, 330 rpm stirrer speed, and 7.1 min extraction time. For hydrous ethanol, an 8.90 wt % oil yield was obtained under the optimum conditions of 27.4:1 g/g hydrous ethanol-to-DCBS ratio, 550 rpm stirrer speed, and 12.1 min extraction time. Therefore, the high yields of CBSO from optimal conditions of hexane and anhydrous ethanol solvents were selected to test the extraction efficiency using RM for saving energy and chemicals in the next step of solvent evaporation. It was found that six cycles of hexane and two cycles of anhydrous ethanol were achieved to extract oil from DCBS with an efficiency of over 80%. These results indicate that hexane has a higher potential for oil extraction than ethanol. Regarding environmental friendliness, oil obtained from CBSs and ethanol could be used as food additives, while the higher protein content of DFCBSs as a byproduct of the oil extraction process could be used as an animal feed. This study recommends ethanol over hexane because it is safer for the environment. For savings in the costs of solvent and energy, the recovered solvent after the distillation process to separate the oil and solvent in RM can be reused in a new cycle of oil extraction. Furthermore, this study recommended a simple technique that could extract oil from DCBSs at room temperature and pressure. As a result, the extractor design was not concerned with high temperature and high pressure during the process, and it was easily scaled up for the extractor to promote cocoa producers in a remote community. Finally, feed pellets were successfully produced from both DCBSs and DFCBSs by using the pelletization technique. This pelletization was accomplished by adding 25 wt % water to DFCBS before being formed by a pellet machine.

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Notes

The authors declare no competing financial interest.

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