EVALUATING KEY PARAMETERS IN COCOA POD HUSK PECTIN ULTRASONIC-ASSISTED EXTRACTION VIA TWO-LEVEL FACTORIAL DESIGN

Arief Huzaimi Md Yusof * and Haslita Ibrahim
Malaysia Cocoa Board, Cocoa Innovative and Technology Centre, Lot 12621 Nilai Industrial Area, 71800 Nilai, Negeri Sembilan, Malaysia
**Corresponding author: ariefhuzaimi@koko.gov.my*

Malaysian Cocoa J. 17: 35-40 (2025)

ABSTRACT – *The extraction of pectin from cocoa pod husk, a valuable by-product of cocoa production, presents an opportunity for sustainable utilization in various industries. This study evaluated the key parameters influencing pectin yield using a two-level factorial design. Temperature, extraction time, and acid concentration were selected as the main factors, and their effects on pectin yield were investigated. The extraction process employed ultrasonic-assisted extraction (UAE), which utilizes high-frequency sound waves to enhance cell wall disruption and improve mass transfer, resulting in higher pectin recovery efficiency. The factorial design allowed for the assessment of both main effects and interaction effects between these factors. Results revealed that temperature and acid concentration had the most significant impact on pectin yield, with strong interaction effects observed. The best conditions for influencing the pectin yield were high temperature, high acid concentration, and longer extraction time. This study highlights the importance of optimizing extraction parameters and demonstrates the effectiveness of factorial design in enhancing the efficiency of pectin extraction from cocoa pod husks. The findings provide valuable insights into the sustainable recovery of pectin, achieving a maximum yield of 12.5%, and adding value to a by-product of the cocoa industry.*

Keywords: Cocoa pod husk, factorial design, pectin, ultrasonic-assisted extraction

INTRODUCTION

Pectin is an intricate polysaccharide predominantly consisting of galacturonic acid units, which are predominantly present in the cellular walls of plants. By functioning as a binding agent between cells, it serves a vital role in preserving the structure and integrity of plant tissues. Pectin is well acknowledged for its ability to form gels, thicken, and stabilize, which makes it very important in many sectors, particularly in food manufacturing where it is employed in the production of jams, jellies, and other similar items that need precise textural characteristics (Raj, 2012). Additionally, it is used in the pharmaceutical industry as a stabilizing agent or emulsifier, and in the manufacturing of dietary fibres because of its recognized health advantages (Roman-Benn *et al*., 2023).

The potential for pectin extraction in cocoa pod husk (CPH) arises from the presence of this polymer in the cell walls of the husk. However, the yield and quality of pectin from CPH are affected by various parameters, including extraction conditions, such as temperature, pH, and extraction duration. Solubilization of pectin and its degree of esterification are influenced by these parameters, which subsequently affect its functional characteristics (Spinei & Oroian, 2022). Compared to well-established sources such as citrus and apple, cocoa pod husk pectin has received limited attention due to its variability in composition, underutilization of cocoa by-products, and the lack of optimized extraction methods, thus presenting a clear research gap for exploration

The application of factorial design in experimental designs enables a more effective investigation of several variables concurrently. Within the realm of pectin extraction, several parameters including temperature, extraction duration, pH, and solvent concentration are recognized as influential in affecting the quantity and quality of the pectin acquired (Chandel *et al*., 2022). To minimize the number of tests needed while capturing the effects of each element and their interactions, a two-level factorial design offers an effective framework for evaluating these parameters (Das & Dewanjee, 2018).

The objective of this work is to investigate these parameters to see their influence on the yield of pectin. Through methodical analysis of the influence of each factor, the outcomes can result in improved application of cocoa pod husk, so enhancing the value of what is conventionally regarded as waste and connecting with sustainability objectives. Furthermore, the knowledge acquired from this research could establish a basis for future investigations and industrial uses of pectin extraction from other agricultural by-products.

MATERIALS AND METHODS

Plant Materials

Cocoa pods were purchased from Pusat Penyelidikan dan Pembangunan Koko Bagan Datuk, Perak. The pods were washed, the seeds were removed, and the pods were sliced using an industrial slicer. The sliced pods were then dried under the sun for 5 days to remove the moisture. The dried cocoa pod husks were ground using an industrial grinder into a powder and then sieved using a 1 mm mesh sieve. The cocoa pod husks were treated with 80% ethanol with ratio 1: 20 of ethanol for 30 minutes at 40 °C to remove tanning and other impurities. The washed cocoa pod husk was then dried in the drying cabinet at 40 °C for 24 hours. The treated cocoa pod husk was labelled as cocoa pod husk powder (CPHP) and stored in a -20°C freezer for further use.

Pectin Extraction

The screening of the parameter of extraction was conducted using a two-level factorial (2LFD). The three parameters-temperature, extraction time, and acid concentration were examined at two different levels (low and high level). Pectin extraction from CPHP was conducted using ultrasound-assisted methods with modification (Pagarra et al., 2019). The extraction started with 50 g of CPHP sonicated in 500 mL of acidic solution (HCL) at different parameters as in Table 1. After sonication, the mixture was filtered and stored at 4°C for further purification. The cooled mixture were precipitated with 95% ethanol for 24 hours, washed three times with 95% ethanol, and then dried in an oven at 50°C until a constant weight was reached, which was recorded as the weight of dry pectin per 100 g of CPHP. Data analysis and statistical evaluation of the factorial design were performed using Design-Expert software version 16.

Table 1: Independent variables of the experimental design for the extraction of pectin from CPHP.

Assay	Ph	Temperature (°C)	Duration (Minutes)	Pectin (g)	Yield (%)
1	2	60	10	2.20	4.40
2	4	60	10	1.83	3.65
3	4	60	30	3.49	6.98
4	2	60	30	5.30	10.60
5	4	80	10	2.49	4.98
6	4	80	30	4.10	8.20
7	2	80	10	3.29	6.58
8	2	80	30	6.25	12.5

Fourier Transform Infrared (FT-IR) Spectroscopy

The extracted pectin samples were analysed using FT-IR spectroscopy (Cary 630 FT-IR spectrometer, Agilent) within the wavelength range of 4000-600 cm^{-1} to observed the existence of the pectin functional groups (Kozioł et al., 2022). Commercial pectin from citrus grade CF 301 supplied by Take It Global Sdn. Bhd. served as a control.

RESULTS AND DISCUSSIONS

Two-Level Factorial Design

The presented data and ANOVA as in table 2 illustrates the impact of three crucial factors, mainly pH, temperature, and extraction duration, on the production of pectin obtained from cocoa pod husk. These parameters have a crucial role in determining the extraction efficiency and overall quality of pectin, a very useful biopolymer widely employed in the food, pharmaceutical, and cosmetic sectors because of its gelling, stabilising, and emulsifying characteristics (Maria *et al*., 2021).

Table 2. P-value for the yield of pectin from CPHP using 2LFD

Source	F-Value	P-Value
Model	683.11	0.0293
A: pH	776.45	0.0228
B: Temperature	377.62	0.0327
C: Time	2656.43	0.0124
AB	33.77	0.0185
AC	251.69	0.0401
BC	2.72	0.3471
$R^2 = 0.9844$		

Analyses were performed at two different pH levels: 2 and 4. The findings firmly demonstrate that a hyperacidic environment (pH 2) significantly improves the production of pectin. The greatest yield in the dataset was obtained in assay 8 (pH 2, 80°C, 30 minutes), with a value of 12.5%. This phenomenon can be attributed to the increased protonation of galacturonic acid units in the pectin structure under low pH conditions. At lower pH, the carboxyl groups (–COOH) of galacturonic acid are fully protonated, which disrupts the electrostatic interactions and calcium cross-linking between pectin and other components of the plant cell wall matrix, such as cellulose and hemicellulose. This leads to a loosening of the middle lamella and enhances the release of pectin (Zykwinska *et al*., 2005; Voragen *et al*., 2009). Additionally, acidic conditions promote partial hydrolysis of the glycosidic bonds linking pectin to the matrix, further facilitating its solubilisation (Dangi dan Yadav, 2020). Although pectin is generally less soluble in highly acidic solutions, the objective during extraction is not solubility, but rather efficient liberation from the plant tissue. Therefore, the enhanced release at pH 2 is due to the acid-induced breakdown of pectin–matrix interactions, not the increased solubility of pectin in the medium. In contrast, experiments carried out at pH 4 produced relatively lower results. Assay 2, conducted at pH 4, 60°C, for 10 minutes, yielded the lowest value of 3.65%. This drop in yield at higher pH is consistent with the reduced ability of less acidic environments to weaken the polymeric structures that bind pectin within the cell wall (Riyamol *et al*., 2023).

Thermal conditions are crucial in the extraction process as they influence both the solubility and hydrolysis rate of pectin. The experiments performed at 80°C displayed greater yields in comparison to those performed at 60°C, therefore showing that increased temperatures improve the efficiency of extraction. As an illustration, assay 8 at 80°C for 30 minutes at pH 2 demonstrated a yield of 12.5%, but assay 4 at 60°C for 30 minutes at pH 2 yielded 10.6%. The larger output at elevated temperatures is attributed to the augmented thermal energy, which expedites the breakdown of pectin from the plant cell walls and enhances the efficiency of disrupting the cell matrix (Wu *et al*., 2018). Moreover, elevated temperatures increase the solubility of pectin in the watery solution, thereby promoting its subsequent release and retrieval. Nevertheless, it is crucial to acknowledge that high temperatures might result in the deterioration of pectin, impairing its molecular weight and functioning. Hence, it is essential to maintain an ideal temperature during the extraction process in order to achieve a balance between the yield and the structural integrity of the extracted pectin.

Another critical determinant of pectin production is the length of the extraction cycle. Longer extraction durations were consistently associated with greater yields in all evaluations. Assays 4 and 8, conducted with extraction durations of 30 minutes, produced yields of 10.6% and 12.5% respectively. In contrast, assay 1 and 7, which lasted 10 minutes, reported far lower yields of 4.40% and 6.58%. These findings indicate that extended periods facilitate a more thorough breakdown of the plant material and a higher liberation of pectin into the water phase. Increased extraction durations enhance the energy and time available for the dissolution of pectin, resulting in increased yields. Significantly prolonged extraction durations can lead to the deterioration of pectin, especially when exposed to high temperatures, therefore reducing the quality and functional characteristics of the extracted pectin (Benito-Román *et al*., 2024). Hence, it is crucial to optimise the extraction time in order to achieve a desirable balance between the yield and the maintenance of pectin's molecular integrity.

There is an interaction when the level of one factor changes how the response is affected by another factor. In other words, a factor does not change the response in the same way at different levels of the other factor. If the value of one variable changes and the effect of the other variable changes, this is called an interaction between two factors. On the other hand, interactions between factors that are not important show the same patterns of response across different levels of another factor. The two-factor interaction effects are looked at in this study, and both significant and insignificant interactions are shown. The relationship between Factor A (pH) to Factor B (temperature) and factor C (time) respectively was significant at $\alpha = 0.05$. On the other hand, the relationship between Factor B (temperature) and factor C (time) was not significant. The important interaction shows that the pectin yield goes up a lot when the acid concentration is high, especially when the temperature is high. On the other hand, the fact that the interaction was not significant suggests that temperature changes have the same effect on pectin yield no matter how long the extraction takes. This shows how different combinations of factors can have different effects on the response variable. For example, some combinations can change the extraction efficiency in big ways, while others stay the same in all conditions.

FTIR Spectroscopy

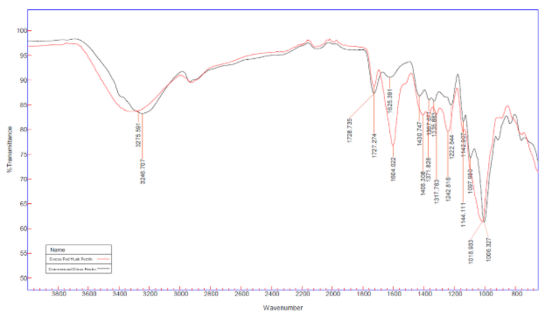

Figure 1: FTIR of Pectin from cocoa pod husk and commercial pectin from citrus

The Fourier Transform Infrared Spectroscopy (FTIR) method is a highly effective analytical approach employed for the identification of functional groups and the characterisation of the molecular structure of diverse substances, such as polysaccharides like pectin (Bayar *et al.*, 2016). Pectin is an intricate polysaccharide present in the cell walls of plants. Its extraction from agricultural by-products such as cocoa pod husk has attracted attention because of its considerable potential uses in the fields of food, medicines, and cosmetics.

The FTIR spectrum of pectin (Figure 1) usually displays distinct absorption bands associate with different functional groups found in the pectin chain (Kozioł *et al.*, 2022). The O-H stretching resonance (3200-3600 cm^{-1}): The prominent peak at 3246.707 cm^{-1} and 3245.591 cm^{-1} is caused by the O-H stretching vibrations of hydroxyl groups, which are plentiful in pectin because of its polysaccharide composition (Hong *et al.*, 2021). An analysis of the magnitude and range of this peak can provide valuable information on the extent of hydrogen bonding and the hydration condition of the pectin that CPH pectin may have stronger intermolecular hydrogen bonding or moisture retention. The absorption bands observed in the range of 2800-3000 cm^{-1} are associated with the C-H stretching vibrations occurring in the methyl and methylene groups included within the pectin backbone (Lu *et al.*, 2005).

The characteristic signal observed at 1700-1750 cm^{-1} corresponds to the esterified carbonyl groups (C=O) that are found in the galacturonic acid units of pectin (Hennessey-Ramos *et al.*, 2021). The spectrum obtained from cocoa pod husk pectin showed a moderate peak at 1727.274 cm^{-1}, suggesting the presence of esterified carboxyl groups. However, the intensity of this peak was noticeably lower compared to that observed in commercial pectin from citrus (1728.735 cm^{-1}) due to its higher level of methyl esterification (Mahmud *et al.*, 2021). Based on this observation, the cocoa pod husk pectin can be classified as low methoxy pectin (LMP)—defined as having a degree of esterification less than 50%, while citrus pectin is generally considered high methoxy pectin (HMP) with degree of esterification above 50% (Jarrín-Chacón *et al.*, 2023). LMPs form gels in the presence of calcium ions and low sugar concentrations, making them suitable for reduced-sugar or calcium-set food formulations. HMPs, by contrast, require high sugar and acidic environments for gelation. The identification of cocoa pod husk pectin as LMP implies distinct advantages for dietary or pharmaceutical applications where low-sugar, high-calcium settings are desired.

The peaks seen in the ranges of 1600-1650 cm^{-1} and 1400-1450 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of carboxylate anions (COO^{+}), respectively. The peaks in the spectrum indicate the level of methylation and the existence of unbound carboxylic acid groups in the pectin structure. Higher absorbance at this band in CPH pectin suggests a stronger interaction or higher concentration of free carboxylate groups in the sample or differences in molecular environment, such as hydrogen bonding or pectin conformation, which can enhance absorption intensity (Boakye-Gyasi *et al.*, 2016). This higher absorbance despite CPH pectin being low methoxy (lower degree of esterification) aligns with the expectation that LMPs have more free carboxyl groups available, resulting in stronger carboxylate signals in FTIR spectra. The absorption bands observed at 1100-1200 cm^{-1} are associated with the stretching vibrations of C-O-C bonds in glycosidic linkages and the stretching vibrations of C-O bonds in alcohol radicals.

In addition, minor peaks below 1500 cm^{-1} observed in cocoa pod husk pectin may be attributed to phenolic compounds or lignin derivatives, reflecting partial co-extraction of polyphenols or incomplete purification common due to the lignocellulosic nature of cocoa pod husks (Boeriu et al., 2004). These differences underline the need for further refinement or processing when aiming to substitute cocoa pod husk pectin for conventional pectin sources.

CONCLUSIONS

This research investigated the extraction of extract pectin from cocoa pod husk using a two-level factorial design (2LFD) to observe how pH, temperature, and extraction time affected the process. The analysis of the data, which includes both yield results and analysis of variance (ANOVA), shows that these factors have a big effect on pectin recovery. It was found that lower pH levels, higher temperatures, and longer extraction times all led to higher pectin yields. The highest yield of 12.5% was achieved at pH 2, 80°C, and 30 minutes of extraction. These results are supported even more by the statistical analysis, which shows that time has the biggest impact on pectin yield, followed by pH and temperature. The relationship between pH and temperature was also important, which suggests that the highest yield is achieved by combining acidic conditions with high temperature in the observation range.

ACKNOWLEDGMENTS

This project was carried out using the Development of Cocoa-Based Vegan Cosmetic Products. Special thanks to those who are involved in collecting data.

REFERENCES

Bayar, N., Kriaa, M., & Kammoun, R. (2016). Extraction and characterization of three polysaccharides extracted from Opuntia ficus indica cladodes. International journal of biological macromolecules, 92, 441–450. https://doi.org/10.1016/j.ijbiomac.2016.07.042

Benito-Román, Melgosa, R., Illera, A. E., Sanz, M. T., & Beltrán, S. (2024). Kinetics of extraction and degradation of pectin derived compounds from onion skin wastes in subcritical water. *Food Hydrocolloids*, *153*(December 2023). https://doi.org/10.1016/j.foodhyd.2024.109957

Boakye-Gyasi, M. E., Ofori-kwakye, K., & Sasu, C. (2016). Physicochemical and Antimicrobial Properties of Cocoa Pod Husk Pectin Intended as a Versatile Pharmaceutical Excipient and Nutraceutical. Journal of Pharmaceutics. https://doi.org/10.1155/2016/7608693

Boeriu, C. G., Bravo, D., Gosselink, R. J. A., & Van Dam, J. E. G. (2004). Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. *Industrial Crops and Products*, *20*(2), 205–218. https://doi.org/10.1016/j.indcrop.2004.04.022

Chandel, V., Biswas, D., Roy, S., Vaidya, D., Verma, A., & Gupta, A. (2022). Current Advancements in Pectin: Extraction, Properties and Multifunctional Applications. *Foods*, *11*(17), 1–30. https://doi.org/10.3390/foods11172683

Dangi, N., & Yadav, B. S. (2020). Characterization of partial acid hydrolysates of citrus pectin for their pasting, rheological and thermal properties. Journal of food science and technology, 57(7), 2681–2692. https://doi.org/10.1007/s13197-020-04304-w

Das, A. K., & Dewanjee, S. (2018). Optimization of Extraction Using Mathematical Models and Computation. In *Computational Phytochemistry*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-812364-5.00003-1

Gawkowska, D., Cybulska, J., & Zdunek, A. (2018). Structure-related gelling of pectins and linking with other natural compounds: A review. *Polymers*, *10*(7). https://doi.org/10.3390/polym10070762

Hennessey-Ramos, L., Murillo-Arango, W., Vasco-Correa, J., & Astudillo, I. C. P. (2021). Enzymatic extraction and characterization of pectin from cocoa pod husks (Theobroma cacao L.) using celluclast® 1.5 L. *Molecules*, *26*(5). https://doi.org/10.3390/molecules26051473

Hong, T., Yin, J. Y., Nie, S. P., & Xie, M. Y. (2021). Applications of infrared spectroscopy in polysaccharide structural analysis: Progress, challenge and perspective. *Food Chemistry: X*, *12*(November),100168. https://doi.org/10.1016/j.fochx.2021.100168

Jarrín-Chacón, J. P., Núñez-Pérez, J., Espín-Valladares, R. D. C., Manosalvas-Quiroz, L. A., Rodríguez-Cabrera, H. M., & Pais-Chanfrau, J. M. (2023). Pectin extraction from residues of the cocoa fruit (Theobroma cacao L.) by different organic acids: A comparative study. Foods, 12(3), 590.

Kozioł, A., Środa-Pomianek, K., Górniak, A., Wikiera, A., Cyprych, K., & Malik, M. (2022). Structural Determination of Pectins by Spectroscopy Methods. *Coatings*, *12*(4). https://doi.org/10.3390/coatings12040546

Lu, R., Gan, W., Wu, B. H., Zhang, Z., Guo, Y., & Wang, H. F. (2005). C-H stretching vibrations of methyl, methylene and methine groups at the vapor/Alcohol (n = 1-8) interfaces. *Journal of Physical Chemistry B*, *109*(29), 14118–14129. https://doi.org/10.1021/jp051565q

Mahmud, S., Hisham, H., Rizki, M., & Erwinda, R. (2021). Microwave-assisted extraction of pectin from pomelo, kinnow mandarin, and citron peels. Food Research, 5(5), 80–88.

Maria, C., Freitas, P., Jane, S., Gomes, V., Souza, L., & Rita, C. (2021). Maria, C., Freitas, P., Jane, S., Gomes, V., Souza, L., & Rita, C. (2021). and Pharmaceutical Industry : A Review. Coatings, 11(922), 1–22. *Coatings*, *11*(922), 1–22.

Pagarra, H., Rahman, R. A., Muis, A., & Novia Arifin, D. A. (2019). Screening of factors influencing Pectin Extraction of Pomelo peels using 2-Level Factorial Design. *IOP Conference Series: Materials Science and Engineering*, *551*(1). https://doi.org/10.1088/1757-899X/551/1/012127

Porto de Souza Vandenberghe, L., Kley Valladares-Diestra, K., Amaro Bittencourt, G., Fátima Murawski de Mello, A., Sarmiento Vásquez, Z., Zwiercheczewski de Oliveira, P., Vinícius de Melo Pereira, G., & Ricardo Soccol, C. (2022). Added-value biomolecules' production from cocoa pod husks: A review. *Bioresource Technology*,*344*(October2021). https://doi.org/10.1016/j.biortech.2021.126252

Raj, S. (2012). *A Review on Pectin: Chemistry due to General Properties of Pectin and its Pharmaceutical Uses*. *March 2021*. https://doi.org/10.4172/scientificreports.550

Riyamol, N., Gada Chengaiyan, J., Rana, S. S., Ahmad, F., Haque, S., & Capanoglu, E. (2023). Recent Advances in the Extraction of Pectin from Various Sources and Industrial Applications. *ACS Omega*, *8*(49), 46309–46324. https://doi.org/10.1021/acsomega.3c04010

Roman-Benn, A., Contador, C. A., Li, M. W., Lam, H. M., Ah-Hen, K., Ulloa, P. E., & Ravanal, M. C. (2023). Pectin: An overview of sources, extraction and applications in food products, biomedical, pharmaceutical and environmental issues. *Food Chemistry Advances*, *2*(January), 100192. https://doi.org/10.1016/j.focha.2023.100192

Spinei, M., & Oroian, M. (2022). The Influence of Extraction Conditions on the Yield and Physico-Chemical Parameters of Pectin from Grape Pomace. *Polymers*, *14*(7). https://doi.org/10.3390/polym14071378

Wu, H. C., Bulgakov, V. P., & Jinn, T. L. (2018). Pectin methylesterases: Cell wall remodeling proteins are required for plant response to heat stress. *Frontiers in Plant Science*, *871*(November), 1–21. https://doi.org/10.3389/fpls.2018.01612