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# Optimization of Pectin Extraction from Dragon Fruit Peel using Cellulase Enzyme and Microwave-Assisted Method

Hoang Nguyen, Thanh Truc Tran*

Department of Biotechnology, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City, Vietnam
ttructhanh@hcmut.edu.vn

Vietnam's tropical monsoon climate provides ideal conditions for cultivating and exporting fruits, particularly dragon fruit (*Hylocereus spp.*). This study aims to utilize agricultural waste from dragon fruit to develop affordable fiber supplement tablets that support Vietnamese health. Pectin was extracted using varying concentrations of cellulase enzyme (0 %, 0.4 %, and 0.8 % (wt %)) combined with microwave treatment durations of 0 s, 30 s, and 60 s, and extraction times of 60 min, 80 min, and 100 min. A fractional factorial design was employed to evaluate the effects of these three factors on pectin yield, and response surface methodology (RSM) was applied to optimize the extraction process. Findings indicated that extraction time had no significant effect on pectin yield, while microwave duration and enzyme concentration were influential factors. The RSM model demonstrated strong predictive accuracy with an $R^2$ value of 0.9334. The optimal conditions were identified as a microwave treatment time of 48.05 s and an enzyme concentration of 0.6396 % (wt %), yielding a predicted pectin recovery of 27.026 %, closely matching the actual yield of 27.24 %. Furthermore, the extracted pectin exhibited inhibitory activity against alpha-amylase with an $IC_{20}$ =1,465.26 µg/mL and shows free radical scavenging activity against ABTS radicals with an $IC_{50}$ = 171.6 µg/mL. The FT-IR spectra indicated that the extracted pectin shares a similar chemical structure with commercial pectin.

## 1. Introduction

The dragon fruit industry produced over 840,000 t in the first 10 months of 2024 (Mai et al., 2025), resulting a massive volume of by-products from dragon fruit being discarded. Despite this, research on their utilization remains limited. Concurrently, the increasing demand for dietary fiber supplements, driven by busy lifestyles and insufficient vegetable intake, presents an opportunity.

Dragon fruit peel contains high concentration of pectin, but extracting it requires breaking down the cellulose wall. This study combines cellulase enzyme to break β-1,4-glucosidic bonds of cellulose wall (Hamid et al., 2015) due to its environmental friendliness (Zaidel et al., 2017) with microwave treatment to break hydrogen bonds between cellulose (Jabareen et al., 2021). By combining both methods, extraction time and enzyme concentration can be reduced, ensuring profitability while protecting the environment.

Research on extracting pectin from dragon fruit peel is opening up a new direction to meet market demand for fiber-enriched products with many potent bioactivities like aiding in weight management and free radical scavenging activity. Utilizing agricultural by-products can offer consumers reasonable prices while also contributing to solving the issue of surplus agricultural waste.

## 2. Material and method

### 2.1 Material

The dragon fruit peel was gathered from local market in Ho Chi Minh City. After, it was cleaned and dried at 60 ℃ until the moisture content dropped below 10 %. The dried peel was then homogenized and sieved using a 0.3 mm mesh. The cellulase enzyme used in the study was derived from *Aspergillus niger* (Sigma-Aldrich).

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### 2.2 Pectin extraction via cellulase enzymatic hydrolysis combined with microwave-assisted treatment

Experimental design for investigating the effects of enzyme concentration, extraction time and microwave time on pectin yield using fractional design. The experiment was arranged using completely randomized design with three factors and three levels: Enzyme concentration = 0 %, 0.4 % and 0.8 % (wt %), microwave time = 0 s, 30 s and 60 s and extraction time = 60 min, 80 min and 100 min. Dragon fruit peel dried powder was mixed with acetate buffer (0.05 M, pH = 5.5) at a ratio of 2 % w/v. The mixture was subjected to microwave treatment (1100 W) before adding cellulase enzyme and leaving at room temperature. Then the enzyme was deactivated, the solution was centrifuged and the supernatant was precipitated with 96 % ethanol (ratio 1:1) at 4 ℃ for 12 h, then dried at 50 ℃ until the weight stabilized. The pectin yield (H %) was determined using Eq(1).

$$H\ (\%) = \frac{m_1 \cdot 100}{m_2} \quad (1)$$

Where: H % is pectin yield, $m_1$ is mass of extracted pectin (g), $m_2$ is initial mass of dried fruit peel (g)
Crude extracted pectin was purified by dialysis using a membrane with a molecular weight cutoff of 14,000 Da against distilled water for 24 h. Following dialysis, the aqueous fraction was concentrated and subjected to pre-precipitation with 96 % ethanol at a 1:1 volume ratio for 12 h, then dried at 50 ℃. The dialyzed pectin was subsequently analyzed and compared to both crude extracted pectin and commercial pectin by Fourier-transform infrared (FT-IR) spectroscopy (Dranca et al., 2019).

### 2.3 Pectin characterization

#### 2.3.1 Degree of Methoxylation - DM

0.5 g pectin was weighed and dissolved in 100 mL distilled water. The solution is titrated with NaOH 0.1 N. Degree of methoxylation was determined using Eq(2) (da Gama et al., 2015):

$$DM\ (\%) = \frac{V_{NaOH} \cdot C_{NaOH} \cdot 3.1}{m} \quad (2)$$

Where $V_{NaOH}$ is final titration volume of NaOH (mL), $C_{NaOH}$ is Concentration of NaOH solution (N), m is Mass of crude pectin (g)

#### 2.3.2 Degree of Esterfication - DE

0.1 g pectin was weighed and dissolved in 20 mL distilled water. The solution is titrated with NaOH 0.1 N. The degree of esterification was determined using Eq(3) (da Gama et al., 2015):

$$DE\ (\%) = \frac{V_2 \cdot 100}{(V_2 + V_1)} \quad (3)$$

Where $V_1$: First titration volume of NaOH (mL); $V_2$: Second titration volume of NaOH (mL).

### 2.4 Bioactivity

#### 2.4.1 The α- amylase inhibition assay

A 0.02 mL aliquot of the test sample was incubated with α-amylase (10 U/mL) at 50 °C for 10 min, followed by the addition of 0.02 mL of 1 % starch solution and further incubation. The enzymatic reaction was terminated by heating at 70 °C for 5 min. Subsequently, 0.007 mL of 5 mM iodine solution was added, and absorbance was recorded at 630 nm. Acarbose (0–25 µg/mL) was used as the positive control (Poovitha et al., 2016).

#### 2.4.2 ABTS free radical scavenging assay

A 0.01 mL volume of test sample was incubated with 0.5 mL of $ABTS^{\bullet+}$ solution, which was prepared by mixing 7 mM ABTS and 2.45 mM $K_2S_2O_8$ in equal volumes for a 24 h reaction. Absorbance was measured at 734 nm after 1 minute of sample incubation. L-ascorbic acid (0–50 µg/mL) was used as the standard antioxidant reference (Arumugam et al., 2006). The free radical scavenging capacity was determined using Eq(4).

$$SC_{ABTS^{\cdot+}} = \left(1 - \frac{A_s - A_b}{A_n}\right).100 \quad (4)$$

Where $SC_{ABTS^+}$ is free radical scavenging capacity (%), As is absorbance of the test sample at 734 nm, Ab is absorbance of the blank sample at 734 nm, An is absorbance of the negative control at 734 nm

### 2.5 Statical Analysis

All experimental data were processed and visualized using R and RStudio (version 4.5.0). Statistical analyses were conducted at a significant level of 5 %. Response Surface Methodology (RSM) was employed for optimization and modeling using the same software environment.

## 3. Result and discussion

### 3.1 Effects of Enzyme Concentration, Extraction Time, and Microwave Duration on Pectin Yield

The analysis for the effects of three factors are shown in Table 1 and Table 2:

*Table 1: Pectin Extraction Yield from Dragon Fruit Peel*

| Run | Enzyme concentration (wt%) | Extraction time (min) | Microwave time (s) | Pectin yield (%) |
|---|---|---|---|---|
| 1 | 0 | 60 | 0 | 18.2% ± 0.9 % |
| 2 | 0.8 | 100 | 60 | 25.4% ± 0.64 % |
| 3 | 0 | 100 | 0 | 19.5% ± 0.35 % |
| 4 | 0.8 | 60 | 0 | 23.79% ± 0.42 % |
| 5 | 0.8 | 100 | 0 | 24.2% ± 0.58 % |
| 6 | 0.8 | 60 | 60 | 25.3% ± 0.20 % |
| 7 | 0.4 | 80 | 30 | 26.1% ± 0.16 % |
| 8 | 0.4 | 80 | 30 | 25% ± 0.13 % |
| 9 | 0.4 | 80 | 30 | 24.1% ± 0.49 % |
| 10 | 0 | 100 | 60 | 24.3% ± 0.83 % |
| 11 | 0.4 | 80 | 30 | 24.3% ± 0.75 % |
| 12 | 0 | 60 | 60 | 23.9% ± 0.1 % |

*Table 2: Linear Regression Model of Two Factors: Microwave Time and Enzyme*

| | Estimate | Std. Error | T-value | P-value |
|---|---|---|---|---|
| Enzyme concentration | 0.015987 | 0.005278 | 3.029 | 0.0291 * |
| Microwave time | 0.016512 | 0.005278 | 3.129 | 0.0260 * |
| Extraction time | 0.002762 | 0.005278 | 0.523 | 0.6231 |
| Enzyme concentration x Extraction time | -0.001487 | 0.005278 | -0.282 | 0.7894 |
| Microwave time x Extraction time | -0.001512 | 0.005278 | -0.287 | 0.7859 |
| Enzyme concentration x Microwave time | -0.009737 | 0.005278 | -1.845 | 0.1244 |
| $R^2$ = 0.8202 | | | | |
| Adjusted $R^2$ = 0.6043 | | | | |

The ANOVA analysis and linear regression model showed the pectin yield is affected by enzyme concentration and microwave time (P-value < 0.05). In contrast, extraction time and second-order factors showed no significant effect. The regression reliability was 0.8202, which means 82.02 % of experimental results support the hypothesis. Extraction time will be eliminated and RSM will focus on microwave time and enzyme concentration.

### 3.2 Optimization of the Pectin Extraction Process

The optimization model is constructed with two factors: Enzyme concentration (0.4 wt%, 0.8 wt% and 0.6 wt%) and microwave time (30 s, 45 s and 60 s) incorporating coded data points. The result of response surface analysis is shown in Table 3 and Table 4, while its visualized diagram is shown in Figure 1.

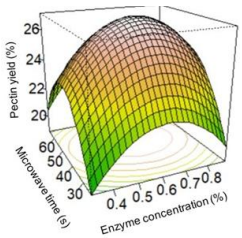


*Figure 1: The visualization of response surface model with the effect of microwave time and enzyme concentration on the pectin yield and the stationary point*

The linear regression model predicting pectin extraction yield (H%) was determined using Eq(5):

$$H\,(\%) = 0.269 + 0.0051757X_1 + 0.0073979X_2 + 0.00125X_1X_2 - 0.0137250X_1^2 - 0.0188250X_2^2 \quad (5)$$

Where $X_1$: Enzyme concentration (%); $X_2$: Microwave time (seconds).
Under optimized conditions, the pectin yield reached 27.24 %, with a degree of methoxylation (DM) of 9.38 % and a degree of esterification (DE) of 90.09 %. Chua et al. (2020) optimized pectin extraction from dragon fruit peel using citric acid (ratio 3.33 % w/v) combined with ultrasound treatment (32 min at 85 °C), obtaining a yield of 23.09 %, a DE of 33.27 %, and an ash content of 6.36 %. These findings suggest that the combination of cellulase enzyme and microwave-assisted extraction offers significant potential to improve pectin recovery from dragon fruit peel.

*Table 3: Experimental design and results of the response surface methodology*

| Run | Coded level for enzyme concentration | Coded level for microwave time | Enzyme concentration (wt%) | Microwave time (s) | Pectin Yield (%) |
|---|---|---|---|---|---|
| 1 | -1 | -1 | 0.4 | 30 | 23% ± 0.49 % |
| 2 | +1 | -1 | 0.8 | 30 | 23.3% ± 0.2 % |
| 3 | -1 | +1 | 0.4 | 60 | 24.5% ± 0.25 % |
| 4 | +1 | +1 | 0.8 | 60 | 25.3% ± 0.68 % |
| 5 | 0 | 0 | 0.6 | 45 | 27.3 ± 0.82 % |
| 6 | 0 | 0 | 0.6 | 45 | 26.4 ± 0.55 % |
| 7 | $-\sqrt{2}$ | 0 | 0.317157288 | 45 | 22.7% ± 0.32 % |
| 8 | $+\sqrt{2}$ | 0 | 0.882842712 | 45 | 24.85% ± 0.75 % |
| 9 | 0 | $-\sqrt{2}$ | 0.6 | 23.78679656 | 21.9% ± 0.3 % |
| 10 | 0 | $+\sqrt{2}$ | 0.6 | 66.21320344 | 23.61% ± 0.92 % |

*Table 4: Response surface model analysis result*

| | Estimate | Std. Error | T value | P value |
|---|---|---|---|---|
| Intercept | 0.2690000 | 0.0038229 | 70.365 | 1.098e-08 * |
| Enzyme concentration | 0.0051757 | 0.0023410 | 2.2109 | 0.078020 |
| Microwave time | 0.0073979 | 0.0023410 | 3.1601 | 0.025093 * |
| Enzyme concentration x Microwave time | 0.0012500 | 0.0033107 | 0.3776 | 0.721253 |
| Enzyme concentration – squared | -0.0137250 | 0.0027864 | -4.9257 | 0.004376 ** |
| Microwave time – squared | -0.0188250 | 0.0027864 | -6.7561 | 0.001079 ** |
| $R^2$ = 0.9334 | | | | |
| Adjusted $R^2$ = 0. 8669 | | | | |
| Stationary points | Enzyme = 0.6396 % | | Microwave time = 48.05 s | |
| Predicted pectin yield | 27.02586 % | | | |
| Experimental pectin yield | 27.24 % ± 0.0072 % | | | |
| Pectin characterization | DM (%) = 9.38 ± 1.74 (%) | | DE (%) = 90.09 ± 1.15 (%) | |

### 3.3 FT-IR Analysis

The FT-IR analysis result of pectin samples are shown in Figure 2. All three samples exhibited characteristic pectin-specific regions, including carbohydrate peaks between 1300 and 800 $cm^{-1}$(Dranca et al., 2019). (Urias-Orona, et al., 2010), acid galacturonic peaks in the range of 1120 to 990 $cm^{-1}$(Dranca et al., 2019) and carboxyl peaks at 1627 $cm^{-1}$(Dranca et al., 2019). Notably, ester peaks at 1738 $cm^{-1}$(Dranca et al., 2019) were detected only in samples in Figure 2b and Figure 2c. The sample in Figure 2a displayed silica-related peaks at 778 $cm^{-1}$ and 693 $cm^{-1}$, as confirmed by comparison with the Thermo Fisher Scientific spectral library, showing 76.98 % similarity to silica flour. In contrast, the sample in Figure 2c contained commercial additives such as Kelzan S and Kelzan D, used for gel stabilization, and exhibited 86.44% similarity to wood polysaccharides. The sample in Figure 2b contained some organic impurities, including hemicellulose and alginic acid, and despite some mineral-related peaks, lacked the distinct silica features observed in Figure 2a. Figure 2b showed 84.20 % similarity to pectin derived from incense cedar, indicating that the dialysis process significantly enhanced the purity of the pectin.

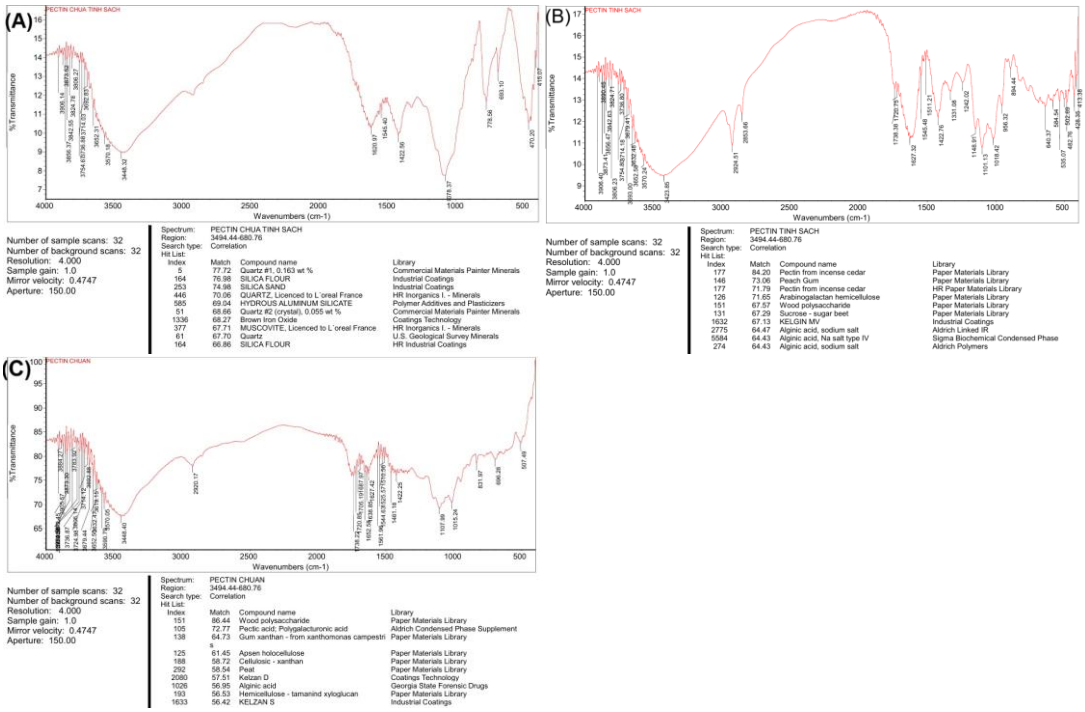


*Figure 2: The FT-IR analysis result of pectin samples: (a) The crude pectin sample; (b) the dialyzed pectin sample; (c) the commercial pectin sample of Sigma-Aldrich*

### 3.4 Bioactivity of pectin

#### 3.4.1 The α- amylase inhibition assay

The α- amylase inhibition results of arcabose and extracted pectin are shown in Figure 3. The α-amylase inhibition capacity of pectin extracted from dragon fruit peel is significantly lower than that of acarbose. According to Bai et al. (2020), pectin modulates α-amylase activity primarily by increasing the viscosity of digestive fluids, thereby controlling starch breakdown. However, crude pectin contains numerous impurities, which necessitate using larger amounts of the sample to effectively enhance viscosity. Furthermore, Bai et al. (2021) highlighted that low-methoxyl pectin (LMP), which lacks ester groups, interacts with and inhibits the enzyme more easily, whereas the pectin extracted from dragon fruit peel is classified as high-methoxyl pectin (HMP).

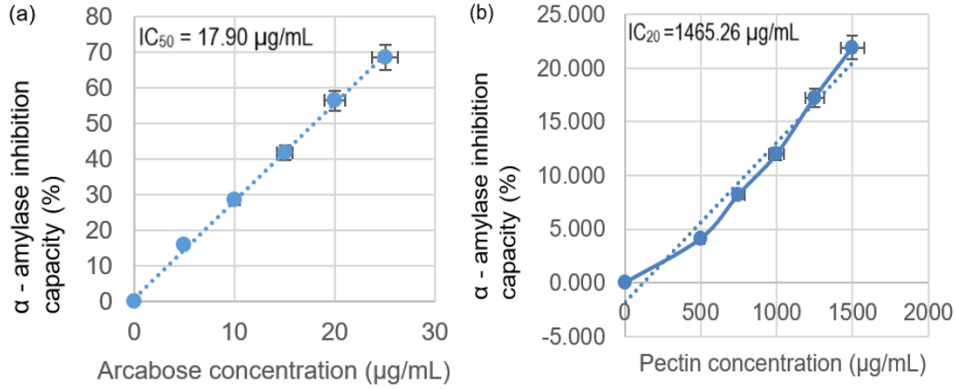


*Figure 3: α-amylase inhibition capacity of (a) acarbose; and (b) pectin extracted from dragon fruit peel*

#### 3.4.2 ABTS free radical scavenging assay

The antioxidant activity results of L-ascorbic and extracted pectin are shown in Figure 4. Dragon fruit pectin exhibits a strong ABTS radical scavenging ability, although it is approximately four times lower than that of L-ascorbic acid. According to Wang et al. (2013), apple pectin also demonstrates notable ABTS radical

scavenging activity with an $IC_{50}$ value of 500 µg/mL. Although the pectin's antioxidiant mechanism is not fully researched, various studies point out that many polysaccharides – including pectin - possess these abilities.

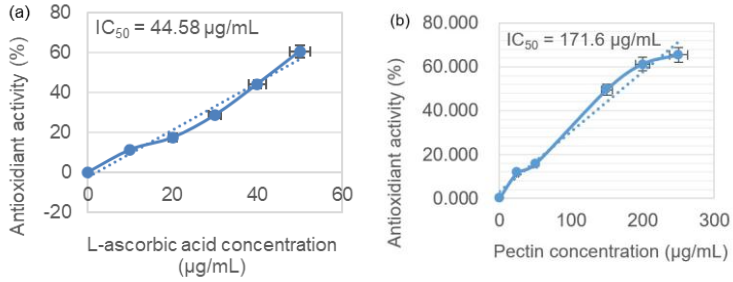


*Figure 4: Antioxidant activity of (a) L-ascorbic acid; and (b) pectin extracted from dragon fruit peel*

## 4. Conclusion

Dragon fruit peel shows great potential as a primary source of pectin when using commercial cellulase enzyme (Aspergillus niger - Sigma-Aldrich) combined with a microwave-assisted extraction method. Among the three factors investigated, enzyme concentration and microwave time significantly influenced the pectin yield, whereas extraction time had minimal impact. The response surface methodology identified optimal stationary points at an enzyme concentration of 0.6396 % (wt%) and microwave time of 48.05 s. The maximum pectin yield achieved was 27.24 % ± 0.0072 %, with a degree of methoxylation (DM) of 9.38 ± 1.74 % and degree of esterification (DE) of 90.09 ± 1.15 %. Additionally, pectin extracted from dragon fruit peel demonstrated bioactivity, including α-amylase inhibition with an $IC_{20}$ of 1465.26 µg/mL and ABTS free radical scavenging with an $IC_{50}$ of 171.6 µg/mL. After dialysis, the pectin sample showed significant removal of inorganic impurities while maintaining a chemical structure similar to that of commercial pectin.

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