

Article

Optimization and Characterization of Pectin Extraction from Cocoa Pod Husks by Ultrasound-Assisted Enzymatic Method

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Abstract

Cocoa pod husk is the main byproduct of cocoa processing and is rich in pectin, holding great potential for high-value utilization. Traditional acid extraction causes severe environmental pollution, while the single enzymatic method is time-consuming, necessitating the development of a green and efficient extraction technology. In this study, an ultrasound-assisted enzymatic extraction method was employed, and the extraction process was systematically optimized via Plackett–Burman design, the steepest ascent method, and Box–Behnken response surface methodology. Under the optimized conditions, the pectin yield, galacturonic acid content, and degrees of esterification were $14.34 \pm 0.39\%$, $67.93 \pm 1.13\%$, and $43.62 \pm 0.6\%$, respectively, indicating low-methoxyl pectin characteristics. Compared with literature data, the present method showed favorable performance in terms of yield, purity, extraction time, and enzyme consumption relative to conventional acid and single enzymatic extraction methods. This approach effectively couples ultrasonic cell wall disruption with specific enzymatic hydrolysis, thereby enabling efficient preparation of high-quality pectin under mild, low-energy conditions. It provides a green and highly efficient alternative for the resource utilization of cocoa pod husk waste.

Keywords: cocoa pod husk; pectin; ultrasound-assisted; enzymatic hydrolysis; response surface

1. Introduction

The cocoa tree is an important tropical cash crop, with global annual cocoa pod production reaching approximately 4.735 million tons [1]. Cocoa pod husk accounts for about 70% of the fruit by weight and represents the major byproduct generated during processing, amounting to millions of tons annually [2]. Currently, only a small fraction is used as animal feed, while most is discarded or incinerated, resulting in resource waste and environmental problems such as soil degradation and greenhouse gas emissions [3–5]. Notably, cocoa pod husk contains 6–13% pectin by dry weight [6] and thus holds significant potential for high-value utilization.

Pectin is a complex polysaccharide primarily composed of D-galacturonic acid residues as the main chain. Owing to its unique gelling properties and health benefits such as appetite regulation and the prevention of coronary heart disease, it is widely applied in the food and pharmaceutical industries [7–9]. Based on the degree of esterification, pectin can be classified into high-methoxyl and low-methoxyl types; the latter can form gels in the



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presence of calcium ions [10]. Cocoa pod husk pectin belongs to the low-methoxyl category. However, it is tightly associated with cell wall components such as cellulose, which renders conventional single extraction methods inefficient in terms of pectin release, often leading to low yield and purity. Therefore, the development of novel extraction techniques capable of effectively disrupting the cell wall barrier is necessitated.

Traditional pectin extraction mainly relies on acid hydrolysis. Although this process is well-established, it suffers from disadvantages such as pectin degradation under high temperature and strong acid, as well as severe environmental pollution [11]. Enzymatic extraction offers mild conditions and high product quality, but when used alone, it often requires long extraction times and yields limited pectin recovery [12–14]. Ultrasound-assisted extraction utilizes cavitation to disrupt cell walls and enhance mass transfer, featuring high efficiency and low energy consumption. However, due to the dense cell wall structure of cocoa pod husk, where pectin is tightly crosslinked and associated with cellulose, hemicellulose, and lignin, the extraction efficiency of ultrasound alone remains limited [15–17]. Ultrasound-assisted enzymatic extraction combines the dual effects of physical cell wall disruption and specific enzymatic hydrolysis, effectively overcoming the limitations of single methods. This technique has been successfully applied to raw materials such as orange peel and sisal waste, achieving significantly higher pectin yields than conventional acid extraction or single enzymatic extraction, while being more environmentally friendly [18,19].

Although ultrasonic-enzymatic extraction has exhibited favorable performance on other agro-byproducts such as orange peel and sisal waste, research gaps exist for cocoa pod husk: few studies systematically optimize this coupled route, and published works only evaluate acid, single-enzyme, or ultrasonic-only extraction separately. The synergistic effects of core interactive parameters remain unclear, and a complete optimized process system for this combined method has not yet been established. In this study, this method was employed to extract pectin from cocoa pod husk. Key parameters including liquid-to-solid ratio, ultrasound time, and enzymatic hydrolysis time were systematically optimized using response surface methodology. Under the optimal conditions, the performance of the proposed method was compared with conventional acid extraction and pure enzymatic extraction in terms of pectin yield, galacturonic acid content, and degree of esterification. The results showed that the proposed method provides a green and efficient alternative for the high-value utilization of cocoa pod husk.

2. Materials and Methods

2.1. Materials and Instruments

Cocoa pod husks were provided by Hainan Xinglong Tropical Botanical Garden, Wanning, China. Ethanol (analytical grade) was obtained from Shanghai YuanyeBio-Technology Co., Ltd., Shanghai, China. An ultrasonic cleaner (KQ-300DE, maximum ultrasonic power 300 W) was purchased from Kunshan Shumei Ultrasonic Equipment Co., Ltd., Kunshan, China. Celluclast® 1.5 L was acquired from Novozymes, Copenhagen, Denmark.

2.2. Pectin Extraction Protocol

Cocoa pod husks were washed, dried at 50 °C, ground, and sieved through a 60-mesh sieve to prepare homogeneous powder; 10 g of the powder was mixed with 200 mL distilled water at a liquid-to-solid ratio of 20:1 (mL/g), and the pH of the suspension was adjusted to 5.0. Ultrasonic pretreatment was carried out using a water-bath ultrasonic cleaner (KQ-300DE, Kunshan Shumei Ultrasonic Equipment Co., Ltd., Kunshan, China) with a fixed frequency of 40 kHz, a maximum rated power of 300 W, and a power density of 50 W/L, where the extraction suspension loaded in a 250 mL conical flask was fully

immersed in the ultrasonic bath, and the integrated heating and temperature control system maintained the reaction temperature within ± 1 °C throughout the whole ultrasonic process; the default ultrasonic parameters were 300 W, 50 °C, and 30 min unless otherwise noted. Two treatment procedures were adopted: Process 1 referred to ultrasound pretreatment prior to enzymatic hydrolysis, in which Celluclast[®] 1.5 L was supplemented at a dosage of 40 μ L/g substrate after sonication, followed by enzymatic reaction at 50 °C and 200 rpm shaking for 1 h, while Process 2 swapped the sequence of ultrasonic treatment and enzymolysis. After extraction, the mixture was centrifuged at 8000 rpm for 10 min to collect the supernatant, which was mixed with twice the volume of anhydrous ethanol and stood for 24 h to precipitate crude pectin; the precipitate obtained by secondary centrifugation was washed twice with anhydrous ethanol and freeze-dried to constant weight to obtain final pectin samples, and the pectin yield was calculated according to the following formula:

$$\text{Yield} = \text{Mass of crude pectin (g)} / \text{Mass of dry raw material (g)} \times 100\%$$

The complete operational steps described above are intuitively displayed in Figure 1.

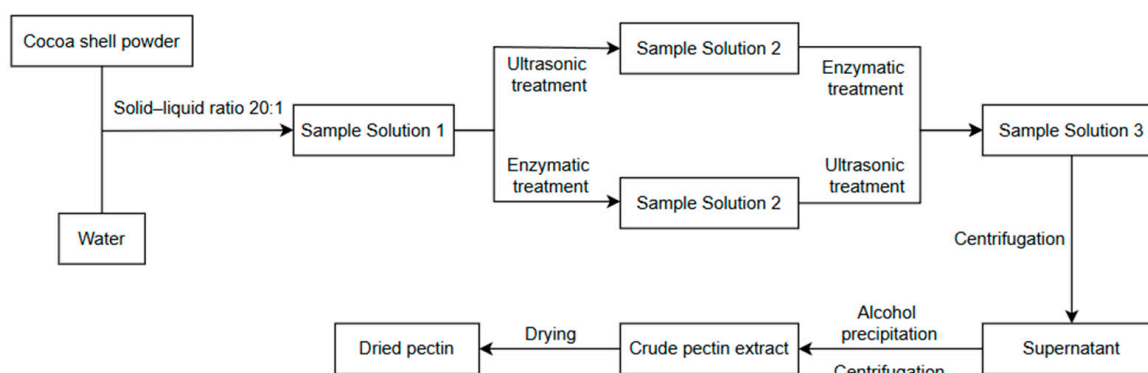


Figure 1. Schematic flowchart of the extraction process.

2.3. Optimization of the Combined Extraction Process

Preliminary experimental results showed that the pectin yield of Process 1 (ultrasound followed by enzymatic hydrolysis) was $9.79 \pm 0.36\%$ (mean $\pm$ SD, $n = 3$), while that of Process 2 (enzymatic hydrolysis followed by ultrasound) was $7.87 \pm 0.28\%$ (mean $\pm$ SD, $n = 3$). Therefore, Process 1 was selected as the optimal treatment sequence for the ultrasound-assisted enzymatic extraction, and all subsequent optimization experiments were conducted using this sequence.

2.3.1. Single-Factor Preliminary Experiments

Prior to the formal statistical optimization, a series of single-factor preliminary experiments were carried out to identify the appropriate ranges of the key parameters and to determine the level settings for the subsequent designs. Eight parameters, namely liquid-to-solid ratio, enzymatic hydrolysis pH, ultrasonic temperature, ultrasonic time, enzyme dosage, enzymatic hydrolysis temperature, enzymatic hydrolysis time, and ultrasonic power, were designated as independent variables (denoted as X_1 to X_8 , respectively). Except for the factor under investigation, the baseline conditions were maintained as follows: liquid-to-solid ratio of 20 mL/g, pH of 5.0, ultrasonic temperature of 50 °C, ultrasonic time of 30 min, enzyme dosage of 40 μ L/g, enzymatic hydrolysis temperature of 50 °C, enzymatic hydrolysis time of 1 h, and ultrasonic power of 300 W. Each factor was examined at 3–5 levels, with the other parameters held constant. Pectin yield was used as the response indicator; all tests were performed in triplicate, and data are presented as mean $\pm$ SD.

2.3.2. Plackett–Burman Design

Based on the results of the single-factor preliminary experiments described in Section 3.1.2, the low (−1) and high (+1) levels for the subsequent Plackett–Burman screening design were assigned for each factor. The selected levels are summarized in Table 1. Ultrasonic power was not included in the PB design and was fixed at 300 W according to the single-factor test results; all tests were performed in triplicate, and data are presented as mean $\pm$ SD. The experimental design is presented in Table 1.

Table 1. Assigned factors and corresponding levels in the Plackett–Burman design.

Factor	Level	
	−1	+1
Liquid-to-solid ratio (mL/g, X_1)	15	25
pH (X_2)	5.0	6.0
Ultrasonic temperature ($^{\circ}$ C, X_3)	55	70
Ultrasonic time (min, X_4)	20	40
Enzyme dosage (μ L/g, X_5)	20	60
Enzymatic hydrolysis temperature ($^{\circ}$ C, X_6)	45	60
Enzymatic hydrolysis time (min, X_7)	90	180

2.3.3. Steepest Ascent Experiment

In this study, pectin yield was used as the response indicator. Significant factors were first screened via Plackett–Burman design, and the steepest ascent experiment was then employed to rapidly narrow the parameter range and locate the optimal process region. The direction of variable changes in the steepest ascent experiment was determined according to the sign of each factor's effect coefficient: factors with positive effects on pectin yield were increased stepwise, while those with negative effects were decreased. The step size for each factor was set proportional to its effect magnitude, and the steepest ascent path was planned accordingly to efficiently approach the optimal parameter region. This intermediate step also provided a rational center point for the subsequent Box–Behnken response surface design; all tests were performed in triplicate, and data are presented as mean $\pm$ SD. The experimental design is presented in Table 2.

Table 2. Levels and factors arrangement for the steepest ascent test.

Run	Liquid-to-Solid Ratio (mL/g, X_1)	Ultrasonic Time (min, X_4)	Enzymatic Hydrolysis Time (min, X_7)
1	15.0:1	20	60
2	17.5:1	25	90
3	20.0:1	30	120
4	22.5:1	35	150
5	25.0:1	40	180
6	27.5:1	45	210

2.3.4. Box–Behnken Optimization Experiment

On the basis of steepest ascent experimental data, a three-factor and three-level response surface model was constructed following Box–Behnken design principles for further systematic optimization. Pectin yield remained the response variable, and previously screened significant factors were optimized in subsequent trials. The experimental factors and their corresponding level ranges are summarized in Table 3. Design–Expert 13.0 software (Stat–Ease Inc., Minneapolis, MN, USA) was used for regression analysis of experimental data. A quadratic polynomial model was established to predict the optimal

extraction conditions, and validation experiments were conducted to verify the reliability of the predicted results.

Table 3. Factors and their corresponding levels in the Box–Behnken design.

Factor	Level		
	−1	0	+1
Liquid-to-solid ratio (mL/g, X_1)	20	22.5	25
Ultrasonic time (min, X_4)	30	35	40
Enzymatic hydrolysis time (min, X_7)	120	150	180

2.4. Determination of Galacturonic Acid Content

The sulfuric acid–carbazole method was employed for the determination of galacturonic acid content. A total of 10 mg of dried pectin sample was moistened with a small amount of anhydrous ethanol, fully dissolved in distilled water, and diluted to 10 mL to prepare a 1 mg/mL stock solution. Standard working solutions with concentration gradients of 0, 50, 100, 150, 200, and 250 µg/mL were obtained by diluting 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the stock solution in 10 mL volumetric flasks. A 1 mL aliquot of each standard solution was transferred into stoppered test tubes, followed by the slow addition of 6 mL of concentrated sulfuric acid in an ice–water bath. The mixtures were uniformly mixed, heated in boiling water for 20 min, and cooled to room temperature. After cooling, 0.2 mL of 0.15% (*w/v*) carbazole–ethanol solution was added, and the mixtures were placed in a dark environment at room temperature for a 2 h color development reaction. Absorbance values were recorded at 530 nm, and a standard curve was established with galacturonic acid concentration as the abscissa and absorbance as the ordinate.

For sample testing, 20 mg of pectin powder was accurately weighed, moistened with anhydrous ethanol, dissolved in distilled water, and fixed to a constant volume of 100 mL after sufficient shaking. A 1 mL portion of the prepared sample solution underwent the same color development procedure as the standard solutions, and the corresponding absorbance was measured. The galacturonic acid concentration of the sample solution was calculated according to the standard curve regression equation, and the final galacturonic acid content of pectin was determined by the corresponding calculation formula.

$$\text{Galacturonic acid content (\%)} = \frac{c \times V \times D}{m \times 10^3} \times 100\% \quad (1)$$

where *c* represents the galacturonic acid concentration of the test solution calculated from the standard curve (µg/mL); *V* represents the constant volume of the sample solution (mL); *D* represents the dilution factor (100 mL/1 mL = 100); *m* represents the weighing mass of the pectin sample (mg); and 10³ represents the unit conversion coefficient.

2.5. Determination of Degree of Esterification

The degree of esterification of pectin was measured via acid–base titration. A total of 0.5 g of pectin sample was accurately weighed and dispersed in 100 mL of distilled water. The solution was titrated with 0.1 mol/L NaOH standard solution using phenolphthalein as the indicator until a stable faint pink color appeared, and the consumed NaOH volume was recorded as *V*₁. Subsequently, 25 mL of 0.1 mol/L NaOH solution was added to the titrated solution for a 2 h room-temperature saponification reaction. Excess alkali was neutralized with 25 mL of 0.1 mol/L HCl solution, and secondary titration with 0.1 mol/L

NaOH solution was performed until the faint pink endpoint reappeared. The volume of NaOH consumed in the secondary titration was recorded as V_2 .

$$DE = \frac{V_2}{V_1 + V_2} \times 100\%, \quad (2)$$

where DE is the degree of esterification of pectin (%); V_1 is the volume of NaOH standard solution consumed in titrating the free carboxyl groups (mL); and V_2 is the volume of NaOH standard solution consumed in titrating the carboxyl groups generated after saponification (mL).

2.6. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Approximately 2 mg of the dried pectin sample was thoroughly mixed and ground with 200 mg of KBr powder, and then pressed into a transparent pellet. FTIR analysis was performed using a Fourier transform infrared spectrometer over the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} , with 32 scans accumulated to complete the collection and recording of the infrared spectral data.

3. Results and Discussion

3.1. Process Optimization Results

3.1.1. Effect of Sequence on Pectin Yield

This study compared pectin yields between two treatment sequences. Process 1 (ultrasound prior to enzymatic hydrolysis) gave a yield of $9.79 \pm 0.36\%$, which exceeded the $7.87 \pm 0.28\%$ obtained from Process 2 (enzymatic hydrolysis prior to ultrasound) (mean $\pm$ SD, $n = 3$). This divergence relates to the structural features of cocoa pod husk cell walls. Existing research confirms that these cell walls form a dense network consisting of cellulose, hemicellulose, lignin, and pectin [20]. A pure enzymatic treatment at the initial stage struggles to drive enzymes deep into intact, compact cell walls to bind with substrates, and therefore lowers hydrolysis efficiency. By contrast, the combined sequence starting with ultrasound takes advantage of acoustic cavitation. The resulting shock waves and shear forces break down dense cell wall structures, loosen tissue, and expose more cellulose skeletons and pectin crosslinking sites. Celluclast[®] 1.5 L added afterwards penetrates cell walls more easily, degrades cellulose backbones, and releases bound pectin. Accordingly, this ultrasound–first sequence was chosen for further parameter optimization.

3.1.2. Results of Single-Factor Experiments

Combined with the yield variation trends observed in single-factor experiments shown in Figure 2, the low (−1) and high (+1) coded levels of each factor listed in Table 1 were reasonably defined. For the liquid-to-solid ratio X_1 shown in Figure 2a, 15 mL/g (−1) and 25 mL/g (+1) were chosen to cover the rising trend before the peak at 20 mL/g and the subsequent mild decline. For the pH X_2 presented in Figure 2b, the range of 5.0 (−1) to 6.0 (+1) centered at the optimal pH of 5.5, capturing yield reductions under acidic or alkaline deviations. For the ultrasonic temperature X_3 displayed in Figure 2c, 55 °C (−1) and 70 °C (+1) were selected instead of 50 °C as the lower bound, because 50 °C only exerted a weak promoting effect on yield; the range of 55–70 °C fully covers the high-yield rising stage of 55–65 °C and the thermal degradation stage above 65 °C, which helps distinguish the dual promoting and inhibitory effects of temperature. The ultrasonic time X_4 illustrated in Figure 2d was set from 20 min (−1) to 40 min (+1) to enclose the optimal 35 min, covering insufficient and excessive ultrasonic treatment conditions. The enzyme dosage X_5 shown in Figure 2e ranged from 20 $\mu\text{L/g}$ (−1) to 60 $\mu\text{L/g}$ (+1) to reflect the slight yield fluctuation below 40 $\mu\text{L/g}$ and gradual yield loss under excessive enzyme

addition. The enzymatic hydrolysis temperature X_6 presented in Figure 2f was set to 45 °C (−1) and 60 °C (+1), taking the optimal value 55 °C as the midpoint to reveal yield reduction caused by temperature deviation. The enzymatic hydrolysis time X_7 displayed in Figure 2g was set at 90 min (−1) and 180 min (+1) to include the yield peak at 150 min, corresponding to incomplete extraction with short hydrolysis and mild pectin degradation with prolonged hydrolysis. All selected parameter intervals were set around the optimal values obtained from single-factor tests and contained both ascending and descending segments of yield, so that the significant influence of each variable on pectin yield could be effectively distinguished within the valid experimental range.

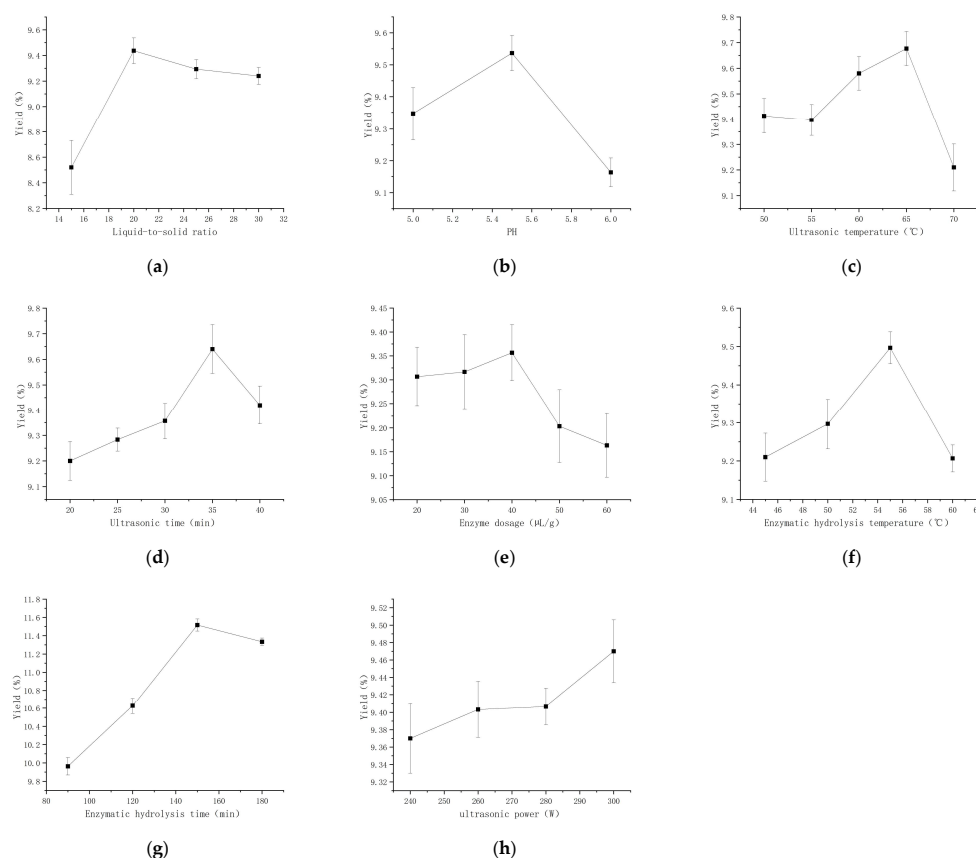


Figure 2. Effects of (a) liquid-to-solid ratio, (b) pH, (c) ultrasonic temperature, (d) ultrasonic time, (e) enzyme dosage, (f) enzymatic hydrolysis temperature, (g) enzymatic hydrolysis time, and (h) ultrasonic power on pectin yield from cocoa pod husks. Data are presented as mean $\pm$ SD ($n = 3$).

The results of ultrasonic power in the single-factor experiment are shown in Figure 2h. The pectin yield increased continuously with increasing power and reached the maximum at 300 W, which was the maximum output of the available equipment, with neither a plateau nor a decline being observed within the tested range. Therefore, the ultrasonic power was fixed at 300 W for all subsequent experiments. It should be noted that, due to equipment limitations, the effects of higher power levels remain to be further investigated.

3.1.3. Plackett–Burman Experimental Design Results

As summarized in Table 4, the yield data of all 12 Plackett–Burman experiment runs were obtained. As shown by the ANOVA results in Table 5, the model p -value was 0.0037 ($p < 0.05$), indicating statistical significance. X_1 (liquid-to-solid ratio) and X_4 (ultrasonic time) exerted highly significant effects on pectin yield ($p < 0.01$), while X_7 (enzymatic hydrolysis time) showed a significant effect ($p < 0.05$). X_2 (pH), X_3 (ultrasonic temperature), X_5 (enzyme dosage), and X_6 (enzymatic hydrolysis temperature) had no significant

influence within the tested ranges. The liquid-to-solid ratio regulates pectin mass transfer through the concentration gradient: at a low ratio, pectin accumulates near the solid–liquid interface, weakening the concentration gradient and limiting diffusion; increasing the ratio enlarges the solvent reservoir, maintains a higher concentration gradient, and promotes continuous pectin dissolution. Meanwhile, the liquid–to–solid ratio also affects ultrasonic cavitation efficiency—moderately increasing the liquid volume enhances cavitation propagation, whereas an excessive ratio reduces the volumetric ultrasonic energy density and dilutes the enzyme, thereby weakening catalytic efficiency. The superposition of these multiple effects results in a nonlinear relationship between liquid-to-solid ratio and yield, consistent with the observed plateau beyond 20–25 mL/g. Prolonged ultrasonic time intensifies cavitation, disrupts the cellulose–lignin network, increases cell wall permeability, and exposes pectin-binding sites. Insufficient enzymatic hydrolysis time leads to incomplete pectin release, whereas excessive time causes pectin degradation, reducing the yield.

By contrast, pH (5.0–6.0) fell within the optimal activity range of Celluclast® 1.5 L, resulting in limited yield variation; within the ultrasonic temperature range (55–70 °C), thermal degradation and cavitation enhancement partially offset each other; enzyme dosage (20–60 µL/g) was already sufficient to hydrolyze accessible cellulose substrates; and enzymatic hydrolysis temperature (45–60 °C) remained within the optimal activity window, exerting a marginal effect. The significance ranking of the factors (ultrasonic time > liquid–to–solid ratio > enzymatic hydrolysis time) indicates that physical disruption of the cell wall is the primary rate-limiting step for pectin release, further highlighting the synergistic nature of the ultrasound-assisted enzymatic extraction process. According to the Plackett–Burman screening outcomes, X_1 , X_4 , and X_7 were chosen for subsequent response–surface optimization, whereas all non-significant variables were assigned fixed levels: pH of 5.0 (the optimum value observed in single-factor pre-tests), ultrasonic temperature of 60 °C (midpoint of the experimental range), enzyme dosage of 20 µL/g (the lower bound of the non-significant range for cost reduction), and enzymatic hydrolysis temperature of 55 °C (the optimal temperature for Celluclast® 1.5 L). It should be acknowledged that these fixed levels only exert negligible effects compared with the three prominent significant factors (ultrasonic time, liquid–to–solid ratio and enzymatic hydrolysis time) within the current experimental range, rather than representing global optimal conditions. Narrower parameter gradients can be adopted in follow-up research to achieve more precise optimization.

Table 4. Results of Plackett–Burman experiment (mean $\pm$ SD, $n = 3$).

Run	Factor							Yield (%)
	Liquid-to-Solid Ratio (X_1)	pH (X_2)	Ultrasonic Temperature (X_3)	Ultrasonic Time (X_4)	Enzyme Dosage (X_5)	Enzymatic Hydrolysis Temperature (X_6)	Enzymatic Hydrolysis Time (X_7)	
1	−1	+1	−1	−1	+1	+1	−1	8.73 $\pm$ 0.19%
2	−1	−1	−1	−1	−1	−1	−1	8.83 $\pm$ 0.26%
3	+1	−1	−1	+1	+1	−1	+1	11.38 $\pm$ 0.33%
4	+1	+1	+1	+1	−1	−1	−1	10.52 $\pm$ 0.42%
5	+1	−1	+1	−1	+1	+1	+1	10.80 $\pm$ 0.13%
6	+1	+1	−1	+1	+1	+1	−1	11.22 $\pm$ 0.35%
7	−1	+1	+1	+1	−1	+1	+1	9.79 $\pm$ 0.36%
8	−1	−1	−1	+1	−1	+1	+1	10.39 $\pm$ 0.63%
9	−1	−1	+1	+1	+1	−1	−1	9.62 $\pm$ 0.39%
10	+1	−1	+1	−1	−1	+1	−1	9.94 $\pm$ 0.37%
11	+1	+1	−1	−1	−1	−1	+1	10.48 $\pm$ 0.42%
12	−1	+1	+1	−1	+1	−1	+1	8.45 $\pm$ 0.38%

Table 5. Analysis of variance table for the Plackett–Burman experiment.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Significance
Model	10.03	7	1.43	25.40	0.0037	**
X ₁	6.06	1	6.06	107.43	0.0005	**
X ₂	0.2611	1	0.2611	4.63	0.0979	
X ₃	0.3040	1	0.3040	5.39	0.0811	
X ₄	0.27	1	0.27	47.80	0.0023	**
X ₅	0.0052	1	0.0052	0.0923	0.7764	
X ₆	0.2107	1	0.2107	3.73	0.1255	
X ₇	0.4921	1	0.4921	8.72	0.0419	*
Residual	0.2258	4	0.0564			
Total	10.26	11				

Note: * indicates $p < 0.05$, representing a statistically significant difference; ** indicates $p < 0.01$, representing a highly significant difference.

3.1.4. Steepest Ascent Experimental Results

Table 6 revealed that pectin yield rose first and then declined as factor levels increased. The fourth test group achieved the maximum yield, so its parameters served as the central point for the following response surface design.

Table 6. Results of the steepest ascent experiment (mean $\pm$ SD, $n = 3$).

Run	Liquid-to-Solid Ratio X ₁	Ultrasonic Time X ₄	Enzymatic Hydrolysis Time X ₇	Yield (%)
1	15.0:1	20	60	8.71 $\pm$ 0.35%
2	17.5:1	25	90	11.38 $\pm$ 0.38%
3	20:1	30	120	12.84 $\pm$ 0.47%
4	22.5:1	35	150	13.98 $\pm$ 0.14%
5	25:1	40	180	9.42 $\pm$ 0.45%
6	27.5:1	45	210	8.12 $\pm$ 0.41%

3.1.5. Response Surface Optimization Results

Liquid-to-solid ratio (X_1), ultrasonic time (X_4), and enzymatic hydrolysis time (X_7) were set as independent variables, and pectin yield was defined as the response value for response surface tests. Table 7 summarized all experimental results. The dataset was fitted via Design-Expert 13.0, and a quadratic multiple regression equation was established as follows:

$$Y = -169.58675 + 5.65220X_1 + 4.81430X_4 + 0.408750X_7 + 0.032600X_1X_4 - 0.000567X_1X_7 - 0.002683X_4X_7 - 0.144760X_1^2 - 0.070990X_4^2 - 0.000966X_7^2, \quad (3)$$

ANOVA data in Table 8 listed a model F -value of 60.58 and a p -value below 0.0001, indicating extremely high statistical significance ($p < 0.01$), which suggested a strong correlation between the selected factors and the response value. The lack-of-fit p -value was 0.3869 ($p > 0.05$), indicating no obvious lack of fit and no systematic error in the regression equation. The model had an R^2 of 98.73%, reflecting excellent consistency between the experimental data and the model prediction, demonstrating that the equation could effectively describe how the three factors affected pectin yield. The adjusted R^2 (R^2_{Adj}) reached 0.9710, meaning the model explained 97.10% of the response variation. The predicted R^2 was 0.8895, which was in reasonable agreement with R^2_{Adj} (difference < 0.2), which could indicate favorable model predictive performance and no significant overfitting. This 0.2 threshold is an empirical rule of thumb commonly used in response surface methodology to assess whether a model is overfitted. The coefficient of variation (CV%)

was 1.82%, indicating good experimental precision and reliability. The Adequate Precision value, which measures the signal-to-noise ratio, was 22.9823, substantially higher than the critical value of 4, indicating adequate signal resolution and confirming that the model can reliably navigate the design space. These diagnostics collectively confirmed that the model is reliable for analyzing and predicting the ultrasound-assisted enzymatic extraction of pectin from cocoa pod husks, consistent with the model evaluation criteria adopted in previous BBD-based pectin extraction research [21].

Table 7. Results of Box–Behnken experiment (mean $\pm$ SD, $n = 3$).

Run	Liquid-to-Solid Ratio X_1	Ultrasonic Time X_4	Enzymatic Hydrolysis Time X_7	Yield (%)
1	22.5	40	120	13.54 $\pm$ 0.51%
2	25	35	120	13.49 $\pm$ 0.12%
3	25	30	150	11.64 $\pm$ 0.48%
4	20	30	150	11.65 $\pm$ 0.42%
5	22.5	35	150	15.23 $\pm$ 0.62%
6	20	40	150	12.35 $\pm$ 0.57%
7	20	35	180	13.21 $\pm$ 0.32%
8	22.5	35	150	14.98 $\pm$ 0.26%
9	22.5	40	180	13.34 $\pm$ 0.22%
10	25	40	150	13.97 $\pm$ 0.43%
11	22.5	35	150	15.14 $\pm$ 0.38%
12	22.5	30	120	10.73 $\pm$ 0.47%
13	22.5	35	150	15.32 $\pm$ 0.59%
14	20	35	120	12.27 $\pm$ 0.10%
15	22.5	35	150	14.74 $\pm$ 0.27%
16	22.5	30	180	12.14 $\pm$ 0.37%
17	25	40	120	14.26 $\pm$ 0.25%

Table 8. Analysis of variance table for the Box–Behnken experiment.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Significance
Model	32.33	9	3.59	60.58	<0.0001	**
X_1	1.88	1	1.88	31.74	0.0008	**
X_4	6.20	1	6.20	104.49	<0.0001	**
X_7	1.07	1	1.07	17.98	0.0038	**
X_1X_4	0.6642	1	0.6642	11.20	0.0123	*
X_1X_7	0.0072	1	0.0072	0.1219	0.7373	
X_4X_7	0.6480	1	0.6480	10.93	0.0130	*
X_1^2	3.45	1	3.45	58.13	0.0001	**
X_4^2	13.26	1	13.26	223.68	<0.0001	**
X_7^2	3.19	1	3.19	53.72	0.0002	**
Residual	0.4150	7	0.0593			
Lack of Fit	0.2057	3	0.0686	1.31	0.3869	
Pure Error	0.2093	4	0.0523			
Total	32.74	16				
R^2	0.9873					
R^2 Adj	0.9710					
Predicted R^2	0.8895					
CV%	1.82					
Adequate Precision	22.9823					

Note: * indicates $p < 0.05$, representing a statistically significant difference; ** indicates $p < 0.01$, representing a highly significant difference.

Four diagnostic graphs were plotted to verify the validity of the established regression model. As shown in Figure 3a, all residual points basically distributed along the straight line, suggesting that random experimental errors followed a normal distribution. In Figure 3b, residuals scattered randomly around the zero baseline without regular funnel or linear trends, indicating homoscedasticity of the model. Figure 3c illustrated that residuals fluctuated randomly up and down the zero line with no obvious systematic drift over experimental runs, suggesting no time-dependent systematic error. In Figure 3d, the predicted values were highly consistent with the actual measured data, and all points were closely distributed near the diagonal, reflecting good fitting performance and reliable predictive capacity of the response surface model [22].

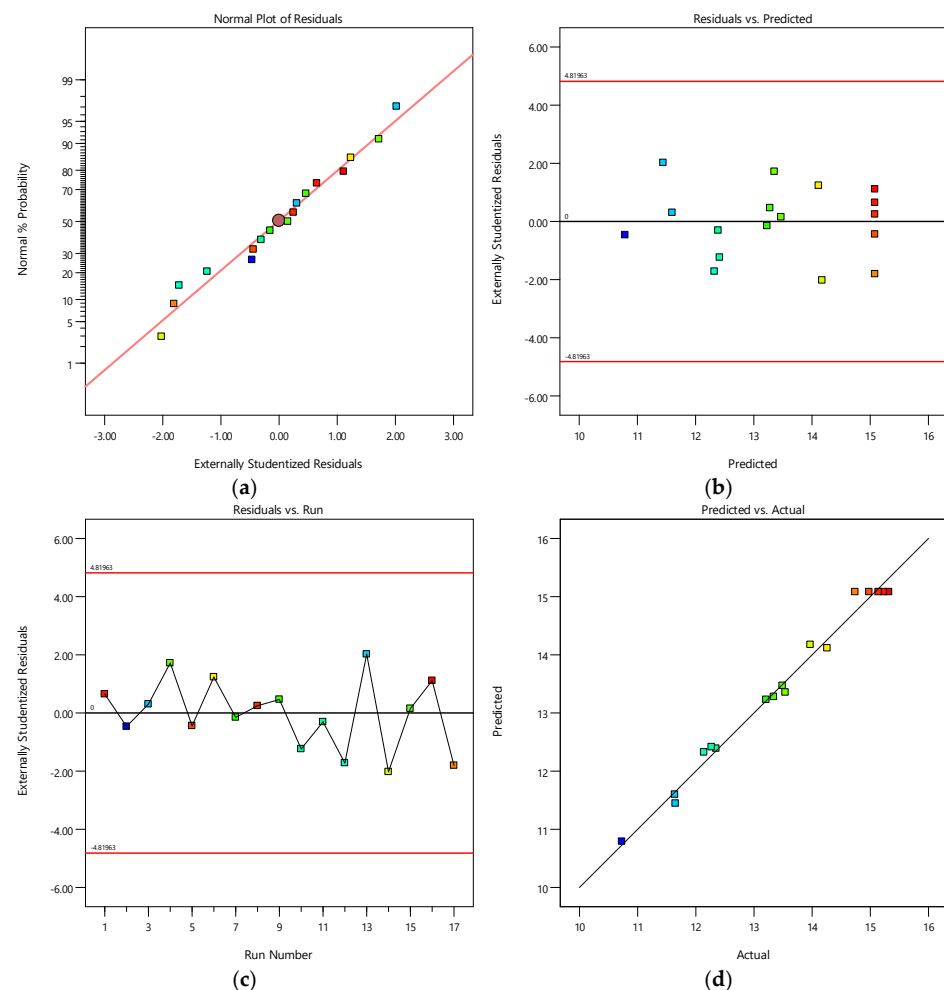


Figure 3. Diagnostic plots of the response surface regression model for pectin yield. (a) Normal probability plot of externally studentized residuals; (b) Externally studentized residuals vs. predicted values; (c) Externally studentized residuals vs. experimental run number; (d) Predicted pectin yield versus actual measured yield.

Figure 4 showed three-dimensional response surfaces and contour plots, which visualized the influences of liquid-to-solid ratio (X_1), ultrasonic time (X_4) and enzymatic hydrolysis time (X_7) on pectin yield. Surface steepness reflected the degree of factor influence: a steeper surface stood for a more significant effect, and the surface apex corresponded to the optimal parameters for maximum yield. Figure 4a,c,e show surface steepness in the order $X_4 > X_1 > X_7$, so ultrasonic time acted as the dominant factor. Acoustic cavitation broke the raw material structure and improved cell wall permeability, making ultrasound

treatment the key rate-limiting step for follow-up enzymatic extraction. This conclusion agreed with the factor significance ranking from ANOVA in Table 8.

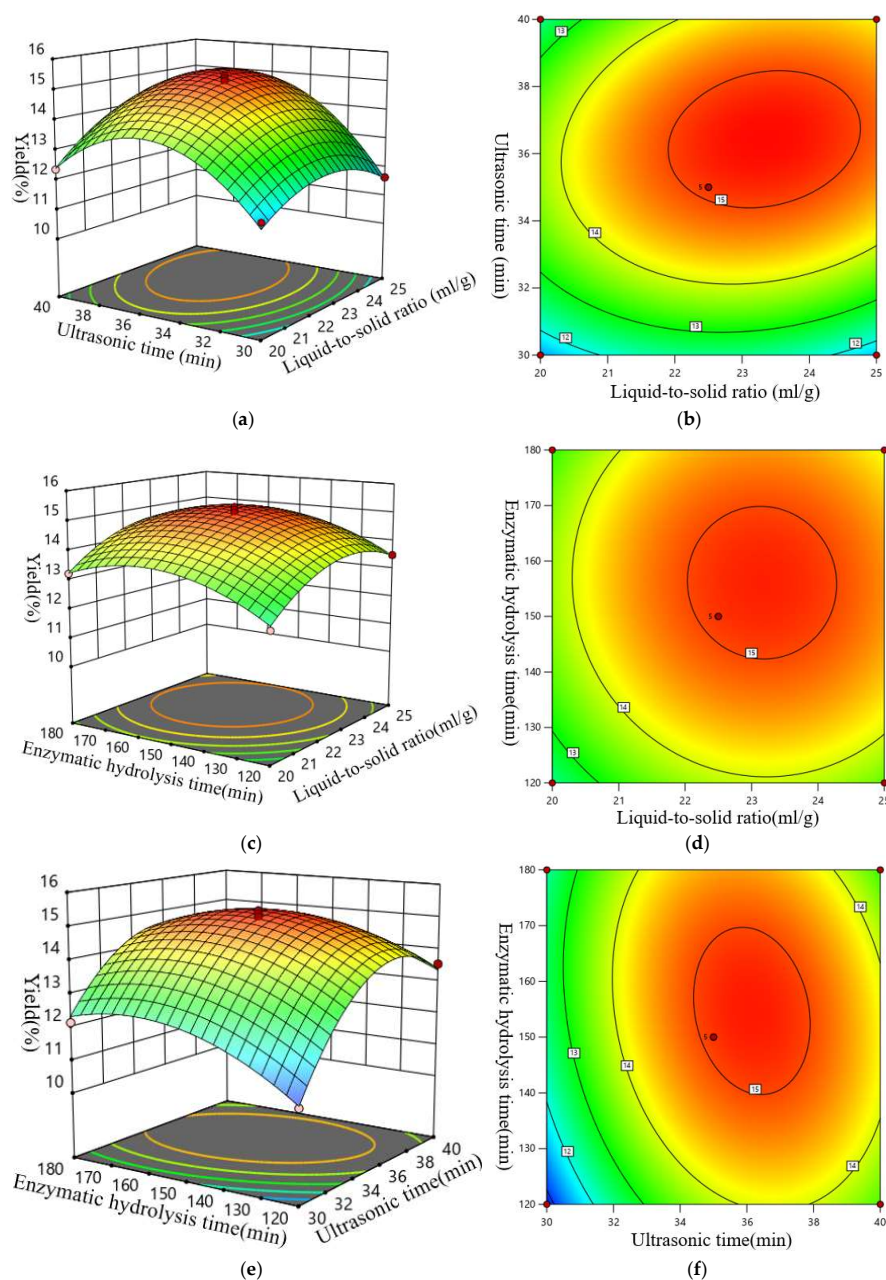


Figure 4. Three-dimensional response surface plots and corresponding contour plots illustrating the interactive effects of extraction variables on pectin yield. (a) Response surface plot of liquid-to-solid ratio (X_1) and ultrasonic time (X_4); (b) Contour plot of liquid-to-solid ratio (X_1) and ultrasonic time (X_4); (c) Response surface plot of liquid-to-solid ratio (X_1) and enzymatic hydrolysis time (X_7); (d) Contour plot of liquid-to-solid ratio (X_1) and enzymatic hydrolysis time (X_7); (e) Response surface plot of ultrasonic time (X_4) and enzymatic hydrolysis time (X_7); (f) Contour plot of ultrasonic time (X_4) and enzymatic hydrolysis time (X_7). All data were obtained from Box–Behnken design experiments.

Contour plot shapes in Figure 4b,d,f directly judge the interaction significance between factors. Circular contours represent insignificant interactions, while elliptical shapes mean significant interactions [23]. Figure 4d displays nearly circular contours for X_1 (liquid-to-solid ratio) and X_7 (enzymatic hydrolysis time), so their interaction shows no statistical significance ($p > 0.05$). The liquid-to-solid ratio mainly governed mass transfer equilibrium [24], whereas hydrolysis time controlled catalytic reactions. An overly high

ratio diluted enzyme concentration and weakened the function of prolonged hydrolysis. By contrast, obvious elliptical contours appeared for X_1 & X_4 (Figure 4b) and X_4 & X_7 (Figure 4f), suggesting strong interactions within both pairs. The combination of X_1 and X_4 showed the strongest interaction. A low liquid-to-solid ratio cut system viscosity and strengthened cavitation; meanwhile, ultrasound-loosened cell walls accelerated solution permeation, and the two effects generated positive synergy. The interaction between X_4 and X_7 ranked second. Ultrasound broke cell walls and exposed more pectin, so enzymes contacted substrates more easily in the hydrolysis stage. Nevertheless, excessively long ultrasonic and enzymatic treatments triggered degradation of released pectin. Degradation loss exceeded newly released pectin, and finally reduced total yield. All visual analysis matched the ANOVA results. Combined analysis from Figure 4 and Table 8 ranked interaction intensity as $X_1X_4 > X_4X_7 > X_1X_7$. The interaction between liquid-to-solid ratio and ultrasonic time was the strongest, followed by ultrasonic time and enzymatic hydrolysis time, while liquid-to-solid ratio and enzymatic hydrolysis time showed the weakest interaction.

3.2. Predicted Optimal Process Conditions and Verification Experimental Results

Figure 4 presents an upward convex response surface, which implies optimal extraction conditions and maximum yield values within the tested variable ranges. Design-Expert 13.0 calculated the theoretical optimal parameters: liquid-to-solid ratio of 25.00 mL/g, ultrasonic time of 36.76 min, and enzymatic hydrolysis time of 153.12 min, with a predicted pectin yield of 14.91%. Importantly, the optimal liquid-to-solid ratio of 25.00 mL/g corresponded to the upper limit of the Box-Behnken design range. When the predicted optimal point falls on the boundary of the experimental domain, it indicates that the actual global optimal extraction conditions are likely to lie outside the current test interval, which is a well-recognized inherent limitation of response surface methodology. To suit practical operation, the parameters were adjusted to a 25 mL/g liquid-to-solid ratio, 37 min ultrasonic treatment, and 153 min enzymatic hydrolysis. Three parallel verification tests produced an average pectin yield of $14.34 \pm 0.39\%$ (mean $\pm$ SD, $n = 3$), with an RSD (relative standard deviation) of 2.72%. The deviation between experimental and predicted values remained below 5%, indicating that the optimized parameters were reliable within the investigated range. For reference, the pectin yield, total extraction duration, and enzyme consumption data reported by Hennessey-Ramos et al. [13] were adopted for indirect horizontal comparison. From numerical trends, the yield of the optimized process in this study was 4.14 percentage points higher (14.34% vs. 10.20%), the total extraction time was shortened by 10 h, and the enzyme dosage was reduced by half. It should be noted that the above comparison merely serves as a literature-based reference instead of parallel experiments conducted under identical conditions. Distinctions in raw materials, pretreatment protocols, extraction parameters, and analytical detection methods exist among various published studies.

3.3. Analysis of Galacturonic Acid Content

D-galacturonic acid content acts as a core index to evaluate pectin purity. The Food and Agriculture Organization (FAO) stipulates that qualified pectin requires a D-galacturonic acid content above 65% [25]. In this work, the sulfuric acid-carbazole method was applied. A standard curve was established with D-galacturonic acid, yielding the regression equation $y = 0.0015x + 0.0642$ ($R^2 = 0.9959$). Spectrophotometric measurement showed that the final product contained $67.93 \pm 1.13\%$ galacturonic acid, a value substantially higher than the results from single enzymatic extraction and traditional acid extraction.

Traditional acid extraction breaks cell walls and dissolves pectin via strong acid, high temperature, and long treatment [26]. Such harsh conditions tend to degrade pectin backbones [27,28] and co-extract starch, pigments, and neutral heteropolysaccharides, thus lowering galacturonic acid content [29]. Chan et al. [30] used citric acid to extract pectin from cocoa pod husk and obtained galacturonic acid content between 31.19% and 59.36%. Hydrochloric acid extraction delivered only 32.58–54.69%. Both datasets were much lower than the $67.93 \pm 1.13\%$ obtained in the present work.

Enzymatic extraction uses cellulase, hemicellulase, and other enzymes to selectively degrade cell wall polysaccharides. It releases pectin under mild conditions [31,32], minimizing molecular degradation and impurity dissolution, so it generally produces pectin with higher galacturonic acid content than acid extraction [31]. Nevertheless, single enzymatic extraction faces drawbacks including poor mass transfer, slow enzyme diffusion, and long processing time. Extended hydrolysis breaks down cellulose but also leaches extra cell wall substances such as cellulose fragments and hemicellulose derivatives. These impurities co-precipitate with pectin and decrease product purity [12]. Hennessey–Ramos et al. [13] only adopted single enzymatic hydrolysis without ultrasonic assistance, and their optimal process yielded merely 10.21% pectin with a galacturonic acid content of 52.06%. Their experimental conditions differed greatly from the ultrasound–enzyme synergistic system in this study: they relied on an extremely long hydrolysis period of over 18 h. Excessively prolonged hydrolysis induced massive co-extraction of cell wall impurities, which largely reduced the purity of their pectin product.

The comparison with acid and single enzymatic extraction methods described above indicates that conventional approaches have inherent limitations in improving pectin purity. The purity advantage of the pectin extracted in this study is attributed to the synergistic mechanism of ultrasonic cavitation and enzymatic hydrolysis, as supported by previous studies on pectin extraction from lignocellulosic biomass, which have demonstrated that the combination of enzymatic and ultrasonic treatments can synergistically disrupt the lignocellulosic matrix and reduce the co-dissolution of impurities, thereby yielding pectin with higher purity [18,19]. Ultrasound enhances substrate accessibility through cavitation, while enzymes selectively release pectin under mild conditions; the combined application of these two techniques effectively reduces the co-extraction of non-pectin components such as neutral sugars.

3.4. Analysis of Degree of Esterification

The degree of esterification (DE) reflects the molecular characteristics of pectin and largely determines its gelation behavior. Per industrial standards, pectin with DE below 50% is categorized as low-methoxyl pectin. We determined the DE of the pectin from optimized ultrasound–assisted enzymatic extraction via acid–base titration, obtaining a value of $43.62 \pm 0.6\%$. This reading lies within the DE range (7.17–57.86%) reported by Chan et al. [30] for cocoa pod husk pectin extracted using acid and hot water methods. While there are inevitable variations in raw materials and experimental setups across different research works, this consistent numerical range lends credibility to our test results. A DE value of $43.62 \pm 0.6\%$ confirms the extracted product is low-methoxyl pectin. As documented in previous reports [10,33], this kind of pectin can form gels with calcium ions without extra sucrose, which endows it with great potential in low-calorie food production.

3.5. Fourier Transform Infrared Spectroscopy Analysis

Figure 5 displayed the FTIR spectrum of the pectin extracted under the optimized conditions. A broad O–H stretching peak was observed at 3408 cm^{-1} [34], and a typical alkyl C–H absorption signal appeared at 2929 cm^{-1} [35], both of which are characteristic of

polysaccharide structures. The ester C=O stretching peak at 1740 cm^{-1} [36], together with carboxylate signals at 1620 cm^{-1} and 1417 cm^{-1} [37], confirmed the presence of esterified carboxyl groups in the pectin molecule. The relatively weak absorption intensity of the ester carbonyl peak compared to the carboxylate signals indicated a low degree of esterification, which is consistent with the DE value of $43.62 \pm 0.6\%$ determined by acid–base titration. A distinct absorption band at 1040 cm^{-1} [38] was assigned to the pyranose ring structure of galacturonic acid. These spectral features collectively confirmed that the extracted product possessed the characteristic functional groups of pectin with a low-methoxyl structure, consistent with the titration results.

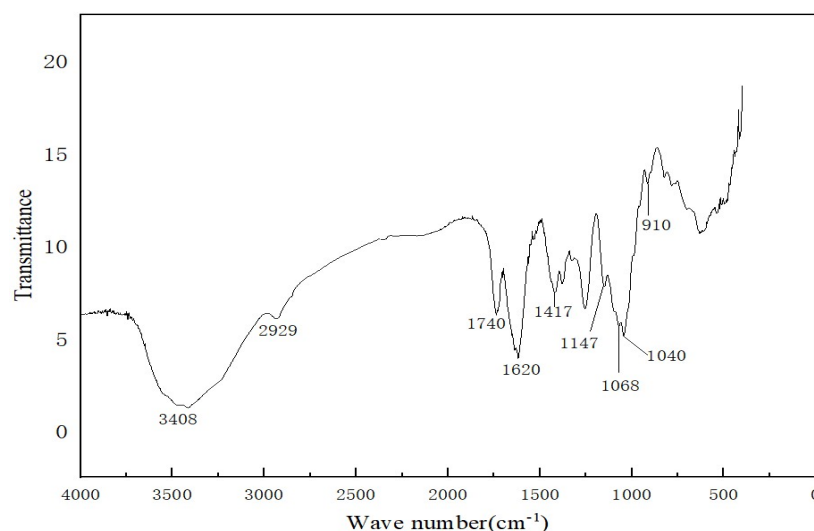


Figure 5. FTIR spectrum of cocoa pod husk pectin.

4. Discussion

Each conventional method suffers from a distinct limitation: acid extraction degrades pectin structure and generates pollution; enzymatic extraction is excessively time-consuming; ultrasound alone cannot fully penetrate the dense lignocellulosic matrix. These issues converge on a common bottleneck—the rigid cell wall that shields pectin from efficient release. Our ultrasound-assisted enzymatic strategy breaks this bottleneck through a sequential “wall-breaking first, hydrolysis second” synergy. Ultrasound fragments the lignocellulosic framework, creating pathways for enzyme penetration, while the subsequent cellulase treatment releases pectin under mild conditions. This approach not only improves yield and purity, but also shortens processing time and reduces enzyme consumption—key factors for industrial viability.

From an industrial application perspective, the optimized low enzyme dosage and mild reaction temperature help cut production costs and energy consumption. Nevertheless, the high upfront cost of ultrasonic equipment hinders large-scale industrial deployment. In addition, limited by available equipment, we only tested ultrasonic power up to 300 W; higher power levels were not investigated, so it remains unknown whether further yield enhancement or unintended pectin molecular degradation would occur. Apart from the incomplete characterization of extracted pectin without comparisons to commercial counterparts, this study also fails to screen various compound enzymes, while the scale-up feasibility of the extraction process has not been verified via pilot trials. All these unresolved points require follow-up research to fully evaluate the industrial practicability of this ultrasound enzymatic synergistic extraction route, and enzyme recycling technology can be developed in subsequent work to further reduce manufacturing costs.

For future research, multi-response optimization strategies like the desirability function can be adopted to synchronously optimize three core indicators—pectin yield, galacturonic acid content, and degree of esterification—so that both high extraction efficiency and satisfactory product quality can be guaranteed simultaneously.

5. Conclusions

In this study, an ultrasound-assisted enzymatic extraction process for pectin from cocoa pod husks was established and optimized. Under the optimal conditions, the pectin yield reached $14.34 \pm 0.39\%$, with a galacturonic acid content of $67.93 \pm 1.13\%$ and a degree of esterification of $43.62 \pm 0.6\%$, indicating that the extracted pectin falls into the low-methoxyl category. FTIR analysis confirmed the characteristic polysaccharide structure and low-esterification features of the extracted product. Compared with literature-reported data, the present method showed favorable performance compared with single enzymatic extraction in terms of yield, and compared with traditional acid extraction in terms of purity, along with shorter extraction time and lower enzyme consumption. Based on its low-methoxyl nature, the extracted pectin may have potential applications in low-calorie food products where calcium-induced gelation is desired. However, direct experimental validation of its gelling behavior and application performance is still required before practical utilization can be realized. Overall, this process offers a feasible and potentially green alternative for pectin preparation from cocoa pod husk waste, with the advantages of high efficiency, satisfactory product quality, and environmentally friendly operating conditions.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of Variance
BBD	Box–Behnken Design
DE	Degree of Esterification
FAO	Food and Agriculture Organization
FTIR	Fourier Transform Infrared Spectroscopy
PB	Plackett–Burman
SD	Standard Deviation

References

1. Krumbiegel, K.; Tillie, P. Sustainable practices in cocoa production: The role of certification schemes and farmer cooperatives. *Ecol. Econ.* **2024**, *222*, 108211. [\[CrossRef\]](#)
2. Anoraga, S.B.; Shamsudin, R.; Hamzah, M.H.; Sharif, S.; Saputro, A.D. Cocoa by-products: A comprehensive review on potential uses, waste management, and emerging green technologies for cocoa pod husk utilization. *Heliyon* **2024**, *10*, e35537. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Vásquez, Z.S.; Neto, L.P.D.; Pereira, G.V.M.; Vandenberghe, L.P.S.; de Oliveira, P.Z.; Tiburcio, P.B.; Rogez, H.L.G.; Neto, A.G.; Soccol, C.R. Biotechnological approaches for cocoa waste management: A review. *Waste Manag.* **2019**, *90*, 72–83. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Kaba, J.S.; Yamoah, F.A.; Acquaye, A. Towards sustainable agroforestry management: Harnessing the nutritional soil value through cocoa mix waste. *Waste Manag.* **2021**, *124*, 264–272. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Balentic, J.P.; Ackar, D.; Jokic, S.; Jozinovic, A.; Babic, J.; Milicevic, B.; Subaric, D.; Pavlovic, N. Cocoa shell: A by-product with great potential for wide application. *Molecules* **2018**, *23*, 1404. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Barrios-Rodríguez, Y.F.; Salas-Calderón, K.T.; Orozco-Blanco, D.A.; Gentile, P.; Girón-Hernández, J. Cocoa pod husk: A high-pectin source with applications in the food and biomedical fields. *ChemBioEng Rev.* **2022**, *9*, 462–474. [\[CrossRef\]](#)
7. Saki, G.; Kohji, K.; Tomio, Y. Involvement of the complex polysaccharide structure of pectin in regulation of biological functions. *Rev. Agric. Sci.* **2021**, *9*, 221–232. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Frosi, I.; Balduzzi, A.; Moretto, G.; Colombo, R.; Papetti, A. Towards valorization of food-waste-derived pectin: Recent advances on their characterization and application. *Molecules* **2023**, *28*, 6390. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Dinani, S.T.; Broekema, N.L.; Boom, R.; van der Goot, A.J. Investigation of the potential of hydrocolloids in meat analogue preparation. *Food Hydrocoll.* **2023**, *135*, 108199. [\[CrossRef\]](#)
10. Wu, C.-L.; Liao, J.-S.; Wang, J.-M.; Qi, J.-R. Gelation behavior and mechanism of low methoxyl pectin in the presence of erythritol and sucrose: The role of co-solutes. *Int. J. Biol. Macromol.* **2024**, *271*, 132261. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Zoghi, A.; Vedadi, S.; Esfahani, Z.H.; Gavlighi, H.A.; Khosravi Darani, K. A review on pectin extraction methods using lignocellulosic wastes. *Biomass Convers. Biorefinery* **2021**, *13*, 5577–5589. [\[CrossRef\]](#)
12. De Laet, E.; Bernaerts, T.; Morren, L.; Vanmarcke, H.; Van Loey, A.M. The use of different cell wall degrading enzymes for pectin extraction from carrot pomace, in comparison to and in combination with an acid extraction. *Foods* **2025**, *14*, 435. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Hennessey-Ramos, L.; Murillo-Arango, W.; Vasco-Correa, J.; Astudillo, I.C.P. Enzymatic extraction and characterization of pectin from cocoa pod husks (*Theobroma cacao* L.) using Celluclast® 1.5 L. *Molecules* **2021**, *26*, 1473. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Ye, Z.; Lu, Q.S.; Lv, D.H.; Yang, C. Enzymatic extraction of hawthorn pectin under ultrahigh pressure: Optimization and characterization. *Molecules* **2025**, *30*, 2210. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Dadhwal, J.; Choudhary, I.; Kumar, S.; Bhagawan, D. Optimization and characterization of pectin extraction from lemon peels using heating and ultrasound assisted methods: A sustainable approach to fruit waste valorization. *Int. J. Biol. Macromol.* **2026**, *339*, 149950. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Minjares-Fuentes, R.; Femenia, A.; Garau, M.C.; Meza-Velázquez, L.A.; Simal, S.; Rosselló, C. Ultrasound-assisted extraction of pectins from grape pomace using citric acid: A response surface methodology approach. *Carbohydr. Polym.* **2014**, *106*, 179–189. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Hernandez-Hernandez, O.; Ferreira-Lazarte, A.; Tiwari, B.; Villamiel, M. Update of high-intensity ultrasound applications for the extraction of pectin from agri-food by-products. *TrAC Trends Anal. Chem.* **2024**, *175*, 117728. [\[CrossRef\]](#)
18. Bosch, R.; Malgas, S. Ultrasound-assisted enzymatic extraction of orange peel pectin and its characterisation. *Int. J. Food Sci. Technol.* **2023**, *58*, 6784–6793. [\[CrossRef\]](#)
19. Yang, Y.S.; Wang, Z.M.; Hu, D.; Xiao, K.J.; Wu, J.Y. Efficient extraction of pectin from sisal waste by combined enzymatic and ultrasonic process. *Food Hydrocoll.* **2018**, *79*, 189–196. [\[CrossRef\]](#)
20. Dimawarnita, F.; Indriyanti, P.D.; Faramitha, Y.; Perwitasari, U. Isolation and characterization of α -cellulose from cocoa pod husk using peracetic acid. *IOP Conf. Ser. Earth Environ. Sci.* **2023**, *1187*, 012043. [\[CrossRef\]](#)
21. Suleiman, J.; Shamsudin, R.; Hamzah, M.H.; Basri, M.S.M.; Jimoh, K.A. Extraction optimization and characterization of durian (*Durio zibethinus*) rind pectin extracted by subcritical water. *Food Chem.* **2025**, *474*, 143123. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Baş, D.; Boyacı, İ.H. Modeling and optimization I: Usability of response surface methodology. *J. Food Eng.* **2007**, *78*, 836–845. [\[CrossRef\]](#)
23. Shivamathi, C.S.; Gunaseelan, S.; Soosai, M.R.; Vignesh, N.S.; Varalakshmi, V.; Kumar, S.R.; Karthikumar, S.; Kumar, V.R.; Baskar, R.; Rigby, S.P.; et al. Process optimization and characterization of pectin derived from underexploited pineapple peel biowaste as a value-added product. *Food Hydrocoll.* **2022**, *107*, 107141. [\[CrossRef\]](#)
24. Sant’Ana, A.S.; Pereira, E.R.; Sobral, T.R.S.; Fernandes, M.S.; Viridiana, F.; Pereira, B.E. Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: A critical review. *Biotechnol. Biofuels* **2020**, *13*, 58. [\[CrossRef\]](#) [\[PubMed\]](#)

25. Freitas, C.M.P.; Coimbra, J.S.R.; Souza, V.G.L.; Sousa, R.C.S. Structure and applications of pectin in food, biomedical, and pharmaceutical industry: A review. *Coatings* **2021**, *11*, 922. [[CrossRef](#)]
26. Dang, G.; Li, J.; Yin, C.; Wang, W.; Zhang, K.; Zhong, R.; Chen, L.; Zhang, H.; Schroyen, M. Deciphering pectin: A comprehensive overview of its origins, processing, and promising utility. *ACS Omega* **2025**, *10*, 11–15. [[CrossRef](#)] [[PubMed](#)]
27. Mansor, M.H.; Williamson, L.; Ludwikowski, D.; Howard, F.; Muthana, M. Pectin extraction from citrus waste: Structural quality and yield with mineral and organic acids. *Physchem* **2025**, *5*, 32. [[CrossRef](#)]
28. Lin, D.; Lin, J.; Li, Z.; Zhao, S.; Wang, L.; Ran, Y.; Ramaswamy, H.S.; Duan, H.; Bai, W.; Wang, C. Impact of extraction methods on the Physicochemical and structural characteristics of pomelo peel pectin. *J. Agric. Food Res.* **2026**, *26*, 102631. [[CrossRef](#)]
29. Nagel, A.; Mix, K.; Kuebler, S.; Bogner, H.; Kienzle, S.; Elstner, P.; Carle, R.; Neidhart, S. The arabinogalactan of dried mango exudate and its co-extraction during pectin recovery from mango peel. *Food Hydrocoll.* **2015**, *46*, 134–143. [[CrossRef](#)]
30. Chan, S.Y.; Choo, W.S. Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. *Food Chem.* **2013**, *141*, 3752–3758. [[CrossRef](#)] [[PubMed](#)]
31. Adetunji, L.R.; Adekunle, A.; Orsat, V.; Raghavan, V. Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocoll.* **2017**, *62*, 239–250. [[CrossRef](#)]
32. Willats, W.G.T.; McCartney, L.; Mackie, W.P.; Knox, J.P. Pectin: Cell biology and prospects for functional analysis. *Plant Mol. Biol.* **2001**, *47*, 9–27. [[CrossRef](#)] [[PubMed](#)]
33. Abdullah Tan, M.F.B.M.F.; Yuliarti, O.; Wong, A.I.C.; Wong, J.J.L. Structural and in vitro starch digestion of wheat flour noodles by calcium mediated gelation of low methoxyl pectin. *Food Struct.* **2024**, *42*, 100394. [[CrossRef](#)]
34. Guo, H.; He, M.; Huang, R.; Qi, W.; Guo, W.; Su, R.; He, Z. Changes in the supramolecular structures of cellulose after hydrolysis studied by terahertz spectroscopy. *RSC Adv.* **2014**, *4*, 57945–57952. [[CrossRef](#)]
35. Haj Romdhane, M.; Beltifa, A.; Mzoughi, Z.; Rihouey, C.; Ben Mansour, H.; Majdoub, H.; Le Cerf, D. Optimization of extraction with salicylic acid, rheological behavior and antiproliferative activity of pectin from *Citrus sinensis* peels. *Int. J. Biol. Macromol.* **2020**, *159*, 547–556. [[CrossRef](#)] [[PubMed](#)]
36. Manrique, G.D.; Lajolo, F.M. FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biol. Technol.* **2002**, *25*, 99–107. [[CrossRef](#)]
37. Cui, J.; Zhao, C.; Feng, L.; Han, Y.; Du, H.; Xiao, H.; Zheng, J. Pectins from fruits: Relationships between extraction methods, structural characteristics, and functional properties. *Trends Food Sci. Technol.* **2021**, *110*, 39–54. [[CrossRef](#)]
38. Muñoz-Almagro, N.; Valadez-Carmona, L.; Mendiola, J.A.; Ibáñez, E.; Villamiel, M. Structural characterisation of pectin obtained from cacao pod husk: Comparison of conventional and subcritical water extraction. *Carbohydr. Polym.* **2019**, *217*, 69–78. [[CrossRef](#)] [[PubMed](#)]

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