ARTICLE

Valorization of cocoa bean shell waste through sustainable extraction of phenolics: Process optimization and characterization

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ABSTRACT

This research aimed to optimize and characterize the phenolics extraction from cocoa bean shells (CBS), a cocoa processing byproduct. Seven factors were studied, including (1) temperature: 30–70 °C, (2) extraction time: 5–90 minutes, (3) solvent pH: 2–12, (4) sample to solvent ratio (SSR): 0.020–0.060 g/mL, (5) concentration of solvent water/ethanol 0–75%, (6) sonication time: 10–60 minutes, and (7) agitation: 0–200 rpm. The Plackett-Burman (PBD) and Box-Behnken design (BBD) of experiments were utilized to establish the significant extraction parameters and their optimum levels, with total phenolic content (TPC) as the response parameter. The model generated was validated and the optimized CBS extract was characterized for its bioactive and antioxidant activities. Results showed that three parameters (extraction time, incubation temperature, and SSR) significantly impacted the extraction of phenolics from CBS. The optimum condition for extraction of phenolics from CBS was achieved with the use of 0.060 g/mL SSR and extracts incubated at 30 °C for 5 minutes. Additionally, the optimized CBS extract was determined to possess considerable quantities of bioactive compounds (total flavonoid content = 13.83 ± 0.84 mg CE/g; TPC = 10.86 ± 0.22 mg GAE/g), and exhibit antioxidant activity ($ABTS^{\bullet+}$ = 23.76 ± 0.59 mg TE/g). This research leverages the capability of CBS as a valuable pool of bioactive compounds with significant antioxidant capacities. The developed method for phenolic extraction from CBS is both environmentally friendly and economically viable, and presents

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KEYWORDS

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a promising prospect for industrial application due to its efficiency and sustainable processing conditions.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a primary ingredient utilized in the production of chocolates and other various cocoa products. It holds economic significance due to its contribution in export revenues, income, and employment (Gayi & Tsowou, 2016). The International Cocoa Organization (ICCO) (2023) reports an annual increase of approximately 2.6% in the cocoa year 2022/2023 from 2021/2022. The increase in demand and production of cocoa corresponds with the surge in waste products being generated during cocoa processing, with significant economic and environmental repercussions resulting from the accumulation of byproducts such as husks, mucilage, and bean shells. On a global scale, approximately 700,000 tons of CBS residues are produced annually (International Cocoa Organization (ICCO) (2017)), and it constitutes crudely 70–80% of the fruit weight (Martínez et al, 2012; Panak Balentić et al, 2018; Lessa et al, 2018; Nair, 2010).

CBS comprises roughly 10–20% of the entire mass of the bean and is typically isolated from the bean after roasting at higher temperatures (Sánchez et al., 2023). Several researches have focused on the potential application of CBS, such as the microencapsulation and the determination of predominant bioactive compounds present in CBS (Djali et al., 2023; Grassia et al., 2021) and exploring CBS as an alternative to wheat flour and its potential as a dietary fiber powder (Nogueira Soares Souza et al., 2022; Handojo et al., 2019). Moreover, some research regarding the characterization of the chemical profile of CBS and its role in human health has been investigated (Younes et al., 2023; Martínez et al., 2012; Domínguez-Pérez et al., 2020; Sánchez et al., 2023; Lessa et al., 2018).

Polyphenols in CBS have been well studied due to several health benefits that they can offer, such as their antioxidant activity, low-density lipoprotein (LDL) oxidation-inhibiting agents, inhibition of proinflammatory mediators, reduction of lipid peroxide, and elevation of endothelium functionality (Wiyono et al., 2020; Okiyama et al., 2017). Over the past years, several meta-analyses associated polyphenols from CBS and cocoa products with reduced risk of coronary artery disease (Kwok et al., 2015), stroke, cardiovascular diseases (CVD) (Buitrago-lopez et al., 2011), heart failure, and heart disease (Yuan et al., 2017; Larsson et al., 2016). Therefore, extracting the bioactive compounds and utilizing them as food ingredients will be valuable to the food industry.

Today, numerous techniques for extracting phenolic compounds using organic solvents have been utilized to acquire maximum extraction capacity, such as microwave extraction (Buntic et al., 2013), heat-assisted extraction (Rebollo-Hernanz et al., 2021), and ultrasonic extraction (Severini et al., 2017). However, certain drawbacks of using organic solvents as the extracting medium are evident, such as increased cost and energy consumption, flammability of organic solvents, and other environmental burdens (Cui et al., 2018; Azmir et al., 2013; Solomakou et al., 2022; Zannou et al., 2022; Panzella et al., 2020). With regards to sustainable development, emerging green extraction methods replaced organic solvents with deep eutectic solvent (DES) and other extracting mediums, such as water, coupled with Response Surface Methodology (RSM) to minimize the use of time, energy, and resources without the risk of toxicity and flammability of organic solvents (Ruesgas-Ramón et al., 2019; Rebollo-Hernanz et al., 2021; Pavlovic et al., 2020). During the determination of variables that could significantly impact the extraction of phenolics from CBS, greener extraction methods should be considered to develop a green circular economy concept which holds a greater impact on sustainable development.

Various factors are being studied that greatly influence the phenolic extraction from CBS. Previous studies demonstrated the influence of pH, extraction time, incubation temperature, and sample to solvent ratio (SSR) on both the polyphenolic compounds and antioxidant capacities of the resulting optimized extracts (Mellinas et al., 2020; Rebollo-Hernanz et al., 2021). In this study, a total of seven factors, with two levels, were considered (extraction time, solvent concentration, incubation temperature, pH, sonication, agitation, and SSR). The optimization of these factors would help determine significant parameters and their optimal levels, ultimately maximizing the amount of solubilized polyphenolic compounds in the extracts from CBS.

The utilization of statistical designs is important to determine which factors and levels will give the highest amount of phenolics from different byproducts (Alavarsa-Cascales et al., 2022; Andres et al., 2020; Mellinas et al., 2020; Balicki et al., 2020; Belwal et al., Liu et al., 2018; 2016; Das & Dewanjee, 2018). Currently, there is limited data concerning the optimization studies focused on extracting phenolics from CBS that utilizes green extraction methods. The results of this research can be used industrially to establish an efficient and economical extraction method of phenolics from CBS. It will not only promote the utilization of CBS but also other byproducts of the cocoa processing industry without environmental burdens. Additionally, this study provides further support for the advancement of functional food products and ingredients through the utilization of CBS. Thus, the main objective of this research is to valorize CBS through sustainable extraction of phenolics and characterize the resulting extract.

MATERIALS AND METHODS

This research aimed to determine the significant factors affecting the valorization of cocoa bean shell through sustainable extraction of polyphenolic compounds using the Plackett-Burman design (PBD) of experiment. Then, the optimum levels of the significant factors were determined via the Box-Behnken design (BBD) of experiment. Afterward, the optimized extracting conditions were verified by replicating the conditions generated by the model five (5) times. Lastly, the optimized extract was characterized for its bioactive profile and antioxidant activity.

Raw material

The cocoa bean shell (CBS) waste utilized for this research was supplied by Malagos Agri-Ventures Corporation (Davao City, Philippines). These were byproducts of their chocolate processing. The samples were stored in a vacuum-sealed plastic pouches and kept in a freezer (-20 °C) until use. The proximate composition of CBS used was predetermined using AOAC methods (5.75% moisture, 9.59% ash, 23.34% crude protein, 11.60% crude fiber, and 9.96% crude fat).

Sample and chemical preparation

CBS was finely ground and sifted through a #20 mesh sieve, and placed in a centrifuge tube wrapped with foil. All solvents/reagents used in this study were of analytical grade. Ethanol (RCI Labscan Ltd., Bangkok) was used for extraction of phenolic compounds, while NaOH (Loba Chemie Ltd., India) and HCl (RCI Labscan Ltd., Bangkok) were used to adjust the pH of the solvents in the PBD.

Phenolic compounds extraction from CBS
Extraction combinations based on the generated PBD and BBD of phenolics from CBS were accomplished using various solvent, incubation temperatures (30–70 °C), incubation times (5–90 minutes, Biobase Meihua Trading Co., Ltd., China), sonication times (10–60 minutes, Cole-Palmer, U.S.A.), and agitations (0–200 rpm, Benchmark Scientific, Inc., U.S.A.). The mixture was incubated and then sonicated. Afterwards, it underwent centrifugation for 10 minutes and the resultant extract was strained through a Whatman® no. 1 filter paper.

Optimization of phenolic extraction from CBS
The optimum parameters for the green phenolics extraction were identified through RSM. Initial screening using the PBD of the experiment revealed significant parameters impacting the yield of phenolic extracts. Subsequently, the BBD was applied to establish the optimal levels of the significant factors identified from PBD. The experimental designs were generated by utilizing Design Expert software version 10 (Stat-Ease 2016).

Screening for significant factors of phenolic extraction by Plackett-Burman design (PBD)
Seven factors were considered (temperature, extraction time, pH, sample: solvent ratio, solvent concentration %, sonication, and agitation). A total of 15 runs, with 3 center points, were tested. The solvents' pH was adjusted with NaOH (0.1N and 0.4N) and HCl (0.1N and 0.4N) solutions. The selection of high and low values for each factor was done based on previously published literature on phenolic extraction from various sources (Alavarsa-Cascales et al., 2022; Grassia et al., 2021; Dewi et al., 2022; Muñiz-Márquez et al., 2013; Rebollo-Hernanz et al., 2021; Hernández-Hernández et al., 2018, 2019). The total phenolic content (TPC), reported as milligram gallic acid equivalents per liter of extract (mg GAE/L), was the analyzed response.

Optimizing significant factors through Box-Behnken design (BBD)
The factors that significantly affected the response were identified according to the results of screening. The identified significant factors were used to generate a set of treatments using the BBD of experiment. A total of 15 runs, with three center points, were analyzed. The response evaluated was the TPC of the extract, presented as milligrams Gallic acid equivalents per liter extract (mg GAE/L).

Model validation
The model was validated five times using the established optimum parameters for the extraction of phenolics from CBS, where the TPC of CBS extracts was quantified. A comparison between the experimental and predicted mean values was done to validate the reliability of the model generated.

Polyphenolic profile and antioxidant capacities of CBS extract
The analysis for quantifying the polyphenolic compounds and antioxidant capacities of CBS was completed through a UV-Vis spectrophotometer (Shimadzu Corp., Japan). During optimization, the response variable evaluated for the CBS extract was TPC. Afterward, the CBS extracts obtained using the optimum conditions were characterized for bioactive compounds (total monomeric anthocyanin, total flavonoids, total phenolics) and antioxidant capacities (DPPH•, FRAP, $ABTS^{\bullet+}$).

Total phenolics content
The quantification of TPC was conducted using the Folin-Ciocalteu method, based on the procedure executed by Babaran et al (2023) with slight adjustments. TPC was quantified using gallic acid in 10% methanol as the standard curve. The standard curve plot ranges from 0–150mg/L with $R^2 = 0.9978$. The TPC values of the samples were reported as milligrams gallic acid equivalents per liter of extract (mg GAE/L) during optimization and per gram dry weight (mg GAE/g DW) during characterization. The unit of measurement was converted into a per gram dry basis during characterization for easier comparison with existing literature.

Total monomeric anthocyanin content
The total monomeric anthocyanin content (TMAC) was quantified by the pH differential method, following the protocol executed by Lee et al (2005), with minor adjustments. TMAC was quantified using buffers of sodium acetate and potassium chloride. The absorbance of extracts was read at 520nm and 700nm. TMAC was reported as milligram cyanidin-3-glucoside equivalents per gram dry weight (mg C3GE/g DW) and was calculated using the formula:

$$TMAC\ \left(\frac{mg\ C3GE}{g}\right) = \Delta A * MW * df * 10^3 * \frac{100}{\varepsilon} * l * 1 \quad (1)$$

Where: $\Delta A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$; MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol), df = dilution factor, ε = extinction coefficient of cyanidin 3-glucoside (26,900 L x mol^{-1}x cm^{-1}), l is the cell path length (1 cm), and 10^3 is the factor conversion from g to mg.

Total flavonoid content
The total flavonoid content (TFC) was quantified by the aluminum chloride colorimetric method, following Luximon-Ramma et al (2002) with slight adjustments. TFC was quantified using a standard curve of catechin in absolute methanol. The standard curve plot ranges from 0–500 µg/L with $R^2 = 0.9902$. The TFC of the samples was reported as milligrams catechin equivalents per gram dry weight of extract (mg CE/g DW).

Quantification of antioxidant properties
The quantification of DPPH• assay was accomplished according to Pisoschi & Negulescu (2012) with minor adjustments. The $ABTS^{\bullet+}$ and FRAP assay was based on Tomasina et al (2012) with slight adjustments. All assays were done in triplicates and a standard curve of Trolox was used. (DPPH: linearity range: 0–7.2 µg/ mL, $R^2 = 0.9934$; $ABTS^{\bullet+}$: linearity range: 0–40 µg/ mL, $R^2 = 0.9950$; FRAP: linearity range: 0–40 µg/ mL, $R^2 = 0.9954$). The results were conveyed as mg Trolox equivalents per gram dry weight (mg TE/g DW).

RESULTS AND DICUSSIONS

Screening of factors affecting phenolic extraction by Plackett-Burman design of experiment
The PBD of experiment was utilized in determining the factors that contributed significantly to the extraction of phenolics from CBS. Results showed that among the 7 factors, incubation temperature, SSR, and extraction time are the significant factors that had the greatest effect on the response. Table 1 shows the PBD generated and the results of TPC per run. Values obtained ranged from 0–1843.09 mg GAE/L extract.

Among the seven factors, three factors were found to have a significant effect ($p<0.05$) on the extraction of phenolics from CBS: SSR, incubation temperature, and extraction time. The percentage contribution of these three factors was 69.69%, 9.34%, and 5.51%, respectively. To graphically visualize which factors contributed a considerable effect on the TPC, a Pareto chart was generated. Figure 1 shows that the extraction time, ratio of sample to solvent, and incubation temperature extended beyond the Bonferroni Limit, which is the reference line that indicates which effects are significant (Hu et al., 2016). Factors that are shown in orange indicate that they contributed positive effects to the response, while factors in blue contributed

negatively. Sonication time and agitation had a negative effect on the response, while the SSR, incubation temperature, extraction time, solvent concentration, and pH had a positive effect on the TPC of the CBS extracts.

Table 1: Plackett-Burman design of experiment for screening of factors and corresponding total phenolic content (TPC)

Run	Temperature (°C)	Extraction time (mins)	pH	Sample to solvent ratio (g/mL)	Solvent concentration (%)	Sonication time (mins)	Agitation (rpm)	TPC (mg GAE/L extract)
1	30	90	12	0.02	75	60	200	331.74
2	70	5	12	0.06	0	60	200	1650.97
3	30	90	2	0.06	75	10	200	1627.1
4	30	5	2	0.06	0	60	200	1001.83
5	70	5	12	0.06	75	10	0	1179.98
6	50	47.5	7	0.04	37.5	35	100	1430.9
7	30	5	12	0.02	75	60	0	0
8	70	5	2	0.02	75	10	200	504.07
9	70	90	2	0.06	75	60	0	1843.09
10	50	47.5	7	0.04	37.5	35	100	1552
11	70	90	2	0.02	0	60	0	139.04
12	50	47.5	7	0.04	37.5	35	100	1377.34
13	30	90	12	0.06	0	10	0	0
14	30	5	2	0.02	0	10	0	0
15	70	90	12	0.02	0	10	200	657.76

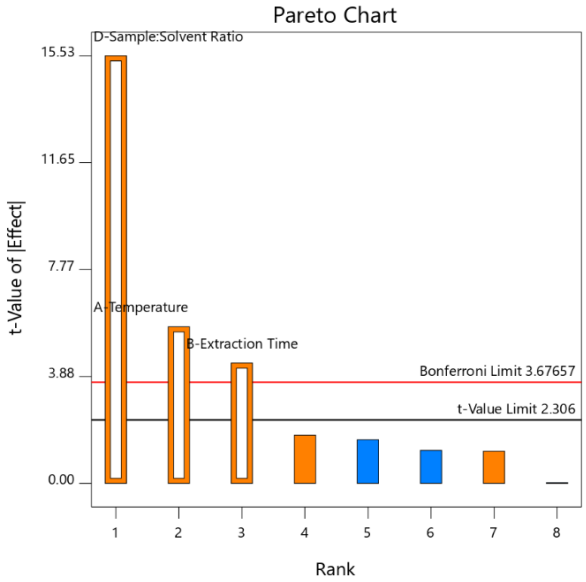

Figure 1: Pareto chart representing the effect of each factor on the total phenolic content (TPC) of cocoa bean shell extracts in descending order using the Plackett-Burman design.

Figure 2 displays the main effects plot of the seven factors (temperature, incubation time, pH, sample: solvent ratio, solvent concentration, sonication time, and agitation). The main effects plot is used together with ANOVA to determine if a factor significantly affects the response. A main effect is present for a factor when the line generated is not horizontal. Figure 2 illustrates that pH, solvent concentration, sonication time, and agitation showed a horizontal line, indicating their negligible impact on phenolic extraction from CBS. However, extraction time, incubation temperature, and SSR showed positive effects to the TPC of extracts, as indicated by the absence of a horizontal line. It can be visually observed that the slope of the line for the SSR is much greater compared to the slope of the line for temperature and incubation time, which increases with its corresponding percent contribution.

Total phenolic content as influenced by sample to solvent ratio (SSR)

As previously indicated, SSR had the highest percent contribution among the three parameters that showed significant effects on TPC of CBS. This suggests that the SSR had the greatest influence on the polyphenolics extraction from the CBS. Melini et al (2023) also noted a comparable result, achieving the greatest value of TPC when the SSR was maximized in the extraction of phenolics from artichoke heads. The mass transfer concept can be used to explain this result, where it states that the variance in concentration gradient between the solid and the solvent facilitated the movement of the solubilized phenolics to the solvent (Belwal et al., 2016).

Total phenolic content as influenced by incubation temperature

According to Jokic et al (2009), the TPC values obtained increased with elevated temperature (from 25–80 °C) with values recorded from 2.21–4.50 mg GAE/g sample respectively. Based on Antony & Farid (2022), the effect of increasing the temperatures up to 80 °C during extraction positively influenced TPC values. This could be associated with the softening of the plant tissue that results in weakened phenol-polysaccharide interactions and the migration of greater amounts of polyphenols into the extracting solvent (Mokrani & Madani, 2016). This result can also be credited to the enhanced solubility and diffusion of phenolic compounds at higher extraction temperatures (Zhang et al., 2018).

Total phenolic content as influenced by extraction time

Time also positively influenced the phenolic extraction from CBS. In the study of Mokrani & Madani (2016) and Sulaiman et al (2017), the bioactive compounds extracted were higher upon increasing incubation time up to 180 and 120 minutes, respectively. In contrast, Mokrani & Madani (2016) observed that increasing time above 180 minutes was counter-effective since the phenolics had the risk of being oxidized. This is explicated by Fick's second law of diffusion, which refers to the existence of balance between the solute concentration in the solvent and the solid matrix. (Paul et al., 2014; Babaran et al., 2023). Meanwhile, Sulaiman et al. (2017) suggest that at a lower temperature of extraction, migration of the phenolic compounds

was allowed under prolonged periods of time, which is not applicable at higher temperatures.

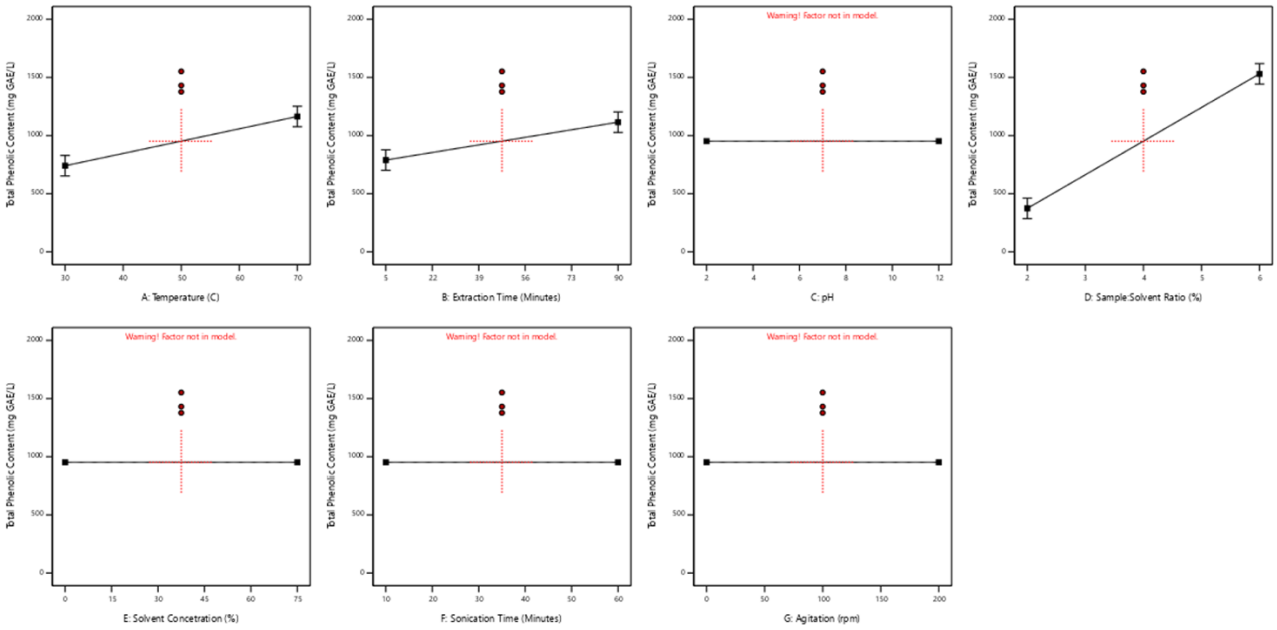

Figure 2: Main effects plot of the factors on the total phenolic content (TPC) of cocoa bean shell extracts using Plackett-Burman design. **A:** Temperature; **B:** Extraction time; **C:** pH; **D:** Sample to solvent ratio; **E:** Solvent Concentration; **F:** Sonication time; **G:** Agitation

Statistical analysis of the model generated by the Plackett-Burman design of experiment

The very low *p*-value ($p<0.0001$) of the generated model indicates that it was highly significant. In addition, the *F*-value of 1.72, for the lack of fit, suggests non-significance. Meanwhile, a *p*-value of 0.4075, exceeding the threshold of 0.05, further suggests insignificant lack of fit. The acceptability of the model generated is based on the non-significance of the lack of fit. For a model to be deemed acceptable, it is necessary for the generated model to exhibit a lack of fit that is insignificant. The high values of determination coefficient (R^2 = 0.9808) and adjusted determination coefficient (R_{adj}^2 = 0.9725) suggest that 97.25% of the variances can be accounted for by the model, indicating a strong fit of the regression equation. The R_{pred}^2 of 0.9341 demonstrates a satisfactory agreement with the R_{adj}^2 of 0.9725, given their discrepancy is below 0.2 (Babaran et al., 2023). The ratio of 26.834 of the adequate precision also indicates an adequate signal, which measures the signal to noise ratio. This suggests that the model is suitable for navigating the design space. The order of the factors in which TPC was greatly affected was as follows: SSR > incubation temperature > extraction time. Subsequently, these three significant parameters were selected for the determination of their optimum levels via the Box-Behnken design (BBD), while the other factors (pH, solvent concentration, sonication time, agitation) were kept constant. The model generated also indicates a positive effect for factors incubation temperature, extraction time, and SSR. The regression equation between the extraction factors and TPC was as follows:

$$\text{TPC} = 952.184 + (211.789 * \text{temperature}) + (162.739 * \text{extraction time}) + (578.564 * \text{SSR}) \quad (2)$$

Optimization of the significant factors by Box-Behnken design

The three factors with significant effect on the TPC values of CBS extracts from the PBD of experiment were then used in the BBD of experiment. This generated 15 base runs with 3 center points. The factors considered for optimization were incubation temperature, SSR, and extraction time. The factors that were held constant were the pH of the solvent (5.73 or the pH of water that was used), solvent concentration (water), agitation (0 rpm), and sonication time (0 minutes).

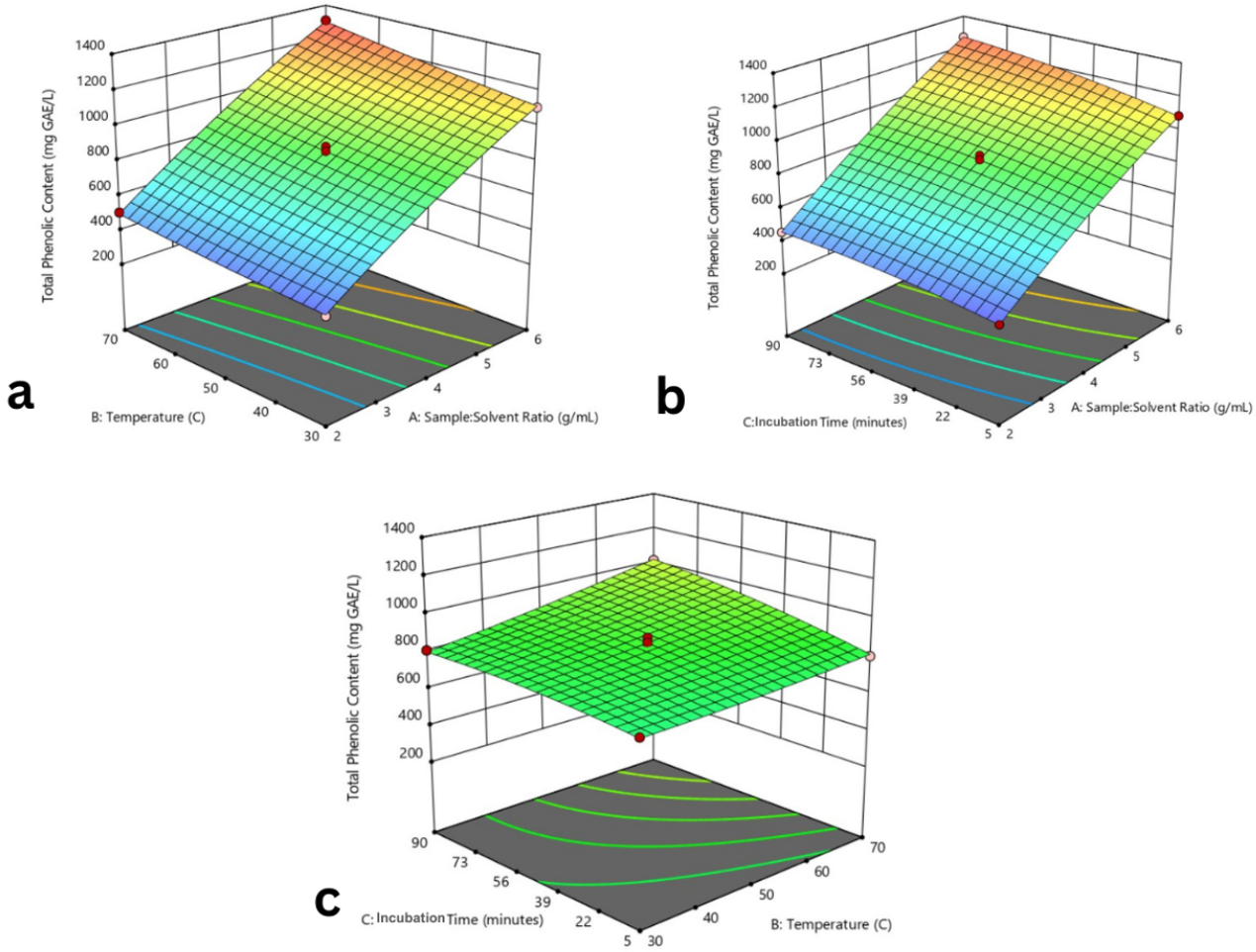

Figure 3: Surface plots of the interaction effects of **(a)** temperature and sample to solvent ratio, **(b)** incubation time and temperature, and **(c)** incubation time and sample to solvent ratio.

Green extraction techniques have been the focus of recent studies with regard to sustainable development (Zainal-Abidin et al., 2017; Value et al., 2020; Tang et al., 2015; Paiva et al., 2014). In this regard, water is the ideal solvent that can be utilized in acquiring desirable compounds from postharvest byproducts. Utilizing water as an extracting solvent satisfies the requirements from an environmental and economic standpoint due to its availability, recyclability, non-toxicity, and non-flammability (Bubalo et al., 2018; Andres et al., 2020). Moreover, there is previous research that utilized water in extracting desirable bioavailable compounds from apple pomace (Reis et al., 2012), bignay pomace (Babaran et al, 2023) and brewer's spent grain (Leite et al., 2019).

Table 2 shows the BBD generated and the TPC per run. The TPC values obtained ranged from 381.26–1308.78 mg GAE/L extract. The least amount was observed on run number 3, while the highest value was observed on run number 13. The run with the lowest value had the following parameters: A – 0.02 g/mL SSR, B – 50 °C incubation temperature, and C – 5 minutes extraction time. The run with the highest value had the following parameters: A – 0.06 g/mL SSR, B – 30 °C incubation temperature, and C – 47.5 minutes extraction time. A similar result on the effects of time and sample concentration was obtained by Melini et al (2023) where short time and low energy were utilized in extracting bioavailable compounds from artichoke heads with optimized parameters of 60 °C for 20 minutes and 0.02 g/mL solvent to sample ratio. For temperature, the results of Sulaiman et al (2017) corroborate with the data found in this research, where lower extraction temperature was employed to avoid the degradation of phenolics due to the heat sensitivity of these compounds. Conversely, the influence of SSR on the CBS extracts behaved similarly with the results from the research of Melini et al (2023) and Belwal et al (2022).

Response surface regression analysis showed an R^2 value of 0.9982, an adjusted R^2 value of 0.9949, and a predicted R^2 value of 0.9902. The predicted R^2 of 0.9902 reasonably corresponds with the adjusted R^2 of 0.9949 since their deviation is below 0.2. The R^2 value of 0.9982 suggests that 99.82% of the variances in the response can be described by the model. The adequate precision pertains to the measurement of the ratio of signal to noise. A ratio exceeding 4 is deemed acceptable, and the ratio of 53.277 suggests that there is signal adequacy. The lack of fit (*F*-value = 0.1995) represents the variation due to the inadequacy of the model which is statistically not significant. Hence, there is no indication that the model ineffectively accounts for the variability of the responses. Moreover, the model *F*-value of 306.41 indicates the significance of the model. The model contains 3 linear effects. The *p*-values of the extraction time (0.0003), SSR (< 0.0001), and incubation temperature (0.0002) were less than 0.05. Thus, a significant linear impact was observed for SSR, incubation time, and extraction temperature, i.e., the total polyphenolic content differed depending on the incubation temperature, extraction time, and SSR.

The model contains three two-way interactions that allow the determination of which factors had significant interaction with each other. The *p*-values of the interactions between the temperature and SSR (0.0769), and extraction time and SSR (0.0591) were above the threshold of 0.05, suggesting interaction effects that were not significant. Meanwhile, the interaction between incubation temperature and extraction time (0.0120) had a *p*-value of not greater than 0.05, demonstrating a

significant interaction effect, i.e., the TPC's correlation with incubation temperature depended on the extraction time.

Table 2: Box-Behnken design for the optimization of the significant factors and the total phenolic content (TPC) of each run

Run	Sample to solvent ratio (g/mL)	Incubation temperature (°C)	Incubation time (mins.)	TPC (mg GAE/L extract)
1	0.04	50	47.5	827.693
2	0.06	50	90	1265.97
3	0.02	50	5	381.259
4	0.02	30	47.5	396.751
5	0.02	70	47.5	506.015
6	0.04	50	47.5	859.494
7	0.06	50	5	1087.4
8	0.02	50	90	456.683
9	0.04	30	90	808.123
10	0.04	30	5	755.122
11	0.04	50	47.5	886.402
12	0.04	70	5	798.746
13	0.06	70	47.5	1308.78
14	0.06	30	47.5	1105.34
15	0.04	70	90	1014.83

As for the squared effects, it can be used to evaluate whether or not a curvature (quadratic) is present in the response surface. The *p*-value of the sample: solvent ratio (0.0192) was less than 0.05, and thus implied significant quadratic effects. This implies that the specific correlation between the SSR and the TPC follows a curved line rather than a straight line. The equation formulated in terms of the actual factors allows for predictions of the response at a specified parameter level; however, it is important to note that the levels should be displayed in the original units for each factor. This equation should not be employed in assessing the relative influence of each parameter, as the coefficients are adjusted to match the units of each parameter, and the intercept is not in the center of the model design space.

$$\begin{aligned} \text{TPC} = {} & 18.67373 + 220.34952(\text{SSR}) \\ & - 3.33071(\text{temperature}) \\ & - 0.888574(\text{extraction time}) \\ & + 0.58862(\text{SSR} * \text{temperature}) \\ & + 0.303384(\text{SSR} * \text{extraction time}) \\ & + 0.047965(\text{temperature} \\ & * \text{extraction time}) - 9.37715(\text{SSR}^2) \\ & + 0.022169(\text{temperature}^2) \\ & - 0.012471(\text{extraction time}^2) \end{aligned} \quad (3)$$

The impact of several parameters on the TPC was evaluated using 3-dimensional response surface curves against any two independent variables, which is shown in Figure 3. For instance, Figure 3a displays the graphical visualization of the influence of interaction between SSR and temperature on the TPC. There is an observed decline in the value of the TPC when both the temperature and SSR were decreased. Meanwhile, the TPC is high when the values of these factors were elevated.

Verification of the optimized CBS extract

The verification process involves conducting experiments utilizing the projected ideal processing conditions. The parameters for verification were determined using the solution provided by the model generated. The solution with the highest desirability index (0.885) was selected. This solution was selected since it presented the maximum SSR and minimum incubation time and extraction temperature, where higher amounts of CBS will be used while utilizing minimal amounts of resources. A predicted TPC of 1040.72 mg GAE/L extract could be achieved with an extraction time of 5 minutes, incubation temperature of 30 °C, and SSR of 0.06 g/mL.

To verify the suitability of the model, a total of five independent runs using the optimum parameters were conducted. The model indicated that at 95% confidence, the predicted data mean would be between the range 976.34 and 1105.06 mg GAE/L extract. A mean TPC value of 1085.85 mg GAE/L extract was obtained during verification; therefore, the model is considered valid. This indicates that the TPC extracted from CBS was successfully predicted by RSM.

Characterization of the optimized CBS extract

The optimized CBS extract was evaluated for its total flavonoid (TFC), total monomeric anthocyanin content (TMAC), and total phenolic (TPC). The antioxidant capacities were also evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), ABTS$^{\bullet+}$, and ferric reducing antioxidant power (FRAP) assays. Table 3 presents a summary of the results obtained from the characterization of the optimized CBS extract.

Table 3: Bioactives profile and antioxidant activity of the optimized phenolic extract from cocoa bean shell.

RESPONSE	EXPERIMENTAL VALUE
Chemical Analysis	
Total Phenolic Content (mg GAE/g DW)	10.86 ± 0.22
Total Flavonoid Content (mg CE/g DW)	13.83 ± 0.84
Total Anthocyanin Content (mg C3GE/g DW)	0.22 ± 0.02
Antioxidant Activity	
DPPH (mg TE/g DW)	8.58 ± 0.04
ABTS$^{\bullet+}$ (mg TE/g DW)	23.76 ± 0.59
FRAP (mg TE/g DW)	0.01 ± 0.0001

Expressed as mean ± SD (n = 3)

The TPC (10.86 mg GAE/g) of CBS extracts in this study was slightly higher compared to nine Philippine indigenous fruits, which ranged from 2.34–7.58 mg GAE/g (Recuenco et al, 2020). Additionally, the TPC of CBS extract was greater in comparison to fresh kiwi fruit (3.32 mg GAE/g), fresh orange peel (7.57 mg GAE/g), and fresh pineapple peel (0.95 mg GAE/g). The comparable amounts of TPC presented can be attributed to the migration of polyphenols from the seed cotyledons to the CBS during fermentation and roasting (Sánchez et al, 2023). However, results for CBS extracts were lower when compared to the findings of Mellinas et al (2020), using an optimized method for microwave-assisted (22.2 mg GAE/g) and traditional Soxhlet extraction (22.1 mg GAE/g) of phenolics from CBS. Several factors such as the extraction temperature (<97.0 °C) and pH of

solvent (9–12) can be accounted for the variation in the yield of phenolics. Elevated temperatures and pH usually degrade the plant cell walls, hastening the transfer of bioactive compounds into the solvent during extraction (Chan et al., 2011; Đurović et al., 2018; Yang et al., 2019). In this study, 30 °C was used as incubation temperature to facilitate green extraction, and minimal use of resources, whereas the pH of the extracting solvent is not a significant factor during the initial screening using PBD.

The TFC (13.83 mg CE/g DW) of CBS extracts in this study was also higher compared to avocado peel (1.37 mg GAE/g) and seed (0.54 mg GAE/g), kiwi fruit (0.11 mg GAE/g), orange peel (3.8 mg GAE/g), pineapple peel (0.19 mg GAE/g), and pomegranate skin (8.63 mg GAE/g) (Saleh et al., 2021). A high amount of TFC was recorded for CBS because the predominant polyphenolic compound present in CBS is flavonoids. As demonstrated by Rebollo-Hernanz et al (2021), the TFC of CBS was evaluated using an optimized aqueous extraction method with an extraction time of 100 °C and SSR of 0.02 g/mL at 39–90 mins incubation time. The results obtained ranged from 12.77–13.77 mg CE/g, which is marginally less than the TFC documented in this research. However, the TFC of CBS extracted using organic solvent (73.90 mg CE/g) is significantly higher. This large gap between extraction using organic and aqueous solvents can be attributed to the enhanced solubility of flavonoids to organic solvents like methanol, chloroform, and others (Zhao et al., 2019).

On the other hand, a low TMAC for optimized CBS extracts was recorded (0.22 mg C3GE/g DW). The TMAC found in this research is significantly lower compared to the TMAC of optimized extracts from black mulberry (12.3 mg C3GE/g) (Koyu et al., 2018) and fruits of acai palm (153.63 mg C3GE/g) (Pompeu et al., 2009). This could be attributed to the drastic reduction of anthocyanins from cocoa beans during fermentation (Melo et al., 2021). The TMAC from cocoa samples from various origins and processing stages was evaluated by Bordiga et al (2015). Results displayed TMAC for pre-roasted nibs ranged from 0.0049–0.12 mg C3GE/g while the TMAC of the final product dark chocolate ranged from 0.0020–0.0034 mg C3GE/g. The very low recorded TMAC of CBS could be credited to the losses of anthocyanin and changes in phenolic composition during roasting and fermentation (Wollgast & Anklam, 2000).

The optimized extracts exhibited antioxidant activities, as evidenced by the quantified values of $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP. For the $ABTS^{\bullet+}$ value (23.76 mg TE/g) of CBS, it is comparably higher than gooseberry peel (4.0 mg TE/g) and seed (10.0 mg TE/g), plum peel (9.0 mg TE/g) and seed (10.0 mg TE/g), melon peel (9.5 mg TE/g) and seed (10.0 mg TE/g), and white grape seed (2.0 mg TE/g) (Duda-chodak & Tarko, 2014). Moreover, the value of $ABTS^{\bullet+}$ measured in this research is slightly larger than the values acquired by Rebollo-Hernanz et al., (2021) for the optimized aqueous extraction method from CBS which ranged from 21.15–21.48 mg TE/g. Comparable results of $ABTS^{+}$ can be attributed to its solubility in both organic solvents and water, which participated in the determination of the antioxidant capacity of CBS extracts using this assay (Opitz et al., 2014). On the other hand, a near-negligible value of FRAP was recorded for this study. This could be due to the pH of the extracting solvent as FRAP necessitates an acidic medium to ensure the solubility of iron and facilitate electron transfer (Zhong & Shahidi, 2015). In this study, the pH of the extracting solvent of the optimized parameters is near neutral. This can explain the extremely low value of FRAP that was obtained for CBS extracts. Meanwhile, the $DPPH^{\bullet}$ (8.58 mg TE/g) of CBS is lower compared to the by-products of industrial fruits such as apple (25.69 mg TE/g) (Barbosa et al., 2021), orange (20.13 mg TE/g), and lemon (33.17 mg TE/g). The low $DPPH^{\bullet}$ value recorded for CBS extracts could be due to the utilization of water as the extracting solvent because water hampers the mechanism of $DPPH^{\bullet}$ assay due to its solubility with organic solvents (Kedare & Singh, 2011).

CONCLUSION

According to the findings of this study, the use of response surface methodology successfully determined and optimized the significant factors affecting the extraction of phenolics from CBS. The model generated was able to predict the optimum extracting conditions. In addition, the study was able to optimize a sustainable and economical extraction procedure that can be achieved at room temperature within a short timeframe. It is also noteworthy that the optimized extraction method is highly accessible for implementation in large scale industry applications. Thus, the environmental and economic impact of this study presents promising benefits. The utilization of CBS for the extraction of functional compounds holds economic appeal for manufacturers due to its efficient raw material utilization. Overall, this study was able to optimize an extraction condition that is cost-effective, sustainable, and highly accessible for application in the industry.

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CONFLICT OF INTEREST

The authors affirm that they do not hold any conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Sheba Mae M. Duque, as the corresponding author, contributed to the conception of experimental design, experiment supervision, project leadership, acquisition of funding, conducted a thorough review and critical revision of the manuscript, and ensured that all listed authors granted their approval before submitting the manuscript. Keila Cristine B. Quejadas and Argel A. Largado are responsible for the data analysis and interpretation, and also drafted and substantively revised the manuscript. Gilda Melanie O. Babaran contributed to the initial gathering, processing, and interpretation of data, and revised the manuscript critically. John Joseph C. Menia is accountable for the gathering of resources and data during the initial phases of the experiment, as well as analysis and interpretation of data. Romel M. Felismino and Katherine Ann T. Castillo-Israel made considerable inputs in the conception of the design of the experiment, resources, manuscript review, and review of the final paper to be submitted.

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