

OPTIMISATION OF ANTIOXIDANT ULTRASOUND ASSISTED EXTRACTION FROM PINEAPPLE PEEL AND COCOA POD MIXTURE USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT. *This study aimed to optimise the ultrasonic-assisted extraction (UAE) of antioxidants from a pineapple peel and cocoa pod mixture using Response Surface Methodology (RSM). Ethanol concentration and sample ratio showed the greatest influence on antioxidant extraction. Subsequently, extraction time, temperature, and sample ratio were optimised using a Box–Behnken design. The optimum extraction conditions were 20 min extraction time, 70 °C extraction temperature, and 90% mixture proportion. Under these conditions, the extract achieved the highest TPC (42.23 ± 0.30 mg GAE/g DW), TFC (16.80 ± 0.20 mg QUE/g DW), FRAP (4.08 ± 0.10 mg AAE/g DW), and the lowest DPPH IC₅₀ (1.10 µg/mL), indicating strong antioxidant activity. ANOVA showed that the pineapple peel and cocoa pod ratio significantly affected all responses ($p \leq 0.05$). Model validation was performed in triplicates ($n = 3$), with errors <8%, indicating good predictive accuracy of the developed models, while FTIR analysis confirmed the presence of phenolic and flavonoid functional groups. These findings demonstrate the potential of UAE to produce antioxidant-rich extracts from fruit processing by-products, promoting waste valorisation and sustainable resource utilisation.*

INTRODUCTION

In recent years, the management of agro-industrial waste has received considerable attention due to environmental problems related to its disposal. In tropical climates, fruit by-products such as pineapple peels and cocoa pods are prevalent among other wastes. These peels are generally disposed of, despite being abundant in bioactive components, including antioxidants. These compounds exhibit numerous health advantages, including anti-inflammatory, antibacterial, and free radical scavenging activities (Flieger *et al.*, 2021).

Plants that possess secondary metabolites like flavonoids and phenols are recognised for their ability to act as free radical scavengers attributing to their antioxidant characteristics. The pineapple plant (*Ananas comosus* (L.) Merrill) is abundant in antioxidants and is recognised for its elevated levels of phenolic compounds, bromelain, and vitamin C. Meanwhile, cocoa (*Theobroma cacao* L.), a prevalent plant in tropical regions, contains polyphenols including catechins, epicatechins, and theobromine. Pineapples can be preserved, prepared, juiced, and consumed fresh. The pineapple industry generates massive amounts of trash, including the removal of the crown and leaves before the juice is harvested, which accounts for 50% (w/w) of the biomass (Khalid *et al.*, 2016). Pineapple peel waste, a byproduct of pineapple canning, may serve as a source for extracting beneficial bioactive compounds.

Cocoa serves as the primary component of chocolate and cocoa beverages, constituting a significant agricultural crop in Malaysia, where smallholders cultivate 89% of the total area. In 2022, the Malaysian Cocoa Board (MCB) documented 5,985 hectares of cocoa cultivation in Malaysia, with export values amounting to RM7.8 billion (Johnsiul *et al.*, 2023). Cocoa pod husks and other unwanted byproducts are a component of the global production of *Theobroma cacao* beans. Cocoa pods contain a variety of beneficial compounds, including polyphenols, which have long been recognised as a source of dietary polyphenols associated with overall health and the prevention of chronic diseases (Perez *et al.*, 2021).

Recent improvements in green extraction technologies, including ultrasound-assisted extraction (UAE), have enabled the effective recovery of these important molecules (Freitas *et al.*, 2016). Utilising Response Surface Methodology (RSM) to optimise this process offers a statistically sound method to enhance extraction yield and efficiency. The application of RSM in this work facilitates the identification of optimal extraction parameters for maximising antioxidant output from a blend of pineapple and cocoa peels. Food processing businesses produce substantial quantities of agricultural by-products, such as pineapple and cocoa peels, which are frequently regarded as waste. Nonetheless, these by-products are abundant in bioactive chemicals, particularly antioxidants. The extraction of important components through sustainable and efficient approaches is garnering increasing interest. The UAE has emerged as an efficient and environmentally friendly method for enhancing antioxidant extraction, owing to its capacity to shatter cell walls and facilitate mass transfer. Utilising RSM to optimise this process offers a statistically sound method to enhance extraction yield and efficiency.

The objective of this investigation was to enhance the recovery of bioactive compounds from a blend of pineapple rind (*Ananas comosus*) and cocoa pod (*Theobroma cacao*) through ultrasound-assisted extraction (UAE). DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC) were assessed as response variables. The effects of key extraction parameters were determined and optimal conditions were established to maximise the recovery of antioxidant-rich compounds from these agro-industrial residues using response surface methodology (RSM).

MATERIALS AND METHODS

Design of Experiments

The optimisation of ultrasound-assisted extraction (UAE) from a pineapple peel and cocoa pod mixture was carried out in three phases. First, the effects of ethanol concentration (10–90 %), pineapple peel-to-cocoa pod ratio (0.00–1.00), and solid-to-solvent ratio (1:15–1:55 g/mL) on TPC, TFC, FRAP, and DPPH RSA were evaluated. In the second phase of experiments, extraction conditions were optimised using response surface methodology (RSM) with a Box–Behnken design. Finally, in the third phase of experiments, the optimal conditions predicted by the models were validated experimentally, and the IC₅₀ values of the extracts were determined.

Sample Collection and Preparation

Pineapple peel waste was collected from Kingfisher Market, while cocoa pods were obtained from the Cocoa Biotechnology Research Centre KKIP, Sabah. The samples were washed, manually separated, and cut into smaller pieces before being oven-dried at 50°C until constant weight was achieved. The dried materials were then ground into powder, sieved through a 40-mesh sieve following the method of Yahya *et al.* (2020) with modifications, and stored in labelled glass bottles for further analysis.

Preparation of Pineapple Peel and Cocoa Pod Mixture Extracts Using Ultrasound-Assisted Extraction

The sample extraction method was modified from Rahayu *et al.* (2023). In different extraction conditions (20, 40, and 60 min), temperatures (30, 50, and 70 °C), and pineapple peel to cocoa pod ratios (10, 50, and 90%), 3 g of dried pineapple peel and cocoa pod powder were crude extracted. Fisher Scientific ultrasonic bath (FB15065) was used for extraction with the temperature control off, the frequency set at 20% of the bath frequency (40 kHz), and the power set at 150 watts. Mixtures were filtered with Whatman No. 1 filter paper after extraction. A 4 rpm rotary evaporator at 50-60 °C concentrated the filtrates into crystallised solids for analysis.

The Effect of Different Ultrasound Extraction Parameters on Polyphenol Content and Antioxidant Activity of Mixture

Aqueous ethanol concentration

The chosen solvents were analysed based on their concentration range. The solvent concentrations were established at 0%, 30%, 50%, 80%, and 100% for 40 minutes at 40 °C. The solvent concentration exhibiting the highest polyphenol content, and antioxidant activity was determined as the extracting solvent, and this concentration range was employed in the subsequent optimisation process. The extraction was achieved using a 0.50 pineapple peel to cocoa pod ratio (PP:CP) and a solid-to-solvent ratio of 1:25 g/mL.

Pineapple peel to cocoa pod ratio

The extracts mixture were analysed according to their ratio range. The extraction of mixtures was conducted at a pineapple peel to cocoa pod ratio of 0.00, 0.25, 0.50, 0.75, 1.00 for 40 minutes at 40 °C. The pineapple peel to cocoa pod ratio exhibiting the highest polyphenol content, and antioxidant activity was determined as the chosen ratio range and then applied in the second optimisation stage. The extraction was achieved using a 70 % ethanol concentration and a solid-to-solvent ratio of 1:25 g/mL.

Solid to solvent ratio

The solid-to-solvent ratio was slightly adjusted to facilitate the extraction of pineapple peel and cocoa pod. The extraction of the mixture was conducted at solid-to-solvent ratios of 1:15, 1:25, 1:35, 1:45, and 1:55 g/mL for 40 minutes at 40 °C. The total phenolic content, total flavonoid content, and antioxidant activity were assessed in relation to the solid-to-solvent ratio. The extraction was conducted using a 70 % ethanol concentration and a 0.50 pineapple peel to cocoa pod ratio (PP:CP).

Response Surface Methodology (RSM) Design

Response surface methodology optimised different extraction times, temperatures, and pineapple peel-to-cocoa pod ratios. In order to maximise phenolic and flavonoid extraction yield, a three-factor Box-Behnken Design was used. The independent variables were coded -1, 0, and +1, and the extract TPC (mg GAE/g), TFC (mg QE/g), FRAP (mg AAE/g), and DPPH RSA (%) were chosen as responses (Table 1). The Box-Behnken experimental design optimised extraction parameters using three variables and levels in 17 tests. The experimental layout was shown in Table 2.

Validation of Response Models

The ultrasound-assisted extraction was conducted under specified conditions to validate the RSM polynomial models for the responses. The ideal parameters for ultrasound-assisted extraction, as indicated by the RSM, were an extraction duration of 20 minutes, a temperature of 69 °C, and a pineapple peel to cocoa pod ratio of 90 %. The experimental conditions were conducted in triplicate (n=3), and the responses for the dependent variables were calculated as the mean values $\pm$ standard deviation. The data

were modelled using an appropriate polynomial equation for each response. The models' optimised conditions for maximum TPC, TFC, FRAP, and RSA were experimentally validated, and the IC₅₀ values of the resultant extracts were ascertained.

Table 1. Independent variables for extraction.

Independent variables	Symbols	Level		
		-1	0	+1
Time (min)	t	20	40	60
Temperature (°C)	T	30	50	70
Pineapple peel to Cocoa pod ratio (%)	R	10	50	90

Table 2. Encode design experiment for the response of antioxidant activity.

Run No.	Extraction time (min)	Temperature (°C)	Pineapple peel to Cocoa pod ratio (%)
1	40	50	50
2	40	50	50
3	40	50	50
4	40	70	90
5	40	50	50
6	20	30	50
7	60	50	90
8	20	70	50
9	20	50	90
10	60	50	10
11	40	30	90
12	40	70	10
13	60	30	50
14	60	70	50
15	40	30	10
16	40	50	50
17	20	50	10

Determination of Polyphenol Content and Antioxidant Activity

Total phenolic content (TPC)

The Folin–Ciocalteu method, as described by Mokrani *et al.* (2016), was performed to determine the total phenolic content (TPC) with minor modifications. The Folin–Ciocalteu reagent was diluted (1:10, v/v) with distilled water, and a 7.5% (w/v) sodium carbonate solution was prepared. Gallic acid standards (0–100 µg/mL) were prepared in methanol for calibration. For analysis, 100 µL of standard or sample extract was mixed with 800 µL of sodium carbonate solution and 200 µL of Folin–Ciocalteu reagent. The mixture was vortexed, and 200 µL was transferred in triplicate into a microplate. After incubation in the dark for 120 min, absorbance was measured at 765 nm using a microplate reader. A gallic acid calibration curve was used to determine TPC, which was expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW). Equations 1 and Equation 2 were used to calculate the gallic acid equivalent (GAE) and total phenolic content (TPC), respectively.

$$GAE (mg \text{ GAE } mL^{-1}) = \frac{\text{absorbance at } 765 \text{ nm}}{\text{constant}} \quad (1)$$

$$TPC (mg \text{ GAE } / g \text{ DW}) = \frac{mg \text{ GAE } \pm \text{standard deviation}}{\text{mass of extract used } / mL} \quad (2)$$

Total flavonoid content (TFC)

The aluminum chloride colorimetric method, as described by Shraim *et al.* (2021), was employed to determine the total flavonoid content (TFC) with minor modifications. A calibration curve was generated by preparing quercetin standards (0–100 µg/mL) in methanol. The reagents were prepared as solutions of sodium hydroxide (1 M), sodium nitrate (5%), and aluminum chloride (10%). For analysis, 200 µL of standard or sample extract was mixed with 200 µL of 5% NaNO₂, 200 µL of 10% AlCl₃, and 400 µL of 1 M NaOH. The mixture was vortexed, incubated in the dark at room temperature for 30 min, and 200 µL was transferred in triplicate into a microplate. Absorbance was measured at 415 nm using a microplate reader. TFC was calculated from the quercetin calibration curve and expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW). A standard calibration curve of quercetin concentration (µg/mL) against absorbance was used to calculate quercetin equivalent (QE) and total flavonoid content (TFC) using Equation 3 and Equation 4.

$$QE \text{ (mg QE mL}^{-1}\text{)} = \frac{\text{absorbance at 415 nm}}{\text{constant}} \quad (3)$$

$$TFC \text{ (mg QE g}^{-1}\text{)} = \frac{\text{mg QE} \pm \text{standard deviation}}{\text{mass of extract used}} \quad (4)$$

DPPH radical scavenging assay

DPPH radical scavenging activity was determined according to Gulcin and Alwasel (2023) with minor modifications. A 0.1 mM DPPH solution was freshly prepared by dissolving 4 mg DPPH in 100 mL methanol and protected from light. Serial dilutions of each extract (0–10 mg/mL) were prepared. For analysis, 50 µL of sample was mixed with 195 µL DPPH solution in a microplate, swirled, and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm in triplicate using a microplate reader. Radical scavenging activity (%) was calculated using the standard equation, and results were expressed relative to an ascorbic acid calibration curve (0–100 mg/mL). The results were calculated using Equation 5.

$$DPPH \text{ Scavenging (\%)} = 1 - \frac{\text{Abs. Control DPPH} - \text{Abs of sample}}{\text{Abs. Control DPPH}} \times 100\% \quad (5)$$

where the absorbance of the control refers to that of the ascorbic acid.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay was performed with slight modifications based on Yahya *et al.* (2020). The FRAP reagent was prepared by mixing acetate buffer (0.4 M, pH 3.6), TPTZ solution in HCl, and ferric chloride solution, followed by incubation at 37 °C for 30 min. For analysis, 20 µL of ascorbic acid standard or sample extract was added to 180 µL of FRAP reagent in a 96-well microplate. The mixture was incubated in the dark for 30 min at room temperature, and absorbance was measured at 593 nm in triplicate. A calibration curve of ascorbic acid (0–60 µg/mL) was used to express antioxidant capacity as ascorbic acid equivalents (AAE) and FRAP values in µmol dry weight. The results were presented as mean ± standard deviation. The following Equation 6 and Equation 7 were used.

$$AAE \text{ (mg AAE mL}^{-1}\text{)} = \frac{\text{absorbance at 415 nm}}{\text{constant}} \quad (6)$$

$$FRAP \text{ (mg AAE g}^{-1}\text{)} = \frac{\text{mg AAE} \pm \text{standard deviation}}{\text{mass of extract used}} \quad (7)$$

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to study chemical bond vibrations within functional groups quickly and non-destructively. Infrared spectra of the mixture were taken with an Agilent Technologies Cary 630 FT-IR spectrometer. With 16 scans, FT-IR spectra were acquired in Transmittance, T (%) from 4000–450 cm^{-1} at 4 cm^{-1} resolution. A reference background spectrum was collected against air under the same conditions as the samples before reading. Analysis of 5 mg of dried extract was done directly on the FTIR sampling accessory (ATR crystal). To prepare for the next sample, the sampling accessory was cleaned with isopropanol and dried with soft tissue after obtaining the sample spectrum. By identifying functional groups, FTIR spectra detected antioxidant-related compounds. This fast, accurate, and non-destructive method was useful for plant-based extract chemical analysis.

Statistical Analysis

All data were gathered in triplicate and presented as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was conducted to ascertain significant differences among variables and to evaluate the model's validity. The results were analysed using analysis of variance (ANOVA) with Design Expert, followed by Tukey's Test utilizing Graph Pad Prism Software Version 10.4.1 (GraphPad Software Inc, La Jolla, CA, USA). A p-value of less than 0.05 was employed for the statistical test as significant effect.

RESULTS AND DISCUSSION

Effect of Different Extraction Parameters on the Polyphenol Content and Antioxidant Activity of Pineapple peel and Cocoa pod mixture

This subtopic examines how ethanol concentration (10–90% v/v), pineapple peel to cocoa pod ratio also known as PP: CP (0.25–1.00), and solid-to-solvent ratio (1:25–1:55 g/mL) affect TPC, TFC, FRAP, and DPPH RSA. These effects were assessed by selecting independent variables for optimisation using response surface methodology. Preliminary research involves choosing independent variables. To maximise resource use, this phase used a constant pineapple peel and cocoa pod mixture rather than a single clone.

Aqueous ethanol concentration

The effect of aqueous ethanol concentration on polyphenol extraction is presented in Figure 1 (a), (b). The highest total phenolic content (TPC) was obtained with 90% ethanol (21.64 ± 1.09 mg GAE/g DW), followed by 50% ethanol (17.78 ± 0.52 mg GAE/g DW), with significant differences ($p < 0.05$). In contrast, the highest total flavonoid content (TFC) was recorded at 50% ethanol (14.23 ± 0.18 mg QE/g DW), followed by a slight decrease at higher ethanol concentrations. This suggests that moderately polar flavonoids are more effectively extracted using ethanol–water mixtures than highly concentrated ethanol. To summarise, TPC ranged from 5.28–21.64 mg GAE/g DW, while TFC ranged from 2.79–14.23 mg QE/g DW. Although maximum TPC was achieved at 90% ethanol, TFC peaked at 50% ethanol. This is in agreement with previous studies that show ethanol concentrations between 50% until 70% provide an effective balance for extracting both phenolic and flavonoid compounds (Herrero *et al.*, 2010; Vijayalaxmi *et al.*, 2015; Ju-Hui *et al.*, 2014). Therefore, 70% ethanol was selected for subsequent experiments.

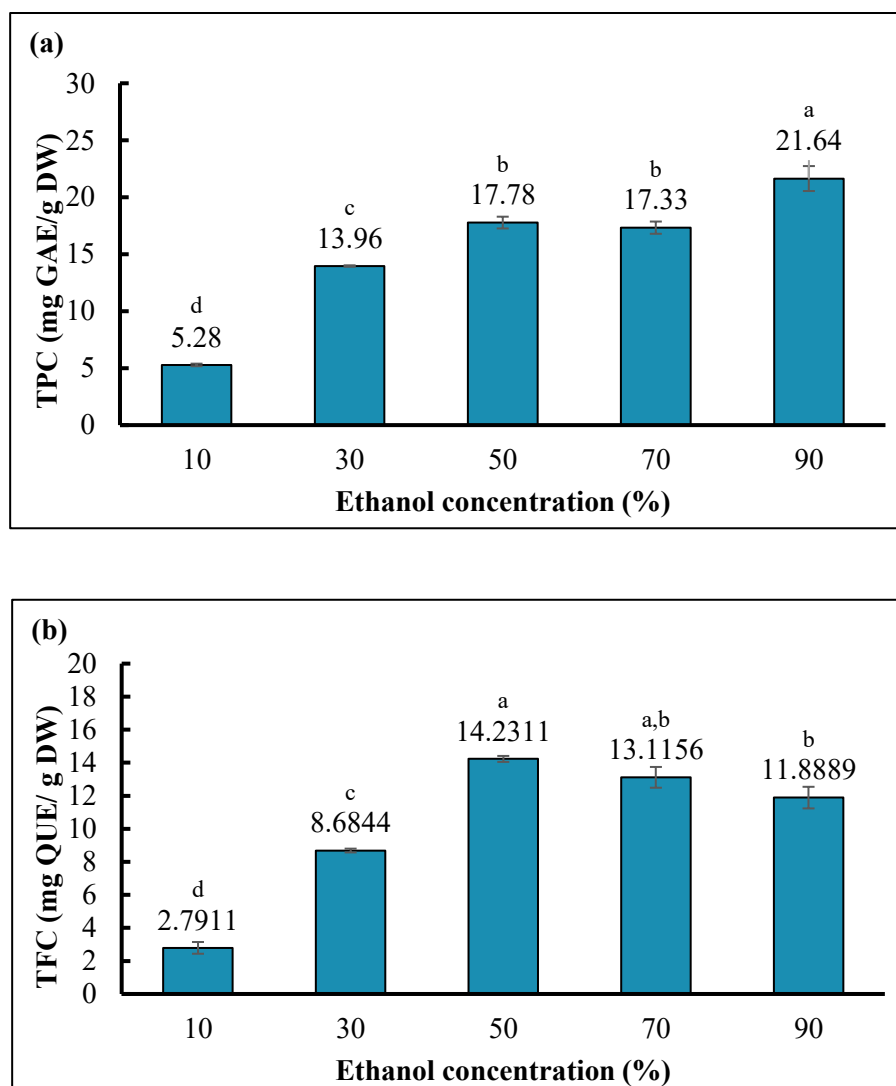


Figure 1. Effect of different aqueous ethanol concentrations on (a) Total Phenolic Content (TPC) and (b) Total Flavonoid Content (TFC) of pineapple peel and cocoa pod mixtures.

As shown in Figure 2(a), the FRAP value increased with increasing ethanol concentration and reached a maximum at 50% ethanol (3.53 ± 0.04 mg AAE/g DW). A decline was observed at 70% (2.90 ± 0.08 mg AAE/g DW) and 90% ethanol (1.83 ± 0.07 mg AAE/g DW), indicating reduced antioxidant activity at higher ethanol concentrations. Similarly, DPPH radical scavenging activity (RSA) as presented in Figure 2(b) peaked at 50% ethanol ($39.70 \pm 0.73\%$) and decreased to 34.01% and 26.76% at 70% and 90% ethanol, respectively. Although the 30% and 90% ethanol extracts showed comparable antioxidant activities, statistical analysis showed significant differences between most treatments ($p < 0.05$). The results align with previous studies reporting that moderate ethanol concentrations (40–60%) are more effective for extracting antioxidant compounds than highly concentrated ethanol (Zhang *et al.*, 2018). Furthermore, both FRAP and DPPH RSA were significantly correlated with TPC and TFC ($p < 0.05$ and $p < 0.001$), indicating that antioxidant activity was largely attributed to polyphenolic compounds. This may be explained by solvent polarity, where ethanol-water mixtures at moderate concentrations provide optimal conditions for extracting phenolics and flavonoids, whereas higher ethanol concentrations reduce the extraction efficiency of these polar compounds (Muhamad *et al.*, 2014).

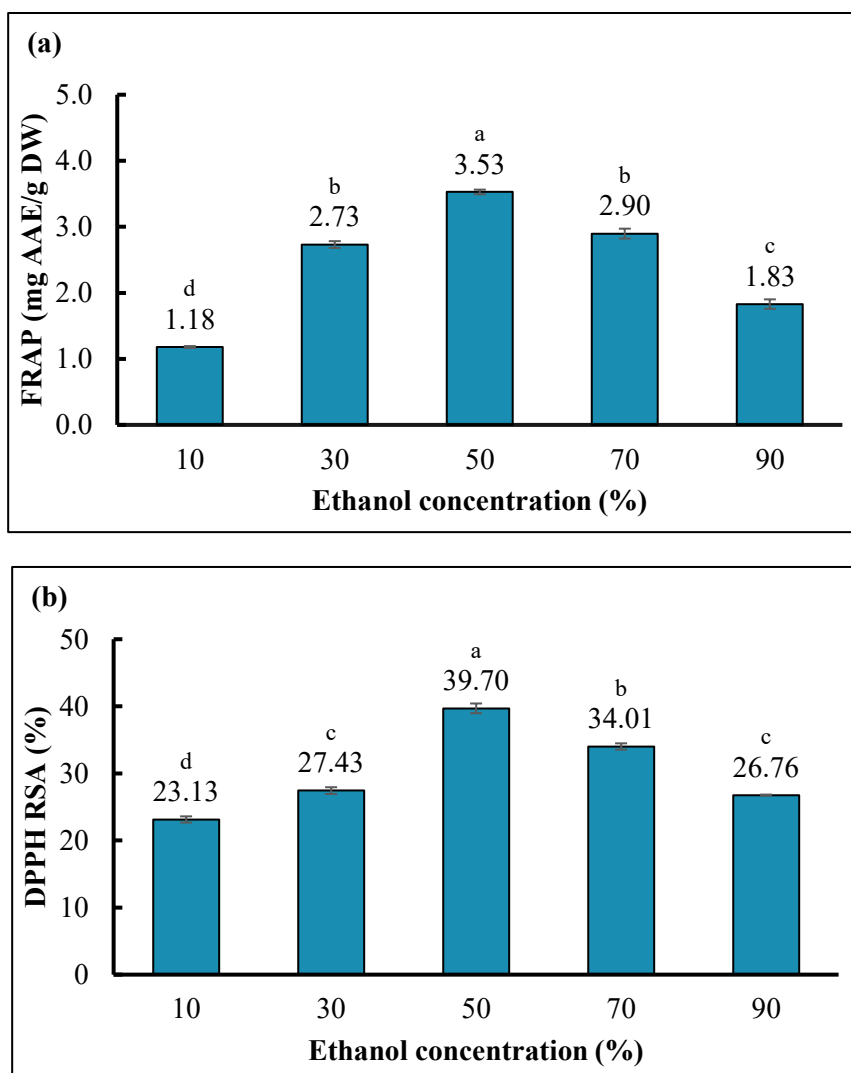


Figure 2. Effect of different aqueous ethanol concentrations on (a) Ferric Reducing Antioxidant Power (FRAP) and (b) DPPH Radical Scavenging Activity (DPPH RSA) of pineapple peel and cocoa pod mixture.

Pineapple peel to cocoa pod ratio

As shown in Figure 4.3(a), total phenolic content (TPC) increased with a higher proportion of cocoa pod in the pineapple peel–cocoa pod mixture, reaching a maximum at a 1.00 ratio (100% cocoa pod) of 30.94 ± 1.73 mg GAE/g DW. This value was significantly higher than 100 % pineapple peel (17.33 ± 0.51 mg GAE/g DW), indicating that cocoa pod contributes substantially to phenolic yield and overall antioxidant potential. A similar trend was observed for total flavonoid content (TFC) in Figure 4.3(b), where the highest value was recorded at the 1.00 ratio (55.96 ± 0.85 mg QE/g DW), compared with 8.25 ± 0.36 mg QE/g DW for 100% pineapple peel and 21.01 ± 0.43 mg QE/g DW at the 0.25 ratio. These findings confirm that cocoa pod, being rich in phenolic and flavonoid compounds, significantly enhances the bioactive content of the extract when its proportion is increased (Soares & Oliveira, 2022).

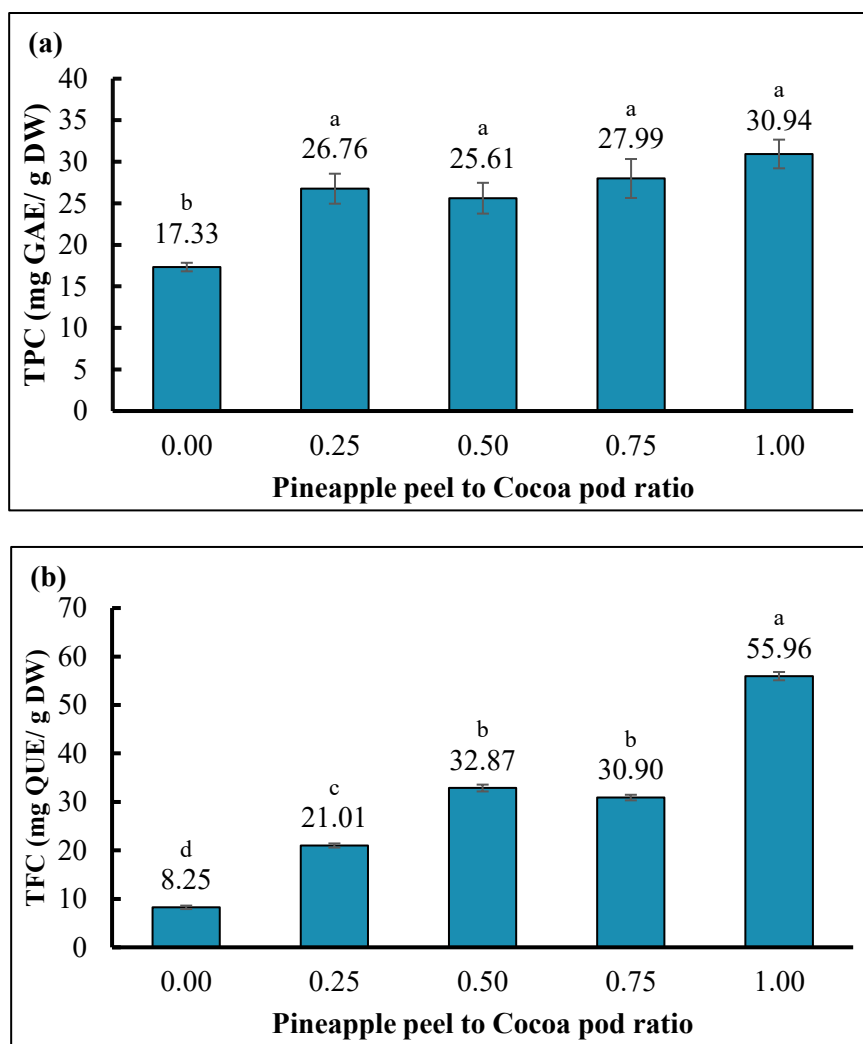


Figure 3. Effect of different pineapple peel to cocoa pod ratios on (a) Total Phenolic Content (TPC) and (b) Total Flavonoid Content (TFC) of pineapple peel and cocoa pod mixture.

Figures 4.4 (a) and (b) show how the pineapple peel to cocoa pod ratio affects ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity (DPPH RSA). For ferric reducing antioxidant power (FRAP), the 1.00 ratio had the highest value (3.53 ± 0.13 mg AAE/g DW), indicating the greatest ability to reduce ferric ions. The 0.75 (75 % pineapple peel, 25% cocoa pod) ratio resulted in a lower FRAP value of 1.76 ± 0.06 mg AAE/g DW, indicating that cocoa pod significantly boosts the extract's antioxidant capacity. Figure 4.4 (b) shows that the 1.00 ratio of cocoa pods had the highest DPPH Radical Scavenging Activity (RSA), with 49.07 ± 0.03 % activity, significantly different from the lower ratios. Scavenging activity at 0.00 ratio (100% pineapple peel powder) was 29.22 ± 0.17 %, while at a 0.25 ratio, it was 25.27 ± 0.09 %. Increased cocoa pod content leads to increased antioxidant activity, suggesting that higher polyphenols and flavonoids may enhance the mixture extract's free radical scavenging potential. The rich and concentrated profile of specific secondary metabolites in cocoa pods explains their superior antioxidant potential relative to pineapple peels. Pineapple peel predominantly contains ferulic acid and vitamin C (Joy *et al.*, 2016), whereas cocoa pod husk is rich in stable polyphenols such as protocatechuic acid and epicatechin (Soares & Oliveira, 2022). Cocoa pods exhibit superior antioxidant properties compared to pineapple peels. The dense phytochemical composition yields lower IC_{50} values, designating it as a 'very strong' antioxidant source in DPPH assays (Muanda *et al.*, 2011), thereby validating its superior performance in the 1.00 ratio (100% cocoa) of this study.

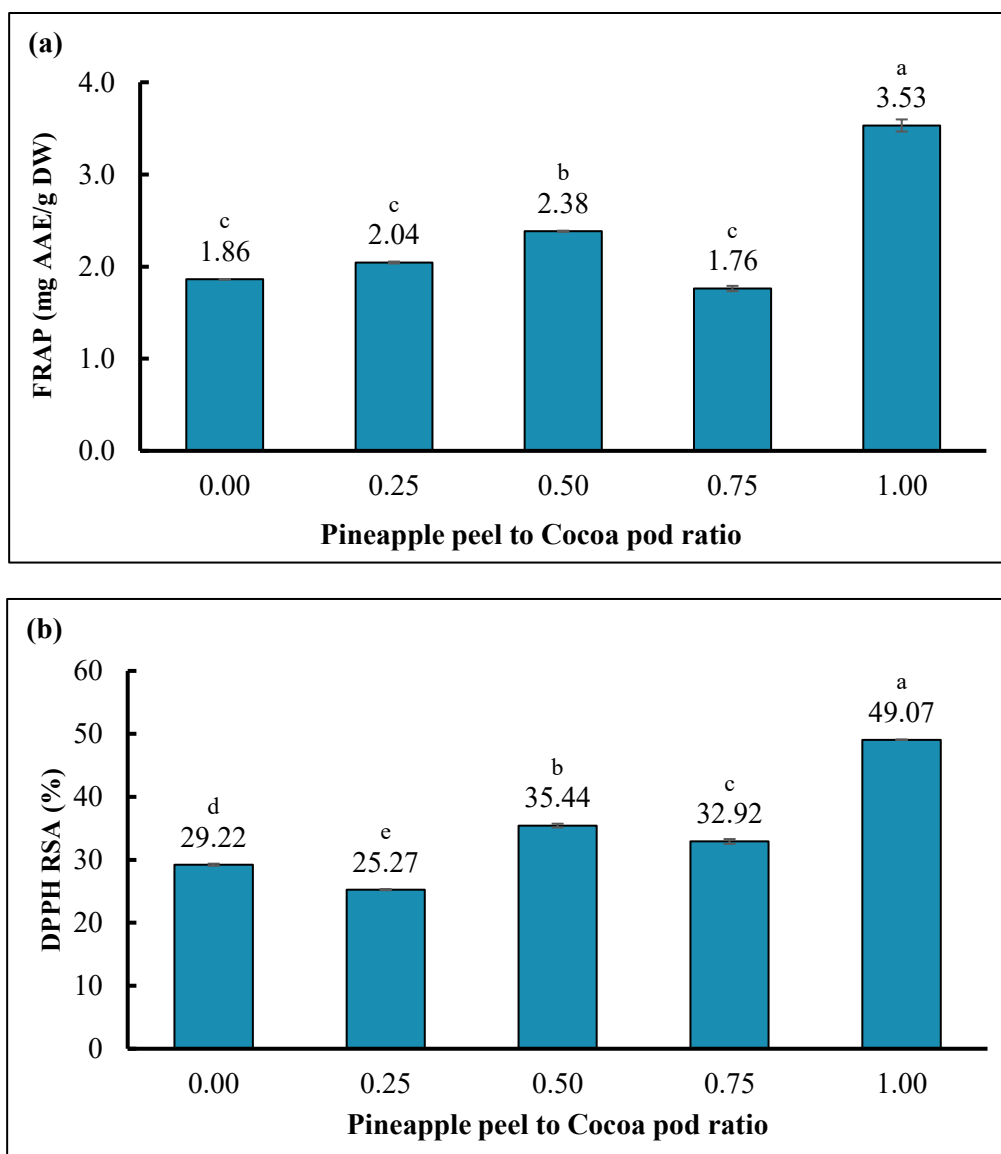


Figure 4. Effect of different pineapple peel to cocoa pod ratios on (a) Ferric Reducing Antioxidant Power (FRAP) and (b) DPPH Radical Scavenging Activity (DPPH RSA) of the pineapple peel and cocoa pod mixture.

Solid-to-solvent ratio

The effect of solid-to-solvent ratio on TPC, TFC, FRAP, and DPPH RSA is presented in Figures 5 and 6. For TPC as observed in Figure 5(a), values ranged from 30.19 ± 0.40 to 36.22 ± 2.24 mg GAE/g DW, with the highest value peaked at 1:45 g/mL. However, no clear trend was observed across the tested ratios (1:15–1:55 g/mL), and differences were not statistically significant ($p > 0.05$), suggesting limited influence of solvent volume beyond an optimum level (Khoddami *et al.*, 2013). Conversely, TFC as presented in Figure 5(b) showed greater variation, with the highest values at 1:25 and 1:55 g/mL (28.03 ± 0.35 and 28.34 ± 0.59 mg QE/g DW), while the lowest was recorded at 1:35 g/mL (5.92 ± 0.49 mg QE/g DW). This indicates that higher solvent volumes may enhance flavonoid extraction due to improved solubility of polar compounds (Tan *et al.*, 2011).

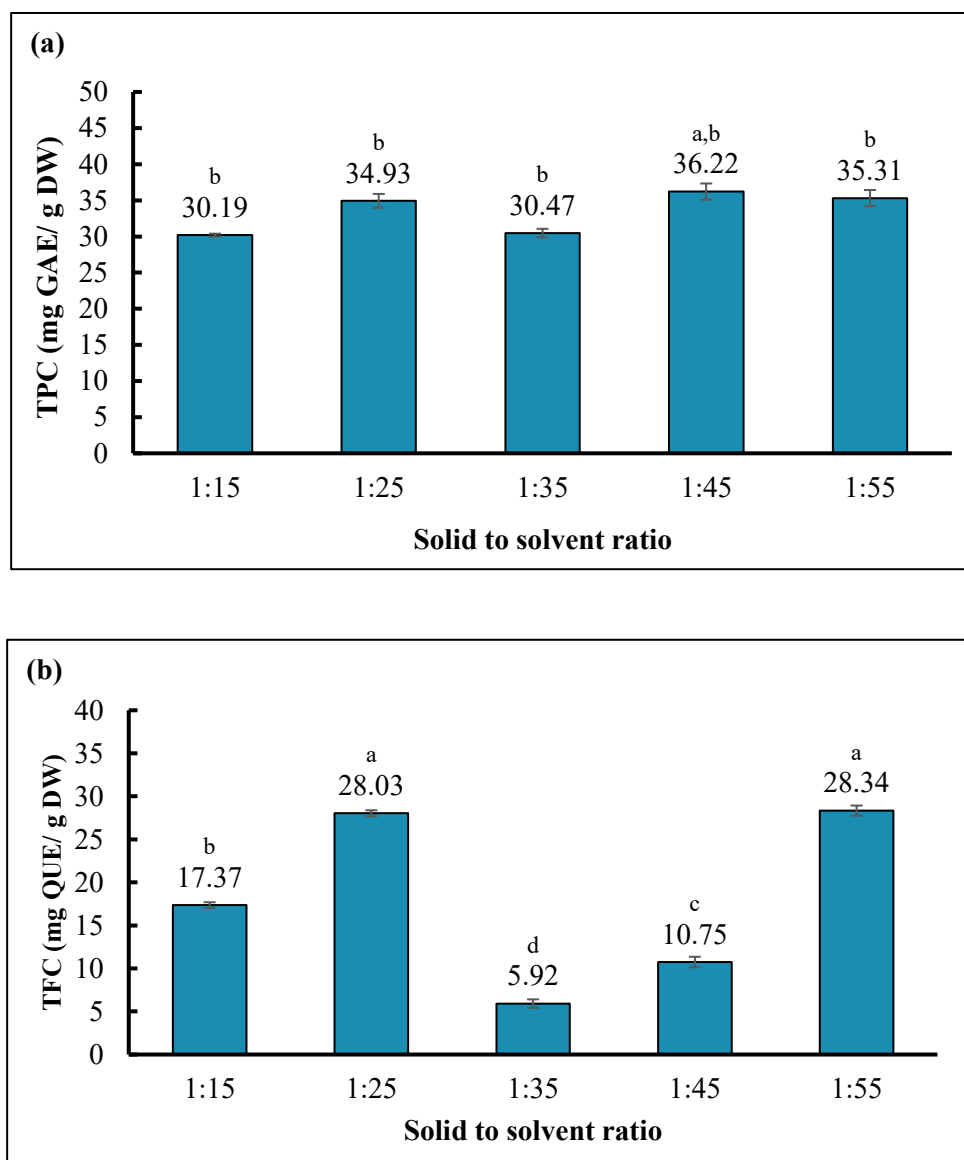


Figure 5. Effect of different solid to solvent ratios on (a) Total Phenolic Content (TPC) and (b) Total Flavonoid Content (TFC) of pineapple peel and cocoa pod mixture.

The effect of solid-to-solvent ratio on FRAP and DPPH RSA is shown in Figure 6. Results of FRAP as presented in Figure 6(a) demonstrate increasing value from 1:15 to 1:35, reaching 1.76 ± 0.03 mg AAE/g DW, decreased slightly at 1:45 (1.58 ± 0.01 mg AAE/g DW), and peaked at 1:55 (2.22 ± 0.001 mg AAE/g DW). A similar fluctuating trend was observed for DPPH RSA (Figure 6(b)), which increased from $18.42 \pm 0.72\%$ (1:25) to $26.35 \pm 0.99\%$ (1:35), dropped at 1:45 ($17.85 \pm 0.41\%$), and reached a maximum at 1:55 ($28.25 \pm 0.49\%$). Statistical analysis confirmed that 1:35 and 1:55 ratios were significantly higher than other treatments ($p < 0.05$). These results suggest that increasing solvent volume enhances mass transfer by improving the concentration gradient between solid and liquid phases, thereby promoting polyphenol diffusion (Wong *et al.*, 2013). However, beyond an optimal range, extraction efficiency may be influenced by dilution effects. Overall, optimal extraction performance was observed within the 1:25–1:55 g/mL range, consistent with previous findings reporting improved flavonoid recovery at similar solid-to-solvent ratios (Wang *et al.*, 2020).

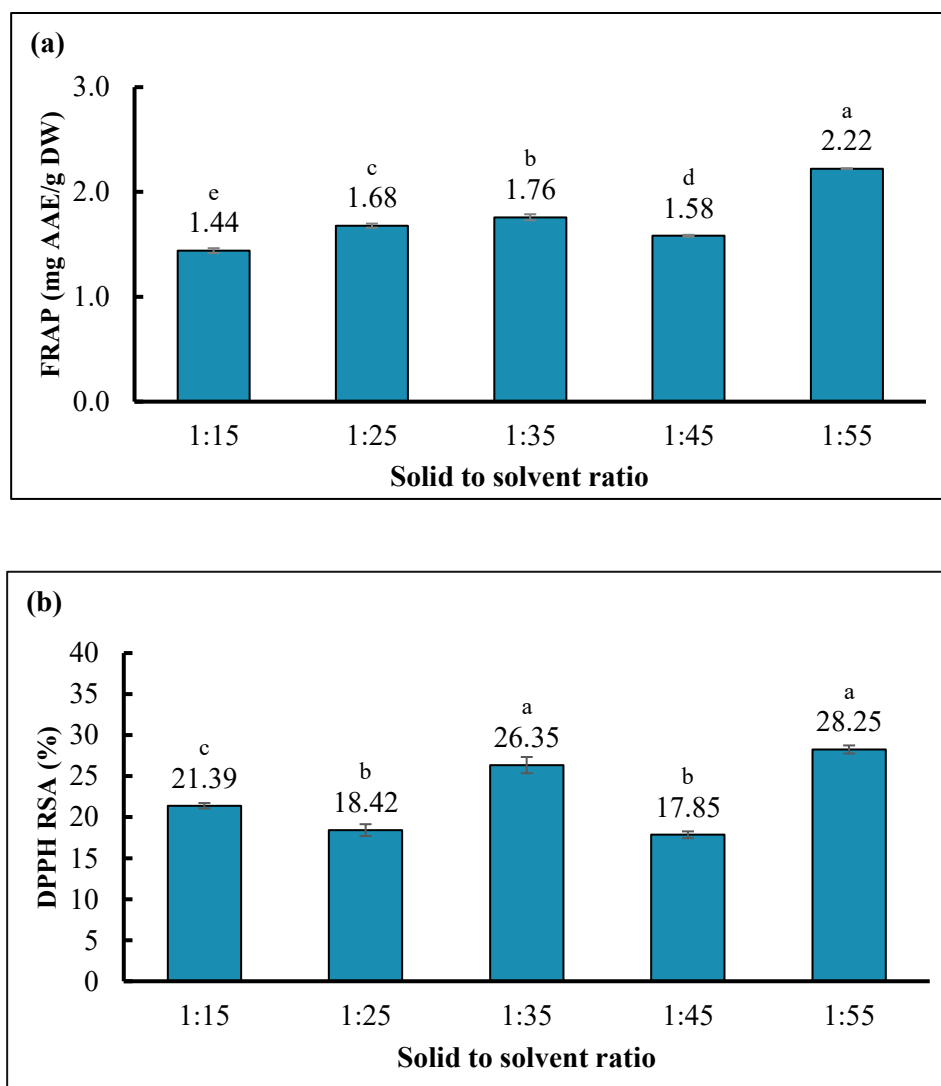


Figure 6. Effect of different solid to solvent ratio on (a) Ferric Reducing Antioxidant Power (FRAP) and (b) DPPH Radical Scavenging Activity (DPPH RSA) of pineapple peel and cocoa pod mixture.

Optimisation of Ultrasonic Assisted Extraction Parameters using RSM

This study optimises ultrasonic-assisted extraction parameters using response surface methodology (RSM) by evaluating the effects of extraction time, temperature, and pineapple peel-to-cocoa pod ratio on polyphenol yield and antioxidant activity, with optimal conditions determined from statistical modelling.

Experimental Results for BBD and Polynomial Models

The Box–Behnken design evaluated three variables, which are extraction time (20–60 min), temperature (30–70 °C), and pineapple peel-to-cocoa pod ratio (10–90% w/w) for optimising polyphenol yield and antioxidant activity (Table 3). The responses showed considerable variation, with TPC ranging from 15.16 ± 0.20 to 42.23 ± 0.30 mg GAE/g DW, TFC from 6.70 ± 0.16 to 16.80 ± 0.20 mg QE/g DW, FRAP from 1.85 ± 0.07 to 4.08 ± 0.10 mg AAE/g DW, and DPPH RSA from $24.57 \pm 0.38\%$ to $75.89 \pm 0.19\%$. These experimental data were used to develop multiple regression models, and the final reduced polynomial equations retained only significant terms ($p < 0.05$) for each response.

Table 3. Box-behnken design and the responses of dependent variables to the extraction conditions.

Run	Time (min)	Temperature (°C)	PP:CP ratio (w/w %)	TPC (mg GAE/g DW)	TFC (mg QUE/ g DW)	FRAP (mg AAE/ g DW)	DPPH RSA (%)
1	40.00	50.00	50.00	15.37 ± 0.16	8.84 ± 0.17	2.11 ± 0.11	38.23 ± 0.05
2	40.00	50.00	50.00	18.78 ± 0.20	7.78 ± 0.28	1.85 ± 0.07	25.28 ± 0.56
3	40.00	50.00	50.00	23.00 ± 0.20	8.07 ± 0.33	1.97 ± 0.09	32.18 ± 0.14
4	40.00	70.00	90.00	19.65 ± 0.16	14.27 ± 0.62	3.36 ± 0.09	62.54 ± 0.44
5	40.00	50.00	50.00	17.42 ± 0.11	9.04 ± 0.16	2.20 ± 0.01	32.80 ± 0.26
6	20.00	30.00	50.00	17.83 ± 0.22	10.41 ± 0.41	2.43 ± 0.07	35.64 ± 0.77
7	60.00	50.00	90.00	22.07 ± 0.33	11.47 ± 0.14	3.02 ± 0.06	75.89 ± 0.19
8	20.00	70.00	50.00	42.23 ± 0.30	10.56 ± 0.44	2.24 ± 0.27	37.14 ± 0.21
9	20.00	50.00	90.00	30.26 ± 0.18	16.80 ± 0.20	4.08 ± 0.10	65.00 ± 0.82
10	60.00	50.00	10.00	16.40 ± 0.24	8.83 ± 0.15	2.53 ± 0.28	24.57 ± 0.38
11	40.00	30.00	90.00	24.27 ± 0.12	15.81 ± 0.96	4.06 ± 0.13	63.63 ± 0.53
12	40.00	70.00	10.00	20.83 ± 0.11	9.22 ± 0.21	2.71 ± 0.04	31.82 ± 0.37
13	60.00	30.00	50.00	16.53 ± 0.10	10.60 ± 0.34	2.42 ± 0.09	43.62 ± 0.96
14	60.00	70.00	50.00	15.94 ± 0.47	9.34 ± 0.14	2.54 ± 0.04	38.74 ± 0.45
15	40.00	30.00	10.00	15.16 ± 0.20	6.70 ± 0.16	2.42 ± 0.07	24.78 ± 0.28
16	40.00	50.00	50.00	17.28 ± 0.09	10.91 ± 0.22	2.61 ± 0.10	42.56 ± 0.79
17	20.00	50.00	10.00	19.64 ± 0.27	8.82 ± 0.11	2.93 ± 0.07	30.72 ± 0.22

Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH Radical Scavenging Activity (DPPH RSA), Ferric Reducing Antioxidant Power (FRAP) presented as mean ± standard deviation of triplicate experiment

Equations (4.1)-(4.4) present the final equation of actual factors, specifically the 2FI model for TPC and quadratic models for TFC, FRAP, and DPPH RSA, expressed in terms of their absolute values (t, T, R). ANOVA analysis in Table 4 indicates that the equation (4.1) for TPC exhibited an F-value of 4.43 and a p-value of less than 0.05. For TFC, Equation (4.2) indicated an F-value of 8.53 and a p-value of less than 0.05. Equation (4.3) for FRAP demonstrated an F-value of 5.85 with a p-value of less than 0.05, whereas Equation (4.4) for DPPH RSA displayed an F-value of 13.32 with a p-value of less than 0.001.

Equation (4.1)-(4.4) shows the final equation of actual factors for TPC, TFC, FRAP, and DPPH RSA in terms of their absolute values (t, T, R);

$$\text{TPC} = -\mathbf{23.43} + \mathbf{0.61*t} + 0.94*T + 0.30*R - 0.02*tT - 1.55 \times 10^{-3} *tR - 3.22 \times 10^{-3} *TR \quad (4.1)$$

$$\text{TFC} = \mathbf{8.09} - 0.04*t - 0.07*T + \mathbf{0.09*R} - 8.81 \times 10^{-4} *tT - 1.67 \times 10^{-3} *tR - 1.27 \times 10^{-3} *TR + 1.60 \times 10^{-3} *t^2 + 1.65 \times 10^{-3} T^2 + 1.20 \times 10^{-3} R^2 \quad (4.2)$$

$$\text{FRAP} = \mathbf{3.84} - 0.03*t - 0.03*T - \mathbf{0.02*R} + 1.98 \times 10^{-4} *tT - 2.07 \times 10^{-4} *tR - 3.10 \times 10^{-4} *TR + 3.25 \times 10^{-4} *t^2 + 3.12 \times 10^{-4} T^2 + 5.38 \times 10^{-4} R^2 \quad (4.3)$$

$$\text{DPPH RSA} = \mathbf{38.58} - 0.77*t + 0.15*T - \mathbf{0.28*R} - 3.99 \times 10^{-3} *tT + 5.33 \times 10^{-3} tR - 2.54 \times 10^{-3} *TR + 9.91 \times 10^{-3} *t^2 + 1.53 \times 10^{-3} T^2 + \mathbf{6.79 \times 10^{-3} R^2} \quad (4.4)$$

*Bold values show significant at least $p < 0.05$ and $p < 0.001$.

Table 4. Analysis of variance (ANOVA) and fit statistics of TPC, TFC, DPPH radical scavenging activity and FRAP models of pineapple peel and cocoa pod extract

Model	Responses			
	TPC	TFC	FRAP	DPPH RSA
	<0.05	<0.05	<0.05	<0.001
Time (t)	<0.05	ns	ns	ns
Temperature (T)	ns	ns	ns	ns
Pineapple peel to Cocoa pod ratio (R)	ns	<0.001	<0.05	<0.0001
Tt	<0.05	ns	ns	ns
tR	ns	ns	ns	ns
TR	ns	ns	ns	ns
t ²	-	ns	ns	ns
T ²	-	ns	ns	ns
R ²	-	<0.05	<0.05	<0.05
Order	2FI	Quadratic	Quadratic	Quadratic
Order Significance	ns	<0.05	<0.05	<0.05
Residual Lack of Fit	ns	ns	ns	ns
Model Fit Statistics				
Std. Dev	4.47	1.22	0.33	5.58
Mean	20.75	10.44	2.68	41.48
C. V. %	21.53	11.68	12.50	13.44
R ²	0.7264	0.9164	0.8827	0.9448
Adj R ²	0.5623	0.8090	0.7319	0.8739

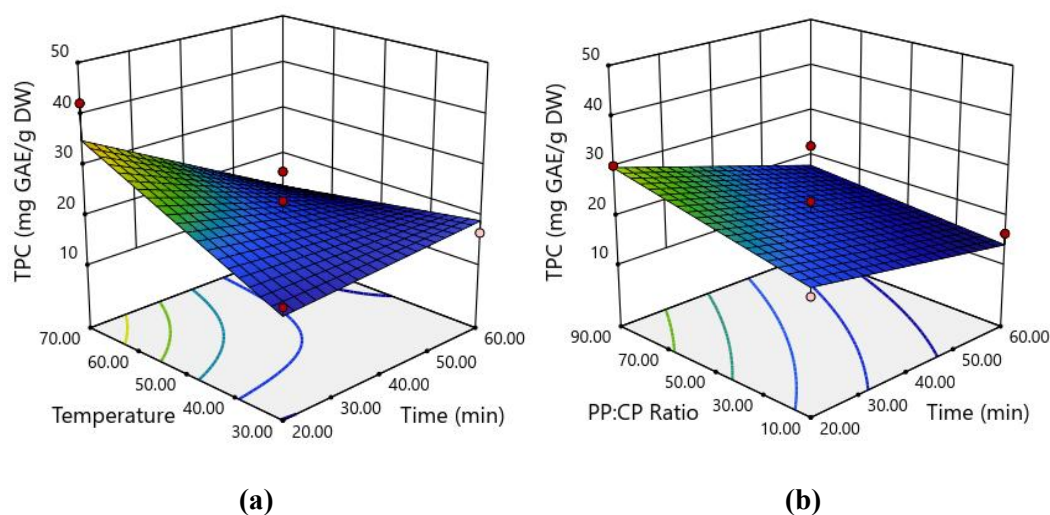
Significant terms ($p \leq 0.05$) nonsignificant terms coded as ns. The final model equation is simplified into only significant terms.

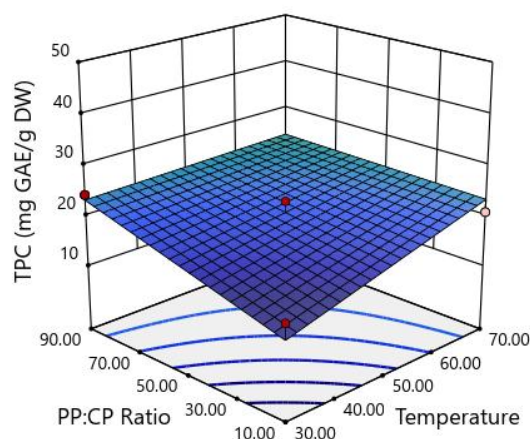
Response Surface Plot of TPC, TFC, FRAP and DPPH RSA influenced by the independent variables

The regression equation produced appears as three-dimensional (3D) response surface plots to illustrate the relationship between any two variables relating a chosen response (Majid & Silva, 2020). The Design Expert software produced 3D response surface plots derived from the RSM polynomial models in Equations (4.1)-(4.4). All response surface plots in Figure 7 through Figure 10 exhibit one response (z-axis) against two factors, while the third factor is maintained at its '0' level, representing the midpoint value of the examined range.

Total Phenolic Content (TPC)

Figure 7 illustrates the plots with the effects of extraction time, temperature, and pineapple peel to cocoa pod (PP:CP) ratio on total phenolic content (TPC). The 2FI model indicated that temperature, extraction time, and PP:CP ratio significantly affected TPC ($p < 0.05$), with significant interaction between temperature and time. At a constant PP:CP ratio, TPC increased with temperature up to 70 °C, particularly after 20 min, with highest TPC observed on Run 8 (42.23 ± 0.30 mg GAE/g DW). Higher temperatures improve phenolic solubility and solvent penetration into the plant matrix (Abdul Karim *et al.*, 2014). However, prolonged extraction at elevated temperatures caused a decline in TPC, suggesting thermal degradation of sensitive antioxidant compounds during UAE (Chemat *et al.*, 2017). Figure 7(b) shows that the PP:CP ratio strongly influenced TPC, with higher cocoa pod proportions producing greater phenolic yields. For example, Run 9 (90% CP, 20 min) yielded 30.26 ± 0.18 mg GAE/g DW, compared with 19.64 ± 0.27 mg GAE/g DW for Run 17 (10% CP, 20 min). The higher cocoa pod content requires optimised extraction time to facilitate the release of bound phenolics from its lignocellulosic structure (Díaz-González *et al.*, 2022). Meanwhile, figure 7(c) shows that at lower temperatures (30–40 °C), changes in the PP:CP ratio had little effect on TPC, as reflected by the relatively flat response surface. TPC remained low (15.16 ± 0.20 to 16.53 ± 0.10 mg GAE/g DW) despite increasing cocoa pod content, likely due to the rigid cell wall structure of cocoa pod husk, which limits phenolic release under mild extraction conditions (Yusof *et al.*, 2019).



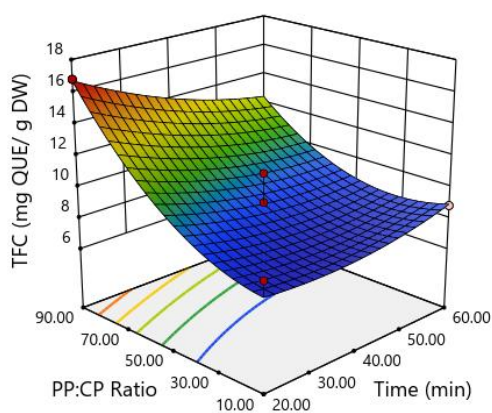


(c)

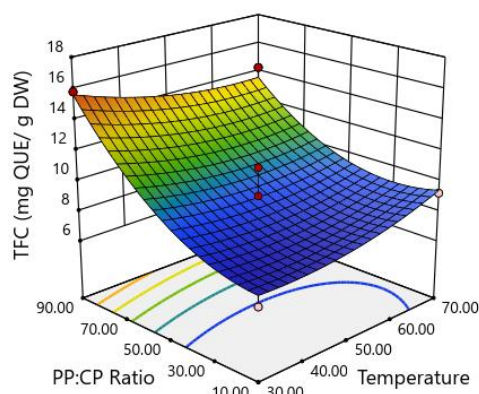
Figure 7. Response surface plots for the effects of extraction time (min), temperature ($^{\circ}\text{C}$), and pineapple peel to cocoa pod ratio on TPC (mg GAE/g DW) (Variables were set the midpoint (40 min, 50°C , and 0.50 ratio).

Total Flavonoid content (TFC)

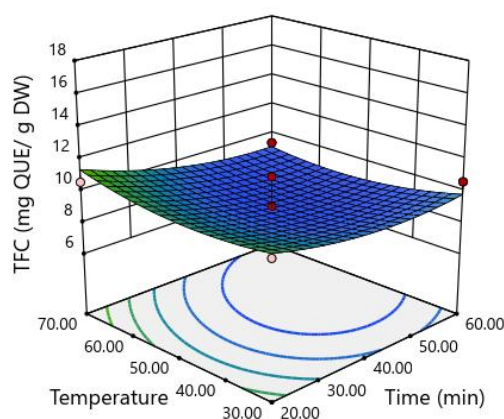
The total flavonoid content (TFC) was best described by a significant quadratic model ($p < 0.05$). ANOVA showed that the pineapple peel to cocoa pod (PP:CP) ratio was the only linear factor that significantly influenced TFC ($p < 0.001$). As shown in Figure 4.8(a) and (b), TFC increased markedly with increasing cocoa pod content, reaching the highest experimental value of 16.80 ± 0.20 mg QUE/g DW in Run 9 (90% CP, 20 min). Furthermore, figure 4.8(b) shows that higher temperatures combined with a high cocoa pod ratio enhanced flavonoid extraction. At a constant extraction time of 40 min, elevated temperatures facilitated flavonoid release by softening the lignin-rich cell walls of cocoa pod, thereby improving the solubility of compounds such as catechins (Abdul Karim *et al.*, 2014). The curved response surface indicates the presence of quadratic effects. In contrast, extraction time and temperature, as well as their interaction terms (T_t , t_R , and T_R), were not significant for TFC. This is reflected in Figure 4.8(c), where the relatively flat surface and circular contour lines indicate weak interactions between these factors. Therefore, maximising TFC mainly depends on maintaining a high cocoa pod ratio, while moderate extraction time and temperature are sufficient to achieve high yields.



(a)



(b)

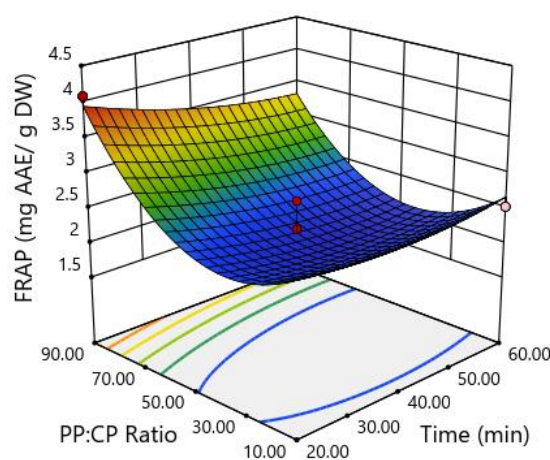


(c)

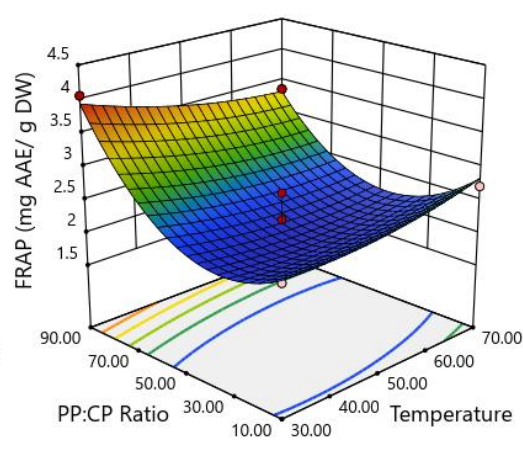
Figure 8. Response surface plots for the effects of extraction time (min), temperature (°C), and pineapple peel to cocoa pod ratio on TFC (mg QUE/g DW) (Variables were set the midpoint (40 min, 50 °C, and 0.50 ratio))

Ferric Reducing Antioxidant Power (FRAP)

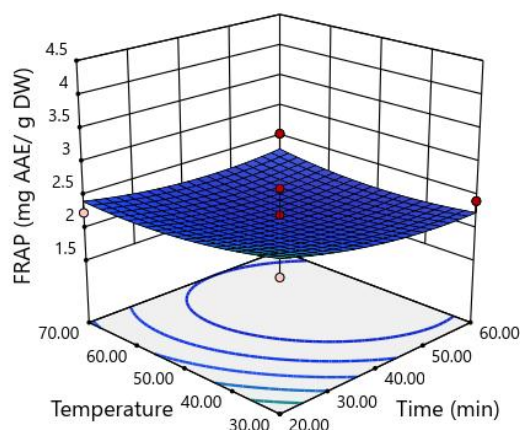
The FRAP response was optimally represented by a significant quadratic model ($p < 0.05$), yielding an R^2 of 0.8827 and an adjusted R^2 of 0.7319. ANOVA revealed that the pineapple peel to cocoa pod (PP:CP) ratio (R) and its quadratic term (R^2) significantly affected FRAP ($p < 0.05$), resulting in the distinctive curvature noted in the response surfaces. Figure 4.9(a) illustrates that FRAP rises with increasing cocoa pod concentration, with Run 9 (90% CP) yielding the maximum value of 4.08 ± 0.10 mg AAE/g DW. While extraction duration was not significant, the surface indicates that shortened extraction periods are sufficient, as extended sonication may degrade reducing agents. Figure 4.9(b) demonstrates that FRAP increased with both cocoa pod ratio and temperature. For example, Run 4 (70°C, 90% CP) produced 3.36 ± 0.09 mg AAE/g DW. Elevated temperatures enhance mass transfer by reducing solvent viscosity and increasing kinetic energy, promoting the release of tannins and lignin-bound phenolics from cocoa pod husks (Bratovic *et al.*, 2021). Cocoa-rich mixtures exhibit superior FRAP values due to their large concentration of phenolic hydroxyl groups, which effectively donate electrons to reduce Fe^{3+} to Fe^{2+} . On the other hand, the relatively flat surface in Figure 4.9(c) indicates that extraction time, temperature, and their interaction (Tt) were not particularly significant.



(a)



(b)

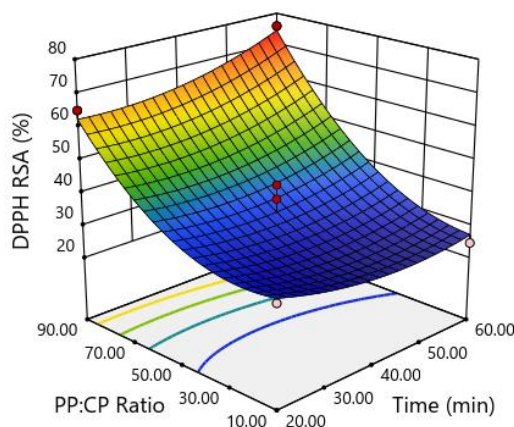


(c)

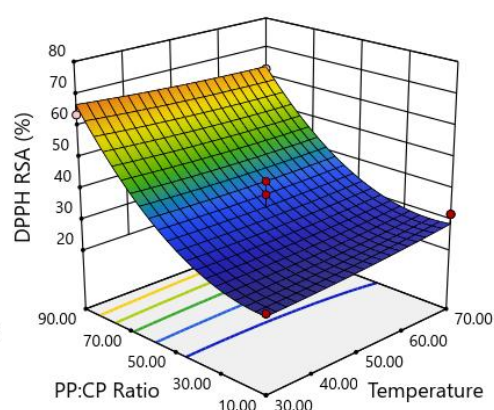
Figure 9. Response surface plots for the effects of extraction time (min), temperature (°C), and pineapple peel to cocoa pod ratio on FRAP (mg AAE/g DW) (Variables were set the midpoint (40 min, 50 °C, and 0.50 ratio))

DPPH Radical Scavenging Activity (DPPH RSA)

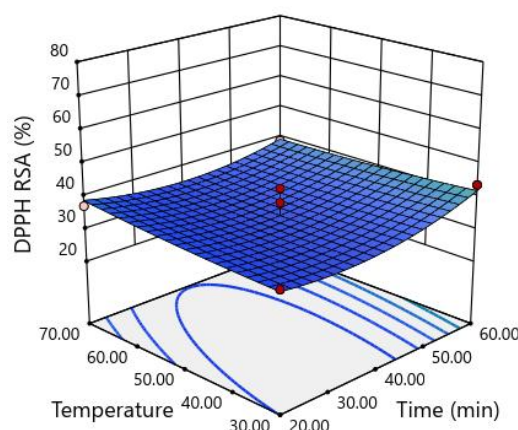
The DPPH radical scavenging activity (RSA) followed a highly significant quadratic model ($p < 0.001$). By referring to Table 4, the pineapple peel to cocoa pod (PP:CP) ratio is identified as the most significant factor ($p < 0.0001$). Figures 4.10(a) and (b) show that RSA increased with cocoa pod content up to 90%, indicating that antioxidant activity is mainly governed by the PP:CP ratio. The highest RSA ($75.89 \pm 0.19\%$) was obtained in Run 7 (90% CP), attributed to the high content of hydrogen-donating polyphenols such as catechins and epicatechins. Although extraction time was not significant ($p > 0.05$), $65.00 \pm 0.82\%$ RSA was still achieved after 20 min (Run 9), suggesting efficient ultrasound-assisted release of antioxidants at high cocoa ratios. Figure 4.10(b) shows that PP:CP–temperature interaction with a curved response due to the significant quadratic term (R^2 , $p < 0.05$). At 70 °C and 90% cocoa pod (Run 4), RSA remained high ($62.54 \pm 0.44\%$), as higher temperature enhances solvent penetration and cell wall disruption, improving antioxidant release (Bugarin *et al.*, 2025). Conversely, Figure 4.10(c) shows a flat surface, indicating a non-significant temperature–time interaction (Tt). Similar RSA values were observed when increasing temperature from 30 °C to 70 °C at a 50:50 ratio. This confirms that RSA is primarily controlled by the PP:CP ratio, while time and temperature have minimal influence within the studied range, enabling a more flexible and energy-efficient process.



(a)



(b)



(c)

Figure 10. Response surface plots for the effects of extraction time (min), temperature (°C), and pineapple peel to cocoa pod ratio on DPPH RSA (%) (Variables were set the midpoint (40 min, 50 °C, and 0.50 ratio))

Experimental Validation of Optimal Conditions Estimated by the RSM Models

The optimum ultrasound-assisted extraction conditions predicted by RSM were a 20 min extraction time, 70 °C, and a pineapple peel to cocoa pod ratio of 90%. These conditions were validated experimentally, and the results for TPC, TFC, FRAP, and DPPH RSA were compared with the values predicted by the polynomial models as presented in Table 5. The relative errors between experimental and predicted values were low for TPC (3.36%), TFC (3.21%), and FRAP (0.16%), while DPPH RSA showed a slightly higher error of 7.92%. Nevertheless, all responses exhibited errors below 8%, indicating that the developed RSM models can reliably predict polyphenol content and antioxidant activity, thereby reducing experimental time and cost. The optimised extract also exhibited stronger antioxidant activity than ascorbic acid ($IC_{50} = 23.38 \mu\text{g/mL}$), with a lower IC_{50} value of $1.10 \mu\text{g/mL}$. This demonstrates the potent antioxidant potential of the pineapple peel–cocoa pod extract.

Table 5. Optimum ultrasound extraction conditions from pineapple peel and cocoa pod mixture for maximum TPC, TFC, FRAP and DPPH RSA.

Optimum conditions for ultrasound extraction	Responses	Maximum values		
		Model prediction	Experimental result ^a	Absolute error(%)
Extraction time = 20 min Temperature = 70 °C Pineapple peel to cocoa pod ratio = 90%	TPC (mg GAE/g)	36.67	40.03 ± 2.06	3.36
	TFC (mg QUE/g)	16.69	19.90 ± 1.03	3.21
	FRAP (mg AAE/g)	3.68	3.84 ± 0.21	0.16
	DPPH RSA (%)	62.88	54.96 ± 4.30	7.92
	DPPH IC_{50} ($\mu\text{g/mL}$)	NA	1.10	NA
	DPPH IC_{50} ascorbic acid standard ($\mu\text{g/mL}$)	NA	23.38	NA

^a Mean ± standard deviation of triplicate experiments for the same processing conditions.

Fourier transform infrared spectroscopy (FTIR) determination

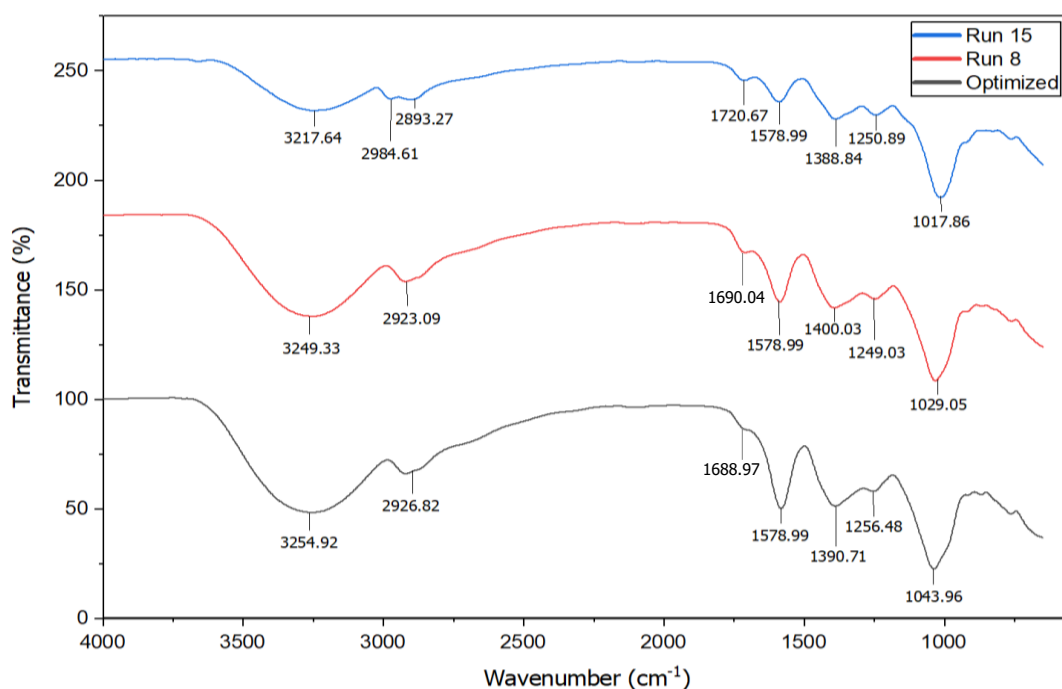


Figure 11. FT-IR spectrum of pineapple peel and cocoa pod mixture.

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups of bioactive compounds in Run 15 (lowest antioxidant activity), Run 8 (highest antioxidant activity), and the optimised extract (Figure 11). FTIR complemented TPC and TFC analyses by providing qualitative information on phenolic and flavonoid structures. All samples exhibited O–H stretching band from 3200 to 3600 cm^{-1} , resembling that of phenolics and flavonoids, including catechins and epicatechins (Batista *et al.*, 2016; Nucara *et al.*, 2013). Bands at 2920–2990 cm^{-1} corresponded to aliphatic C–H stretching, while a C=O band at 1720.67 cm^{-1} was observed only in Run 15. A strong band near 1579 cm^{-1} indicated aromatic C=C stretching, confirming the presence of polyphenols and possibly β -carotene (Leccese *et al.*, 2023). Peaks around 1250 cm^{-1} and 1000–1100 cm^{-1} were characterised to C–O stretch, indicating the presence of phenolic and carbohydrate-derived compounds (Lucarini *et al.*, 2019). Therefore, the results of FTIR spectra confirmed the presence of hydroxyl, aromatic, carbonyl, and ether groups characteristic of phenolics, flavonoids, tannins, and carbohydrates, which contribute to the antioxidant activity of the pineapple peel–cocoa pod extracts (Alean *et al.*, 2022).

Table 6. Summary of FTIR peak values for pineapple peel and cocoa pod mixture extracts.

Functional group	Wavelength (cm^{-1})
Run 15	
O-H stretching	3271.64
C-H stretching	2984.61 - 2893.27
C=O stretch (carbonyl)	1720.67
C=C stretch (aromatic)	1578.99
Run 8	
O-H stretching	3249.33
C-H stretching	2923.09
C=C stretching (aromatic)	1578.99
C-H or C-O bending	1029.05-866.69
Optimized	
O-H stretching	3254.92

(cont.). Summary of FTIR peak values for pineapple peel and cocoa pod mixture extracts.

C-H stretching	2926.82
C=C stretching (aromatic)	1578.99
C-H bending	1300.71
C-O bending	1043.96

CONCLUSION

A three-factor Box–Behnken design was employed to establish suitable ultrasound-assisted extraction conditions for recovering antioxidant compounds from pineapple peel (PP) and cocoa pod (CP) mixtures. The developed models showed good agreement with the experimental data, with R^2 values of 0.9164 for TFC, 0.8827 for FRAP, and 0.9448 for DPPH RSA. The optimal conditions were identified as 20 min of extraction at 70 °C using a mixture containing 90% CP. Validation under these conditions yielded a TPC of 40.03 ± 2.06 mg GAE/g, TFC of 19.90 ± 1.03 mg QUE/g, FRAP of 3.84 ± 0.21 mg AAE/g, and DPPH RSA of $54.96 \pm 4.30\%$, with only minor differences between predicted and experimental values. The optimised extract recorded an IC_{50} of 1.10 $\mu\text{g/mL}$, compared with 23.38 $\mu\text{g/mL}$ for ascorbic acid, demonstrating substantial radical-scavenging capacity. FTIR spectra further revealed O–H, aromatic, and C–O functionalities consistent with phenolic and flavonoid constituents. Overall, the optimised process demonstrates the potential of combining PP and CP as a source of antioxidant-rich extracts while providing a value-added route for the utilisation of these agro-industrial residues.

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