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Maximising pitaya (*Hylocereus polyrhizus*) peel pectin yield through cellulase-assisted extraction: A study on enzyme optimisation

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Received September 15, 2025, Accepted in revised form October 27, 2025
Available online October 28, 2025
DOI: 10.24191/bioenv.v3i3.121

ABSTRACT. Red-purple pitaya (*Hylocereus polyrhizus*) peel is a rich source of plant pigment betacyanins and dietary fibre specifically pectin. Pectin's multifunctional properties enable its use in the healthcare industry. This study investigated the optimum conditions for cellulase enzyme to release maximum pectin from pitaya peel puree. Parameters such as cellulase concentration (50 - 300 µL/100 g of puree), pH (3 - 5.4), time (30 - 180 min) and temperature (25 - 50 °C) were varied for cellulase optimisation followed by pectin extraction using water extraction method and pectin yield determination. The optimal conditions for maximising pectin extraction from pitaya peel were a cellulase concentration of 100 µL per 100 g of puree, a pH of 5.40, a temperature of 37 °C, and a pre-treatment time of 120 minutes. At these optimum conditions for pre-treatment of puree with cellulase, 22.30% of pectin was extracted from pitaya peel pectin using distilled water at pectin extraction conditions reported in our previous study (temperature: 73 °C, time: 67 min, sample to solvent ratio: 1:4). When extraction was performed using varying concentrations of citric acid (0.5 - 2.0%) solutions, a maximum pectin yield (24.63%) at 1.5% citric acid concentration was noted. Based on the proximate composition, functional groups and degree of esterification findings, cellulase-assisted extraction demonstrates strong potential to convert fruit peels into valuable pectin.

Keywords: cellulase, citric acid, enzymatic extraction, pectin, pitaya peel

1. INTRODUCTION

Pectin is a complex heteropolysaccharide that co-exists with cellulose, hemicellulose, lignin, protein and other polysaccharides in the primary cell walls and middle lamella of terrestrial plants (Pang et al., 2024; Riyamol et al., 2023). Pectin is mainly composed of α-1,4-D-galacturonic acid and categorised into four major structural domains, which are homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, and xylogalacturonan (Assifaoui et al., 2024). Pectin's composition and structure are diverse and can vary significantly among different plant species, contributing to a wide array of techno-functional and health properties in food and healthcare applications (Assifaoui et al., 2024; Pang et al., 2024).

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Commercial pectins are extracted from apple pomace, citrus peel and sugar beet pulp, which have consistent characteristics that meet industry demands. However, exploration of novel pectin sources and extraction methods is growing, driven by sustainability initiatives, cost reduction, and the valorisation of agrowaste residues. Particularly, increasing research trend on pectin extraction from fruit pomace, core, seed, peel and rind is noted over the years (Dixit et al., 2025; Sharma et al., 2025). A relatively high pectin recovery (more than 25%) from the waste of tropical fruits such as mango, papaya, watermelon, jack fruit, passion fruit and pitaya fruit has been reported (Marenda et al., 2019; Sharma et al., 2025).

Recent research focuses on green pectin extraction methods, including ultrasound-assisted, microwave-assisted, enzyme-assisted, subcritical water, pressurised carbon dioxide, high-pressure, ohmic heating and any combinations of these methods, as sustainable alternatives (Rawat et al., 2024; Roman-Benn et al., 2023). This is because the conventional method of extracting pectin using mineral acids poses limitations, such as longer extraction time, lower extraction efficiency, degradation of pectin quality and environmental concerns (Dixit et al., 2025; Arumuganainar et al., 2025; Sarangi et al., 2023).

In our previous study, we used a milder extractant (1% of citric acid) and 14.4% pectin was obtained from the fresh whole peel of red-purple pitaya fruit, also known as dragon fruit (*Hylocereus polyrhizus*) (Muhammad et al., 2014). Numerous research studies have underscored the potential of pitaya peel as an alternative source of pectin using individual or combined pectin extraction methods (Chen et al., 2021; Chua et al., 2020; Costa et al., 2022; Nguyen & Pirak, 2019; Thu Dao et al., 2021; Zaid et al., 2020). However, to date, the effect of enzymatic pre-treatment of red-purple pitaya peel using cellulase as a catalyst to maximise pectin yield has not been studied. The cellulase enzyme can disrupt the linkages within the plant cell wall, promoting the release of pectin (Gao et al., 2024). The utilisation of cellulase was found to increase the pectin yield significantly from fruit peels (Costa et al., 2022).

Therefore, in this study, the optimum conditions (temperature, time, pH and enzyme concentration) for the cellulase enzyme to release maximum pectin from red-purple pitaya peel puree were determined for the first time, providing novel insights into enzyme-assisted pectin recovery from this underutilised fruit by-product. Additionally, the identified optimum conditions of cellulase were applied for cellulase-assisted citric acid extraction to compare the pectin yield with that of the cellulase-assisted water extraction method.

2.0 METHODOLOGY

2.1 Material

Commercially matured red-purple pitaya fruits (*Hylocereus polyrhizus*) with an average weight of 350 to 450 g per fruit (Grade A) were purchased from a local farm in Selangor, Malaysia.

2.2 Chemicals

Citric acid (Sigma Aldrich, USA), 95% undenatured ethanol (Merck, Germany) and cellulase from *Aspergillus* sp. with an activity of ≥1000 units/g (C2605, Sigma Aldrich, USA) were purchased from a local chemical supplier in Selangor, Malaysia. The chemicals used for proximate analyses were of analytical grade, with purities ranging from

95% to 99.5%.

2.3 Sample Preparation

Pitaya fruits were washed, dried, and manually peeled. The peels were cut, blanched for 2 min, cooled, and homogenised using a Waring blender. The puree was then packed and frozen for subsequent analysis. The puree was frozen at -20 ℃ to preserve its nutritional, physicochemical and microbiological qualities for extended stability.

2.4 Pre-Treatment of Pitaya Peel Puree with Cellulase Enzyme

Frozen pitaya peel puree was thawed overnight in a chiller at 4 °C before pre-treating with cellulase enzyme (C2605). An aliquot of cellulase was added to the thawed pitaya peel puree and parameters such as cellulase concentration, pH, time and temperature were manipulated to identify the optimum condition for cellulase to release maximum pectin from the cell wall matrix of pitaya peel. The pre-treatment of pitaya peel puree with cellulase at different conditions was carried out in Schott bottles placed in a shaking water bath set at 200 rpm. Deactivation of the cellulase was done at 95 °C for 5 min before the pectin extraction step (Figure 1), as adapted from Gao et al. (2024).

2.5 Determination of Optimum Conditions for Cellulase to Maximise Pectin Yield

In general, cellulase from *Aspergillus niger* is active up to 90 °C within the pH range of 3 and 8. However, cellulase is applied in the pre-treatment step for pectin extraction within a pH range of 3 to 6, and a temperature range of 30 °C to 70 °C (De Laet et al., 2025; Dominiak et al., 2014).

2.5.1 Cellulase Concentration

In this study, the effect of cellulase concentration on the pectin yield was determined by varying the cellulase concentration levels (0, 50, 100, 200 and 300 μL/100 g of pitaya peel puree) while other variables such as temperature (37 °C), time (60 min) and pH (5.4) were kept constant.

2.5.2 pH

The optimal pH was determined by pre-treating the puree with cellulase at different pH levels (3.0, 4.0, 5.0 and 5.4) with other variables kept constant (temperature: 37 °C, time: 60 min, cellulase concentration: 100 uL/100 g of pitaya peel puree). In this study, a pH of 5.4, corresponding to the natural pH of pitaya peel puree, was included alongside the typical pH ranges used in previous studies (pH 3.0 to 5.0). The pH of the thawed puree was adjusted to 3.0, 4.0 and 5.0 using 1% of citric acid. More extreme acidic or alkaline pH levels were not evaluated, as they could potentially reduce the overall quality of the extracted pectin.

2.5.3 Time and Temperature

The optimal combination of extraction times (30, 60, 90, 120 and 180 min) and temperatures (25, 37 and 50 °C) for maximum pectin release was studied at pH 5.4 and a cellulase concentration of 100 uL/100 g of pitaya peel puree. The temperature of 25 °C was selected to represent room temperature for convenience and energy efficiency. The 37 °C condition was included as cellulase exhibits maximum activity around this temperature within a pH range of 3

to 5 for general applications. Meanwhile, 50 °C was chosen as it is the most commonly used temperature in pectin extraction studies employing cellulase.

2.6 Extraction of Pitaya Peel Pectin and Yield Calculation

After pre-treatment of pitaya peel puree with cellulase, pectin extraction using the water extraction method was carried out following the extraction conditions reported in our previous study (temperature: 73 °C, time: 67 min, sample to solvent ratio of 1:4) (Muhammad et al., 2014). Precipitated pectin was washed with 95% undenatured ethanol, centrifuged, and dried in a drying oven (Memmert, Schwabach, Germany) at 50 °C for 16 h. **Figure 1** shows the flow of the pre-treatment of pitaya peel puree with cellulase, followed by the pectin extraction process using the water extraction method. Cellulase-assisted citric acid extraction was also performed to compare the pectin yield obtained when the cellulase-assisted water extraction method was used.

The pectin yield was determined using the following equation:

Y (%) = [Weight of dried pectin (g) / Weight of pitaya peel puree (g)] x 100

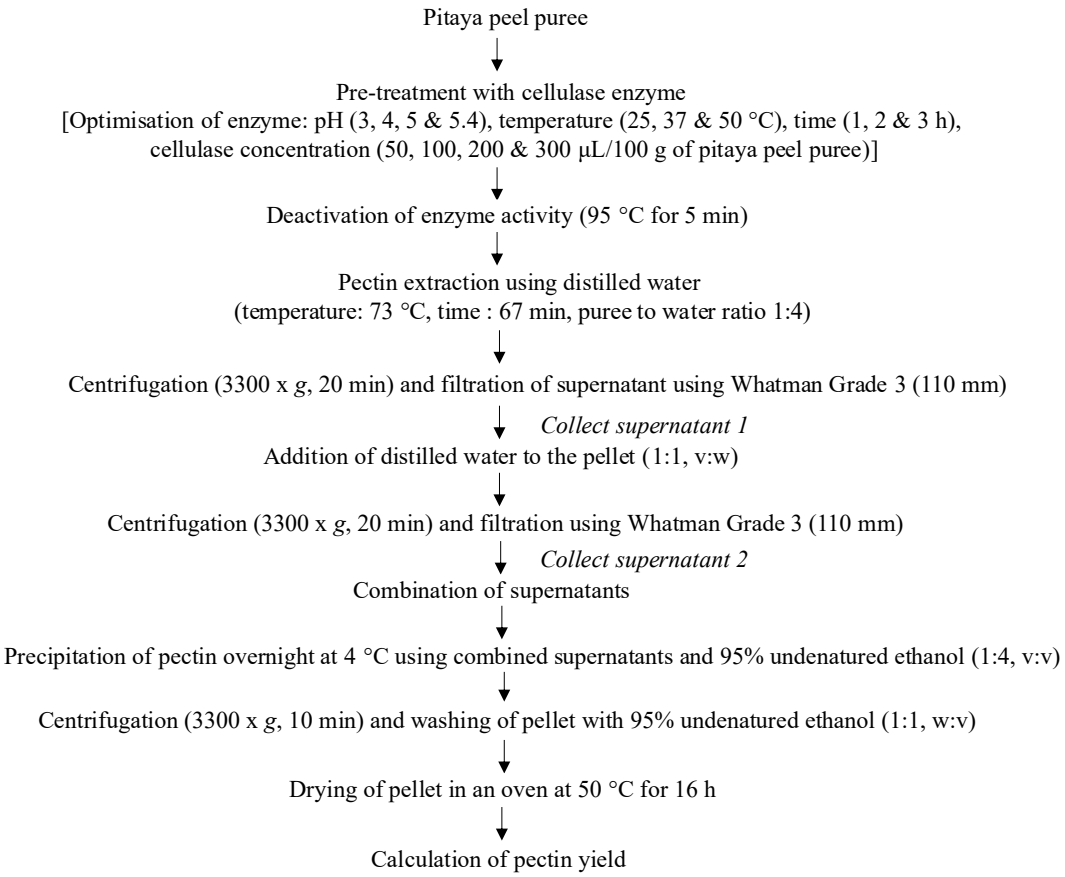

Figure 1. Flowchart of pitaya peel puree pre-treatment with cellulase and the subsequent extraction of pectin using distilled water.

2.7 Functional Groups Identification and Degree of Esterification Determination

The Fourier transform infrared (FT-IR) spectra of water extracted pectin using cellulase pre-treated pitaya peel puree under optimised conditions and commercial citrus pectin (LC-S18) were collected using a Spectrum 100 FT-IR spectrophotometer (Perkin-Elmer, United States) in the wavenumber range from 4000 to 400 cm^{-1}. The degree of esterification (DE) was calculated based on the data from FT-IR spectra according to the method of Gannasin et al. (2015).

2.8 Proximate Analysis of Pitaya Peel and Pectins Recovered via Cellulase-Assisted Extraction

Moisture and ash contents were determined according to AOAC Methods 934.01 and 942.05, respectively (AOAC, 2005). The protein content was estimated as % nitrogen × 6.25 (conversion factor) following the Kjeldahl method (AOAC Method 978.04). The Megazyme Total Starch Assay kit was used to determine the total starch content in accordance with AOAC Method 996.11 (AOAC, 2005). Dietary fibre was measured using the Megazyme Total Dietary Fibre Assay kit, which is consistent with AOAC Method 991.43 (AOAC, 2005). Each experiment was carried out in triplicate.

2.9 Statistical Analysis

Data from triplicate analyses were presented as mean ± standard deviation (SD). Statistical differences among treatment means were evaluated using analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test at a 95% confidence level. Differences were considered significant when p-value < 0.05. The statistical analyses were performed using Minitab statistical package software (Version 16, Minitab Inc., PA, USA).

3.0 RESULTS AND DISCUSSION

3.1 Effect of Cellulase Concentration Levels Used for Pre-Treatment of Pitaya Peels on Pectin Yield

Figure 2 shows the yield of pitaya peel pectin when extracted at four different enzyme concentrations at 37 °C for 60 min, at pH 5.4 (the natural pH of pitaya peel puree). A significant change (p-value < 0.05) in pectin yield was observed when the cellulase concentration was varied, with yield ranging from 17.72 to 20.99%. The highest pectin yield was observed at an enzyme concentration of 100 µL/100 g, indicating this cellulase concentration provided sufficient enzymatic activity to effectively hydrolyse cellulose, thereby loosening the cell wall matrix and enhancing pectin solubilisation. Meanwhile, a lower pectin yield was noted at a lower enzyme concentration (50 µL/100 g) or in the absence of cellulase, which could be explained by an incomplete extraction of pectin. Beyond cellulase concentration of 100 µL/100 g, the yield of pectin levels off with increasing enzyme concentration. This may be due to most of the accessible cellulose, which intertwined with pectin already being hydrolysed (Gao et al., 2024).

3.2 Effect of pH Levels Used for Pre-Treatment of Pitaya Peels on Pectin Yield

Besides the effect of cellulase concentration, the percentage of pitaya peel pectin yield was measured in response to varying pH, ranging from 3 to 5.4, to determine the optimum pH for the pre-treatment of puree with cellulase enzyme. The experiment was carried out at 37 °C for 60 min at a cellulase concentration of 100 uL/100 g of pitaya peel puree. The highest pectin yield of 20.54% (w/w) was obtained at pH 5.4, which is the natural pH of the pitaya peel puree.

The effect of pH on pectin yield was not significant (Figure 3), with the variance in the amount of pectin obtained between pH 3 and pH 5.4 being only 0.98%. This suggests that pH had minimal effect or no significant effect on the pitaya peel pectin yield within the pH range of 3 and 5.4.

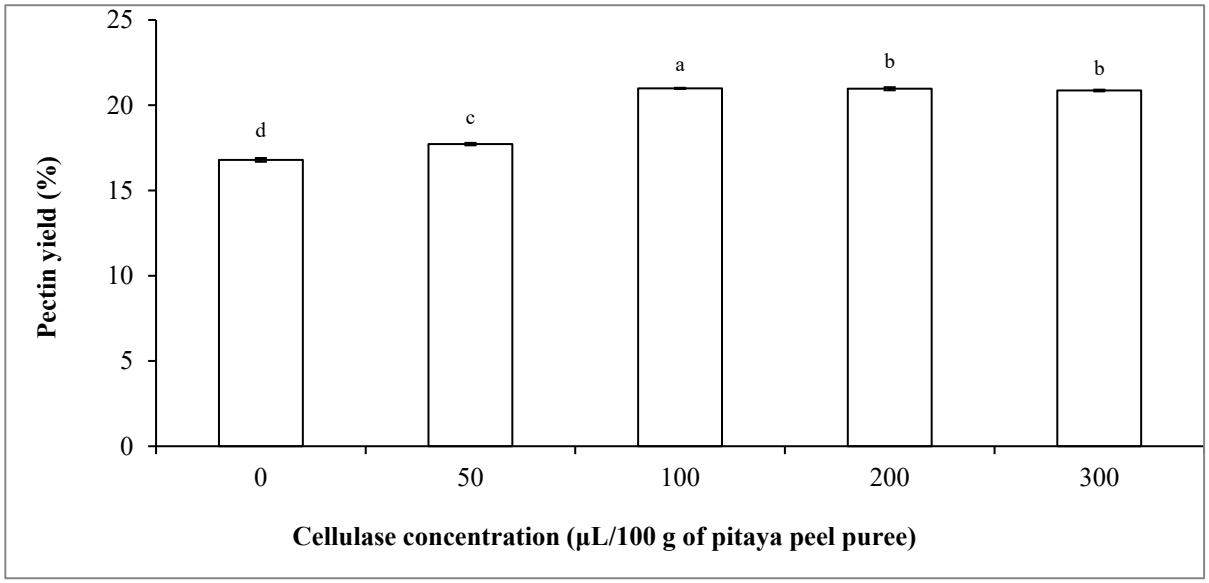

Figure 2. Effect of cellulase concentration on pitaya peel pectin yield with constant variables: temperature (37 °C), time (60 min) and pH (5.4).
Each value is presented as the mean ± standard deviation (n = 3) from triplicate analyses.
[a–d] Different lowercase superscript letters denote significant differences (p-value < 0.05) in pectin yield.

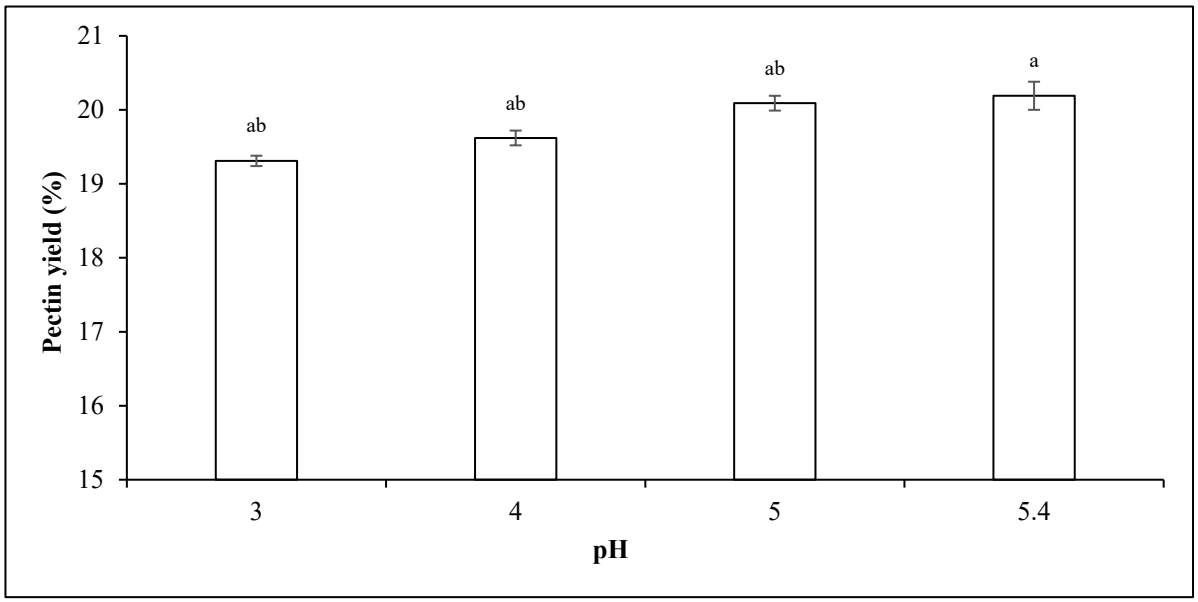

Figure 3. Effect of pH on pitaya peel pectin yield with constant variables: temperature (37 °C), time (60 min) and cellulase concentration (100 uL/100 g of pitaya peel puree).
Each value is presented as the mean ± standard deviation (n = 3) from triplicate analyses.
[a–b] Different lowercase superscript letters denote significant differences (p-value < 0.05) in pectin yield.

3.3 Effect of Time and Temperature Levels Used for Pre-Treatment of Pitaya Peels on Pectin Yield

The optimum temperature and time for the enzymatic pre-treatment of pitaya peel puree for high pectin yield were determined by experimenting with different combinations of temperature (25, 37, 50 °C) and time (30, 60, 90, 120,

180 min), while other variables were held constant (cellulase concentration 100 μL/100 g of peel puree and pH 5.4). In contrast to pH, the variation in temperature and time significantly affected the yield of pitaya pectin (p-value < 0.05), as illustrated in Figure 4. The increase in temperature caused a positive effect on pectin yield up to 37 °C, but showed a reduction when it reached 50 °C. This may have resulted from the degradation of pectin and the denaturation of enzymes at elevated temperature. In terms of time, pectin yield increases with longer time. A maximum yield of 23.13%. (w/w) was achieved from cellulase hydrolysis of pitaya peel in 120 min, as longer times enhance enzyme activity until the substrate becomes limiting, hence releasing more pectin (Mada et al., 2022). However, when the time is extended to 180 min, the yield reduced, possibly due to enzyme inefficiency and pectin degradation at extended time. A moderate temperature of 37 °C and a relatively long incubation (120 min) is optimal for cellulase to release maximum pectin from pitaya peel puree. Enzymatic extraction at lower temperatures can reduce energy consumption and enhance pectin quality compared to the conventional hot mineral acid extraction method.

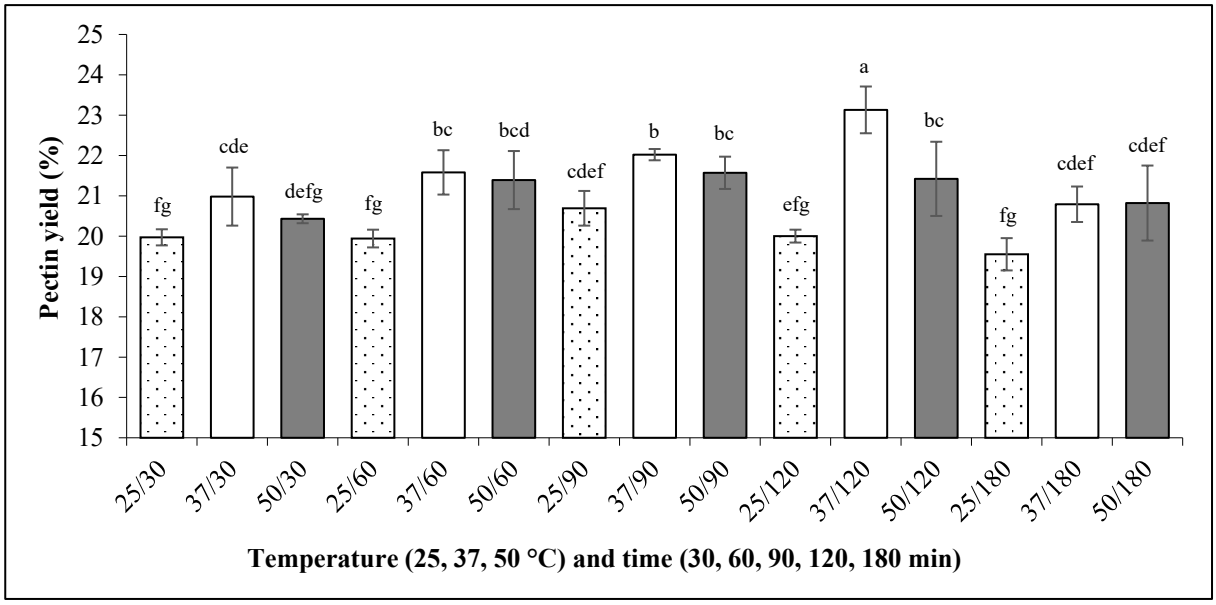

Figure 4. Effect of temperature and time combination on pitaya peel pectin yield with constant variables: cellulase concentration (100 uL/100 g of pitaya peel puree) and pH (5.4).
Each value is presented as the mean ± standard deviation (n = 3) from triplicate analyses.
[a-g] Different lowercase superscript letters denote significant differences (p-value < 0.05) in pectin yield.

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3.4 FT-IR Spectra and Degree of Esterification of Extracted and Commercial Pectins

The FTIR spectrum of the extracted pectin (Figure 5) shows a broad O–H stretching band from 3000 to 3600 cm^{-1}, indicating abundant hydroxyl groups and bound water. The weak ester carbonyl peak near 1735 cm^{-1} and the strong carboxylate band at 1610 cm^{-1} confirmed a lower degree of esterification (26%) compared to that of the commercial low methoxyl pectin (40%). Both samples exhibited characteristic C–O–C and C–O stretching vibrations between 1300 and 800 cm^{-1}, a fingerprint region representing the pectin backbone of galacturonic acid units. Overall, the spectra verified that the extracted pectin possessed the typical structural features of pectin with a lower methoxyl content (Ma et al., 2025).

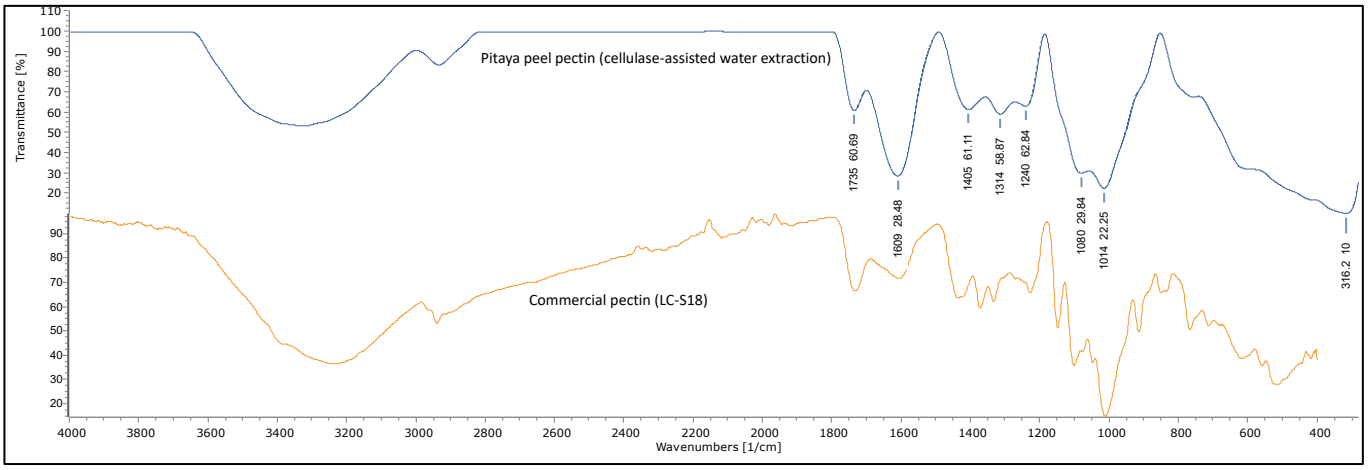

Figure 5. FT-IR spectra of cellulase pre-treated pitaya peel puree pectin extracted using distilled water and commercial pectin (LC-S18).

3.5 Effect of Cellulase Pre-treated Pitaya Peel Puree Under Optimised Conditions on the Yield of Pectin Extracted Using Water and Citric Acid

The optimum conditions identified for cellulase enzyme to facilitate maximum release of pectin from pitaya peel puree are cellulase concentration of 100 uL/100 g of pitaya peel puree, pH 5.4, temperature 37 ℃ and time 120 min. Water extraction of pectin using pitaya peel puree pre-treated with cellulase enzyme under the identified optimum conditions resulted in a pectin yield of 22.3%. Citric acid extraction of pectin from pre-treated pitaya peel puree yielded pectin of approximately 23.5%, 24.1%, 24.6%, and 24.1% for citric acid concentration levels of 0.5%, 1.0%, 1.5%, and 2.0%, respectively. Citric acid concentration of 1.5% resulted in the highest pectin yield of 24.6% when the extraction of pectin was performed using our previously reported conditions (temperature: 73 ℃, time: 67 min, and sample to citric acid ratio of 1:4 (w/v)) (Muhammad et al., 2014). Without the use of cellulase, only 14.4% pectin could be extracted from whole fresh pitaya peel puree using the same extraction conditions.

3.6 Proximate Content of Pitaya Peel and Extracted Pectins

The proximate composition of pitaya peel and its extracted pectin fractions varied depending on the extraction solvent (water or citric acid), as displayed in Table 1. Moisture and dry matter values were relatively consistent, while ash, fat, and protein contents decreased substantially in extracted pectins compared to raw peel, reflecting removal of non-pectic components during extraction. Total starch was drastically reduced in all pectin fractions (< 0.5 g/100 g) compared to the peel (2.92 g/100 g), indicating effective separation of starch. Dietary fibre composition shifted markedly with raw peel containing predominantly insoluble dietary fibre (42.23 g/100 g), whereas extracted pectins were dominated by soluble dietary fibre (69.5–84.0 g/100 g). The highest soluble fibre was obtained at 1.5% citric acid extraction (C1.5CA), while total dietary fibre content was also maximised under this condition (85.41 g/100 g). These results suggest that cellulase-assisted citric acid extraction not only enriches soluble fibre fractions but also influences protein and ash retention depending on acid concentration. Protein bound to pectin can play an important role in stabilising and activating its functional properties, making protein content a key factor for food applications such as emulsions (Mada et al., 2022).

Table 1. Proximate composition of pitaya peel and the extracted pectins based on dry weight basis (g/100g)

Parameter	Pitaya peel (g/100g)	Pitaya peel pectins (g/100g)				
		CDW	C0.5CA	C1.0CA	C1.5CA	C2.0CA
Moisture	7.55±0.03	7.32^{b}±0.08	6.93^{d}±0.12	7.10^{c} ±0.09	7.25^{b}±0.05	7.77^{a}±0.00
Dry matter	92.45 ±0.03	92.68^{c} ±0.08	93.07^{a}±0.12	92.90^{b}±0.09	92.75^{c}±0.05	92.23^{d}±0.00
Ash	9.26±0.09	8.03^{a}±0.04	6.53^{c}±0.14	6.75^{b} ±0.14	5.57^{d}±0.05	6.40^{c}±0.09
Fat	0.70± 0.01	ND	ND	ND	ND	ND
Protein	4.39 ±0.24	0.92^{e}±0.02	1.63^{b}±0.01	1.55^{c}±0.02	1.34^{d}±0.02	0.86^{e}±0.00
Total starch	2.92±0.02	0.19^{e}±0.01	0.41^{b}±0.03	0.49^{b} ±0.01	0.45^{b}±0.01	0.2^{c}±0.00
Soluble dietary fibre	13.85±0.12	69.5^{c}±0.20	76.37^{b}±0.41	76.29^{b}±0.32	84.01^{a}±0.96	60.37^{d}±0.95
Insoluble dietary fibre	42.23±0.10	4.07^{c}±0.79	2.61^{d}±0.1	2.61^{d}±0.1	1.41^{e}±0.79	8.78^{b}±0.12
Total dietary fibre	56.08 ±0.17	73.57^{c} ±0.96	78.98^{b}±0.34	78.96^{b}±0.96	85.41^{a}±0.83	69.15^{c}±0.96

$^{a–e}$ Different lowercase superscript letters denote significant differences (p-value < 0.05) in the composition of pitaya peel pectins.
Note: Pitaya peel pectins were extracted using cellulase-assisted extraction with distilled water (CDW), 0.5% citric acid (C0.5CA), 1.0% citric acid (C1.0CA), 1.5% citric acid (C1.5CA), and 2.0% citric acid (C2.0CA).
ND: not detected.

4.0 CONCLUSION

This study demonstrated that cellulase-assisted extraction effectively maximises pectin yield from pitaya (*Hylocereus polyrhizus*) peel under optimised conditions. The optimum parameters for pitaya peel puree enzymatic pre-treatment were identified as a cellulase concentration of 100 µL/100 g peel puree, pH 5.4, incubation at 37 °C for 120 min. Compared to conventional water or citric acid extraction methods alone, enzymatic pre-treatment of puree significantly improved yield, with citric acid extraction at 1.5% providing the highest pectin recovery (24.6%). The soluble fibre and protein contents of the extracted pectins further demonstrate that the cellulase-assisted extraction method can yield pectins suitable for potential use in food applications such as emulsions and milk gels. Overall, the findings confirm that enzyme-assisted extraction not only improves efficiency and sustainability but also produces good-quality pectin, making pitaya peel a valuable source for functional food ingredients.

5.0 STUDY SIGNIFICANCE, LIMITATIONS AND FUTURE WORK

This study provides the first insight into the optimum cellulase-assisted conditions for maximising pectin recovery from red-purple pitaya peels. Besides red-purple pitaya peels, this study also establishes parameter ranges that can guide future optimisation studies on enzyme-assisted pectin extraction from fruit peels of comparable composition. However, there are two main limitations of this study. First, this study is limited by the focus on cellulase alone, without evaluating other enzymes (e.g., pectinase, hemicellulase, endopolygalacturonase, xylanase) or synergistic multi-enzyme systems that may promote pectin release. Furthermore, this study did not investigate the potential application of other non-conventional pectin extraction techniques, such as ohmic heating, high-pressure, ultrasound, or microwave-assisted methods, following enzymatic pretreatment of the pitaya peel. Future studies should investigate the comparative efficiency of individual and combined enzyme systems, alongside other non-conventional extraction methods, to achieve enhanced pectin recovery and functionality.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Universiti Putra Malaysia, Serdang, Malaysia, for its support of this research.

AUTHOR CONTRIBUTIONS

Majida Al-Ezzi carried out the investigation and prepared the original draft of the manuscript. Kharidah Muhammad contributed to the conceptualisation, provided resources, and supervised the work. Sri Puvanesvari Gannasin was involved in conceptualisation, methodology, supervision, validation, visualisation, writing and editing the final manuscript. Radhiah Shukri provided supervision.

FUNDINGS

Majida Al-Ezzi, the first author, extends her gratitude to the Ministry of Higher Education and Scientific Research in Iraq for sponsoring her doctoral studies.

DATA AVAILABILITY

Not available

COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable

SUPPLEMENTARY MATERIAL

Not available

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