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Response Surface Methodology– Driven Optimization of Pectin Extraction from Peels of an Indigenous *Musa acuminata*

E.J. Balaji[1], U. Shankar K[1*]

1- Department of Life Sciences, Christ University, Bengaluru, India
(*- Corresponding Author Email: uma.shankar@christuniversity.in)

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Abstract

The current study aimed to optimize pectin extraction from the non-AIS (crude) sample variety of *Musa acuminata* (Yelakki) peel, which was conducted by applying Response Surface Methodology (RSM), using a citric acid-assisted extraction method. The study was conducted with RSM to systematically evaluate the individual, quadratic, and interactive effects of process variables (Citric acid concentration, extraction time, and temperature) on the pectin yield. It was carried out based on a three-factor design to determine optimal conditions and statistically significant factors. The optimum conditions for extraction were found to be a concentration of 1.5%, an extraction duration of 90 minutes, and a temperature of 61.59°C with a desirability value of 0.616. In these conditions, the yield of pectin of 2.025% was very lower, when compared with banana peel AIS (8-20%). Concentration, extraction time, and temperature showed a statistically significant effect ($p < 0.05$) on pectin yield. The extracted pectin was categorized as high methoxyl pectin, exhibiting a DE of 53.49%, which is slightly lower than values typically reported for conventional sources. The degree of esterification remains within the acceptable range for HM pectin and contains Methoxyl content of 4.75% and Anhydrouronic acid of 50.45%. Our findings suggest that non-AIS *Musa acuminata* (Yelakki) peel potentially considered as a low-cost raw material for pectin production. Future studies may focus on enhancing pectin yield by using alternative extraction methods and further refining process parameters through optimization techniques using RSM, ANN or other statistical models.

Keywords: Citric acid, Non-AIS, RSM, Statistical modelling, Yelakki banana

Introduction

The fruit processing industry produces significant amounts of by-products, particularly peels and seeds, which are mainly used for low-value applications such as animal feed. These by-products contain a high level of pectin and can serve as an alternative for traditional citrus peel, which has been utilized for the large-scale production of pectin (May, 1990; Min *et al.*, 2011). Pectin consists of a complex group of polysaccharides found in plant cell walls, composed of four structural domains homogalacturonan (HG), xylogalacturonan, rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). HG represents the linear chain of α-1,4-linked galacturonic acid units that could be partially methyl-esterified or acetylated, and intervened by rhamnose units (Baum *et al.*, 2017). The amount of galacturonic acid of pectin influences its physicochemical properties (Sundarraj, Thottiam Vasudevan, & Sriramulu,

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2018). Several studies have explored pectin extraction from a wide range of plant-based residues, including pumpkin, soy hulls, peach pomace, sugar beet, *Cissampelos pareira* (Krueo Ma Noy), apple pomace, mango, Ambarella, banana peel, cocoa husks, citrus peel, jackfruit waste, and Saba banana peel, highlighting the global interest in valorizing agro-industrial by-products as alternative pectin sources (Khamsucharit, Laohaphatanalert, Gavinlertvatana, Sriroth, & Sangseethong, 2018).

Bananas are largely consumed as raw or processed into value-added products such as flour, chips, crackers, and purées. The peel accounts for approximately 30% of the overall weight of the fruit and causes an environmental concern due to nitrogen, phosphorus content and a significant amount of water, making them very susceptible to microbial degradation (Aklilu, 2021). In India, which ranks among the top banana-producing countries, local cultivars like *Musa acuminata* (Yelakki) are particularly prominent in the southern regions of India. A study estimates that global pectin consumption is expected to reach approximately 48,735 metric tonnes, growing at a compound annual growth rate (CAGR) of 3.7%, showing increasing demand across food, pharmaceutical, and industrial applications (Sundarraj *et al*., 2018).

There are different methods proposed by different scientists for the extraction of pectin, extraction providing an environmentally sustainable and non-corrosive alternative to conventional strong acids, with improved suitability for food and pharmaceutical applications (Vriesmann, Teófilo, & Lúcia de Oliveira Petkowicz, 2012). The influence of extraction parameters on pectin recovery from banana peel has been widely investigated, however, to the best of our knowledge, no prior studies have reported the extraction and optimization of pectin yield specifically from *Musa acuminata* (Yelakki) peel using non-AIS crude material. Citric acid and Response Surface Methodology (RSM) was employed to model and optimize this process under such conditions. In many reported studies, pectin extraction was performed on Alcohol Insoluble Solids (AIS), which involves pre-treatment of the plant sample with alcohol to remove soluble impurities and enhance purity (Broxterman & Schols, 2018). However, AIS preparation adds cost and solvent usage, whereas a non-AIS crude sample provides a simpler and more economical alternative, though linked to reduced yield and purity due to residual enzymes.

In the current research, Response Surface Methodology (RSM) was used to model and analyze the impacts of multiple variables, in order to optimize responses by developing multivariate regression equations based on experimental data. According to Giovanni (Giovanni, 1983), the advantage of RSM is related to its ability to reduce the number of experimental trials required to evaluate multiple parameters and their interactions efficiently. Pectin is recognized as a food additive and value-added substance by the Joint FAO/WHO Expert Committee. According to the European Commission standards, pectin with at least 65% galacturonic acid is required to meet the food additive pectin (Müller-Maatsch *et al*., 2016). The level of esterification (DE) greater than 50% classifies pectin as high methoxyl (HM), while a DE below 50% indicates low methoxyl (LM) pectin (Einhorn-Stoll, 2018).

The current study focuses on evaluating the extraction efficiency and quality of pectin obtained directly from non-AIS crude *Musa acuminata* (Yelakki) peel samples, to determine its potential for pectin recovery under such conditions. The RSM model was developed to predict the relationship between input factors and response variable. A Central Composite Design (CCD) was adopted to systematically understand both linear and non-linear interactions among variables, and to create a predictive polynomial model for response optimization. The model was analyzed statistically using the coefficient of determination R^2 and the coefficient of variation (CV) to determine its strength. As a

result, this research was carried out to investigate the impacts of extraction parameters, namely, temperature, extraction time, and citric acid concentration, on both the yield of extracted pectin from non-AIS *Musa acuminata* (Yelakki) peel, and to enhance these conditions through the use of Response Surface Methodology (RSM).

Materials and Methods

Raw Materials

This study utilized ripe banana peels of Yelakki (*Musa acuminata)* as the primary source for pectin extraction. The fruit was acquired from local markets in Bengaluru, India. The peels were removed, thoroughly washed with tap water, and the samples were subsequently rinsed once with distilled water and sliced into pieces using a knife. The peels were subsequently oven-dried at 50°C for 24 h under controlled conditions to reduce residual moisture prior to grinding. Although moisture content was not quantified separately, identical drying conditions were applied to all samples to ensure consistency. The dried samples were then ground into a powder using a grinder, passed through a 1.0 mm mesh sieve, and stored in a polyethylene zip-seal pouch at -20°C in a laboratory freezer until further analysis. Solid-state parameters including drying temperature, particle size (1.0 mm), and solid-liquid ratio (1:20, w/v), were maintained constant throughout all experiments to eliminate their influence on variability and to reflect optimization on chemical and thermal extraction variables.

Pectin Extraction

The method for extracting pectin was carried out following the procedures from Rasco´n-Chu et al. (Rascón-Chu *et al*., 2009) and P. Khamsucharit et al. (Khamsucharit *et al*., 2018) with slight modification. The process of extracting pectin from powdered banana peels was conducted based on the specified parameters of citric acid concentration, time, and temperature outlined in the experimental design. For citric acid-assisted extraction, the Non-AIS crude sample was mixed and suspended in the citric acid solution in a proportion of 1:20 (solid-liquid, w/v). The acidity of the extraction medium was defined by the specified citric acid concentration (%w/v) according to the experimental design, and pH was not independently adjusted during the extraction process. After that, the mixture was heated in a water bath at the specified time and temperature as in the design, and the solution was left to cool down to room temperature. The mixture was then filtered with a muslin cloth and centrifuged at 8000 rpm for 10 minutes. The supernatant was collected and filtered through a muslin cloth to remove the impurities. The filtrate was mixed in an equal volume of 95% ethanol, stirred for 5 minutes to mix and allowed to stand for 24 h at 4°C. The white precipitates were then collected by centrifuging at 6000 rpm for 10 minutes and washed 2–3 times with 95% ethanol. The product obtained was dried at 40 °C in a hot air oven until it reached a stable weight, after which it was ground and stored at 4°C. Pectin yield was calculated as follows:

$$\text{Pectin yield (\%)} = \frac{\text{Amount of extracted pectin (g)}}{\text{Amount of dried peel powder (g)}} \times 100 \quad (1)$$

Experimental Strategies and Statistical Methodology

RSM is a set of mathematical and statistical techniques to identify optimum conditions that leverage data from systematically designed experiments. A Central Composite Design (CCD) under Response Surface Methodology (RSM) was employed to evaluate the individual and interactive effects of three independent variables, namely citric acid concentration (1.5-8.5%), extraction time (30-90 min), and temperature (60-105°C), on pectin extraction efficiency (Table 1). The upper temperature limit (105°C) was selected considering the use of non-AIS sample, which retains native soluble fractions and structural components (Khamsucharit *et al.*, 2018). Elevated temperatures enhance acid-mediated protopectin solubilization, whereas excessive

heating induces pectin depolymerization (De Roeck, Mols, Duvetter, Van Loey, & Hendrickx, 2010). Therefore, the selected range enabled evaluation of both enhanced extraction and potential thermal effects within the CCD framework.

The experimental matrix consisted of 20 runs, allowing for determining the best combination of parameters for the pectin extraction process. The independent variables were considered based on E. G. Aklilu, with some modification (Aklilu, 2021).

CCD of the model involves 3 numeric factors, 14 points at non-center, and 6 points at center, with an alpha factor of 1.68179, was conducted using Design Expert version 22 software (Stat-Ease Inc., Minneapolis, USA, Free trial version). The correlation between the process variables and the response was modelled using a second-order polynomial regression equation suitable for a three-factor system.

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (2)$$

Where **Y** is the predicted response, b_0 is the intercept, b_1, b_2, and b_3 are the linear coefficients, b_{11}, b_{22}, and b_{33} are the quadratic coefficients and b_{12}, b_{13} and b_{23} are the coefficients for the interaction terms x_1, x_2, and x_3 are the coded independent variables. The actual values of the independent variables were converted to coded values using the following standard transformation equation:

$$x = \frac{X_{actual} - X_{center}}{\Delta} \quad (3)$$

Where x is the coded value, X_{actual} is the actual value of the variable, X_{center} is the midpoint of the independent variable, and Δ is half the range of the variable (i.e., $\frac{X_{high}-X_{low}}{2}$). This transformation standardizes the variables and optimizes effective comparison and model fitting in RSM.

Analysis of variance (ANOVA) was conducted using the square root (Linear regression) to assess the statistical relevance of the regression model. The model's quality and predictive reliability were evaluated utilizing the coefficient of determination (R^2), adjusted R^2, and the lack-of-fit test.

Table 1- Values of coded variables

S. No.	Factors	-1	+1	- α	+ α
1	Conc.	1.5	8.5	0.05	9.95
2	Time	30	90	17.57	102.43
3	Temperature	60	105	50.68	114.32

Determination of Equivalent Weight (EW)

The method outlined by Ranganna (Ranganna, 1986) was followed to determine the equivalent weight of the extracted pectin with minor modifications. Extracted pectin of 0.2 g was dispersed in 5 mL of ethanol in a 150 mL conical flask, and dissolved in 40 mL of carbon dioxide-free water. 0.5g of sodium chloride was added to the solution to sharpen the endpoint, and three drops of phenol red indicator were added. The mixture was subsequently subjected to rapid stirring to dissolve the pectin completely. Titration was done with 0.2N sodium hydroxide until a pink coloration, indicative of a pH of 7.5, was observed for 30 s. The neutralized titrate was further used to analyze the Methoxyl Content of pectin. EW was calculated using the equation:

$$\text{EW (g/mol)} = \frac{\text{Weight of pectin (g)} \times 1000}{\text{Vol. of NaOH} \times \text{N of NaOH}} \quad (4)$$

Determination of Methoxyl Content (MC)

MC of extracted pectin was assessed using Ranganna's method (Ranganna, 1986). To the neutralized mixture obtained from the determination of EW, 10 mL of 0.25 N sodium hydroxide was added, following thorough stirring, the mixture was left undisturbed for 30

min at room temperature. After that, 10 mL of 0.25 N hydrochloric acid was added and titrated to the same endpoint as before against 0.2 N sodium hydroxide. MC was calculated using the equation:

$$\text{MC}\ (\%) = \frac{\text{Vol. of NaOH} \times \text{N of NaOH} \times 31 \times 100}{\text{Weight of pectin (g)} \times 1000} \quad (5)$$

Where 31 corresponds to the molecular mass of the methoxyl group.

Determination of Anhydrouronic Acid Content (AUA)

The total AUA value of pectin was estimated using the titration readings from the determination of equivalent weight and methoxyl content. It was obtained following a formula reported by Mohamed and Hasan (Mohamed & Hasan, 1995) as follows:

$$\text{AUA}\ (\%) = \frac{176 \times (0.2)\, z \times 100}{w \times 1000} + \frac{176 \times (0.2)\, y \times 100}{w \times 1000} \quad (6)$$

Where Molecular unit of AUA = 176, the Normality of sodium hydroxide = 0.2, z = mL (titre) of NaOH from EW determination, y = mL (titre) of NaOH from MC determination, w = weight of pectin.

Determination of Degree of Esterification (DE)

The DE of pectin was calculated following a formula reported previously by Lal et al. (Lal *et al*., 2021).

$$\text{DE}\ (\%) = \frac{176 \times \text{MC} \times 100}{31 \times \text{AUA}} \quad (7)$$

Fourier Transform Infrared Spectroscopy (FTIR)

The structural characteristics of Yelakki banana peel pectin, extracted under optimized conditions, were investigated using an FTIR Spectrometer. The pectin samples were kept in a hot air oven to remove moisture and processed into powder. The spectra were recorded within the wavelength range of 4000-400 cm^{-1}, and the resolution was measured at 4 cm^{-1} (Chua, Tang, Ali, & Chow, 2020). The FTIR spectra were analyzed to determine the surface functional groups present on the pectin. The spectra were designed using Origin Pro software (Version 10.25).

Scanning Electron Microscope (SEM)

To examine the morphological structure and elements of the extracted pectin under optimized conditions, SEM was used in conjunction with EDX. The pectin samples were mounted on double-sided carbon tape, secured onto a metal support, and subsequently coated with chromium using a sputter coater and then spotted under a Scanning Electron Microscope (Joel-JSM 6701-F). EDX was analyzed using Hitachi SU6600 FE-SEM. The SEM imaging was performed across a magnification range of 1000 to 35000X, utilizing an accelerating 2 kV voltage (Coimbra *et al*., 2011).

Results and Discussion

Extraction

Citric acid-assisted extraction of pectin from Yelakki banana peel powder (Non-AIS) yielded a range varied from 0.3% to 4.3% on a dry weight basis under various experimentally designed conditions. To elucidate the effects of several experimental factors on these responses within an experimental design, Response Surface Methodology (RSM) was adopted and utilized.

Temperature exhibited a significant influence on pectin recovery. Increasing temperature enhanced extraction efficiency up to an optimum level (82°C) (Chen *et al*., 2021), likely due to improved diffusional transport and enhanced acid-mediated protopectin solubilization. The use of non-AIS sample in the present study may have contributed to this behavior, as the material retains native soluble fractions and structural components unlike AIS-pretreated cell wall residues. The preserved matrix structure can influence mass transfer dynamics during extraction. However, a decline in yield at higher temperatures suggests possible thermally induced depolymerization. The thermal susceptibility of pectin is known to depend on intrinsic structural characteristics such as degree of methylation

(DM) (Fraeye *et al*., 2007), and individual chains may exhibit varying stability under elevated temperature.

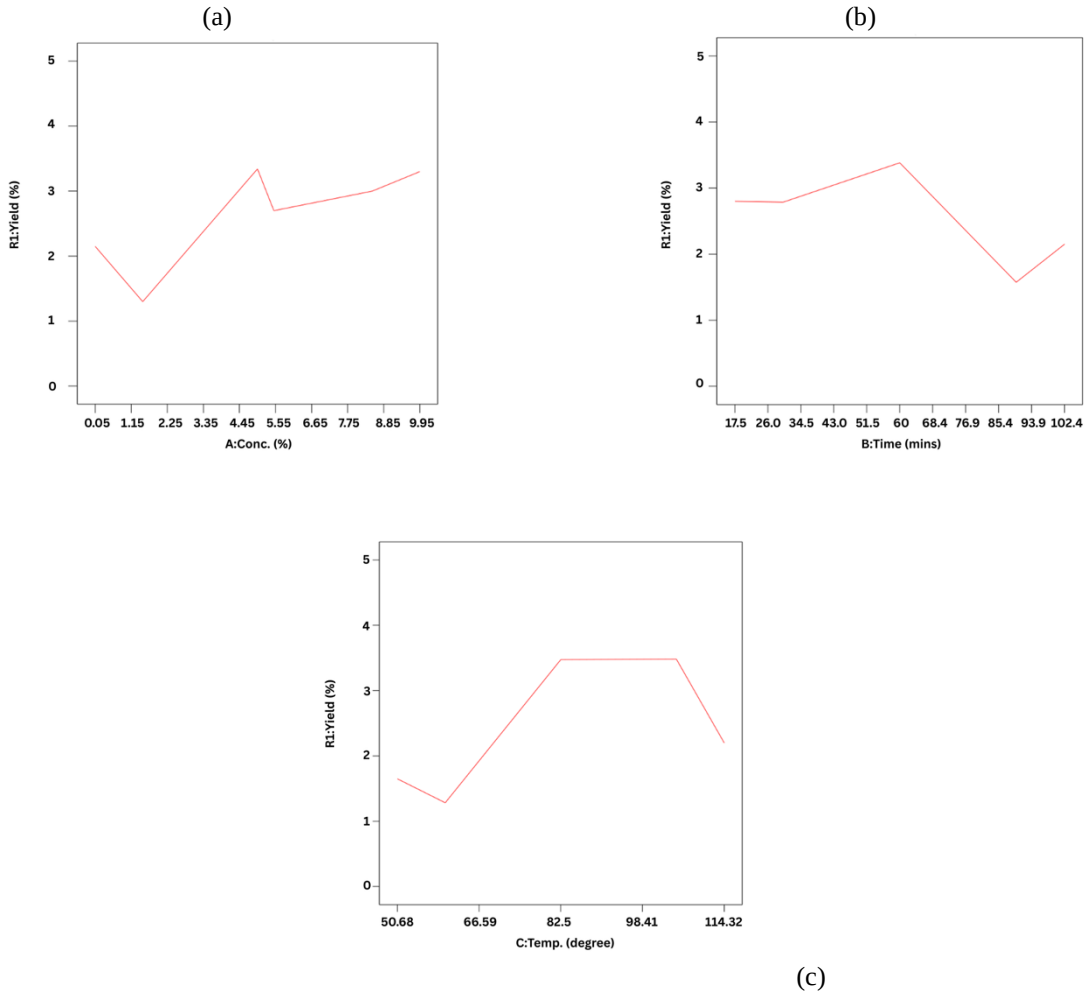


Fig. 1. Effect of citric acid concentration on pectin yield (a), effect of extraction time on pectin yield (b) and effect of temperature on pectin yield (c)

Table 2- Central composite design matrix and experimental yields

Run	Conc.	Time	Temp.	Response	Transformed Sqrt Actual value	Transformed Sqrt Predicted value
1	5	60	114.32	2.2	1.48	1.44
2	5	60	50.68	1.65	1.28	1.31
3	5	102.43	82.5	2.15	1.47	1.42
4	5	60	82.5	3.9	1.97	1.98
5	5	60	82.5	4.15	2.04	1.98
6	1.5	90	105	2.3	1.52	1.57
7	5	60	82.5	3.6	1.90	1.98
8	5.5	30	60	2.7	1.64	1.56
9	0.05	60	82.5	2.15	1.47	1.40
10	1.5	30	60	0.3	0.5477	0.5928
11	8.5	30	105	3.9	1.97	2.02
12	5	60	82.5	3.55	1.88	1.98

13	8.5	30	105	4.25	2.06	2.02
14	5	60	82.5	4.3	2.07	1.98
15	8.5	90	60	0.85	0.9220	0.9319
16	5	60	82.5	3.85	1.96	1.98
17	5	60	82.5	3.95	1.99	1.98
18	5	17.57	82.5	2.8	1.67	1.70
19	5	60	82.5	4	2.00	1.98
20	9.95	60	82.5	3.3	1.82	1.82

Preliminary Data Analysis and Experimental Observations

Following the execution of the Central Composite Design (CCD), a preliminary graphical evaluation of the non-transformed experimental data was performed to understand the underlying interactions among the independent variables concerning the pectin yield response prior to statistical modelling.

The observed pectin yield values across the 20 experimental runs ranged from 0.3% to 4.3% (Table 2). The pectin yield (%) (Fig. 1) presented a nonlinear relationship with rising concentration of citric acid. A decrease in yield was observed initially, followed by a progressive increase, suggesting that moderate to higher concentrations (above ~2.0%) enhance pectin extraction, with the maximum yield at ~5%. This suggests that sufficient acid strength facilitates efficient extraction of pectin. Extraction time (Fig. 1) demonstrated a nonlinear effect on pectin yield. The yield increased moderately up to 60 minutes but declined beyond 70 minutes, possibly resulting from thermal degradation of pectin. Yield (Fig. 1) increased with rising temperature up to ~82.5°C and stabilized between 82.5°C and ~101°C, beyond which a decline was observed. This pattern suggests that elevated temperatures enhance extraction efficiency up to a point; above this threshold, thermal degradation may reduce pectin integrity.

RSM Model Fitting

The statistical analyzes reveal that the quadratic model developed for the pectin yield is highly significant. A smaller p-value and a higher F-value indicate a more significant contribution from the corresponding coefficient in statistical modelling. The results of ANOVA (Table 3) showed that the developed quadratic regression model for pectin yield is highly significant, F-value = 63.94, ($p < 0.0001$); an F-value this large could only be the result of noise in 0.01% of occurrences. This extremely low p-value ($p < 0.0001$) implies that the developed model effectively explains the variability in pectin yield and is statistically significant beyond random chance. The quadratic model showed a statistically insignificant "Lack of Fit" (F-value = 2.63, p-value = 0.1327), relative to pure error. There is a 13.27% chance that a Lack of Fit F-value this large could occur due to noise. Thus, the developed regression model (Eqn. 8) demonstrated adequate predictive capability for pectin yield within the experimental domain, validating their effectiveness in representing the process dynamics over the investigated range of variables. The analysis shows that for pectin yield, A, B, AB, A^2, B^2, and C^2 were found to significantly influence the pectin yield, while C, AC, and BC did not significantly influence the pectin yield (Table 3).

The model's goodness-of-fit (Table 4) showed a strong coefficient of determination (R^2) of 0.9829 and an adjusted R^2 of 0.9675. These values suggest that approximately 98.3% of the total variability in the pectin yield can be linked to the fitted model, reflecting a satisfactory degree of model adequacy for the experimental data. The predicted R^2 of 0.7824 is in reasonable agreement with the Adjusted R^2 (difference < 0.2) as suggested by (Owolabi, Usman, & Kehinde, 2018), further confirming the good predictive potential of the model. An Adeq Precision value of 27.5 (Adeq Precision > 4) also validated the capacity of the model to navigate the design space effectively with a strong signal-to-noise ratio. The Coefficient of Variation (C.V.%) and standard

deviation (Table 4) were reasonably acceptable values for complex experimental systems, indicating good reliability and precision of the experimental data.

Table 3- ANOVA for the response surface quadratic model of pectin yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.10	9	0.3448	63.94	< 0.0001	significant
A-Conc	0.1006	1	0.1006	18.65	0.0015	
B-Time	0.0433	1	0.0433	8.03	0.0178	
C-Temp.	0.0102	1	0.0102	1.89	0.1998	
AB	0.5308	1	0.5308	98.43	< 0.0001	
AC	0.0111	1	0.0111	2.05	0.1823	
BC	0.0070	1	0.0070	1.30	0.2806	
A^2	0.3001	1	0.3001	55.65	< 0.0001	
B^2	0.3918	1	0.3918	72.65	< 0.0001	
C^2	0.8099	1	0.8099	150.19	< 0.0001	
Residual	0.0539	10	0.0054			
Lack of Fit	0.0214	2	0.0107	2.63	0.1327	not significant
Pure Error	0.0325	8	0.0041			
Cor Total	3.16	19				

Table 4- Regression coefficients of the predicted second-order model

S.No.	Response Parameter	Values
1	Std. Dev.	0.0734
2	Mean	1.68
3	C.V. %	4.36
4	R^2	0.9829
5	Adjusted R^2	0.9675
6	Predicted R^2	0.7824
7	Adeq Precision	27.5458

Development of a Regression Model Equation

The regression model equation governing pectin yield was systematically constructed based on experimental data acquired through a Central Composite Design (CCD) under Response Surface Methodology (RSM). A second-order polynomial equation (2) was employed to capture the linear, interaction, and quadratic effects of the independent variables concentration, extraction time, and temperature on pectin yield. The final regression equation, formulated in terms of coded factors whose values are provided in the supplementary material and structured to retain model hierarchy, is presented below

$$\begin{aligned} Y = 1.98 &+ 0.1473x_1 - 0.0966x_2 \\ &+ 0.0468x_3 \\ &- 0.1866x^2{}_1 \\ &- 0.2123x^2{}_2 \\ &- 0.3053x^2{}_3 \\ &- 0.4711x_1x_2 \\ &- 0.0689x_1x_3 \\ &- 0.0499x_2x_3 \end{aligned} \quad (8)$$

In this model, positive coefficients indicate synergistic effects, whereas negative coefficients denote antagonistic effects on pectin yield. To further interpret the response surface geometry, the stationary point of the fitted second-order quadratic model was determined by equating the first-order partial derivatives of Eqn. 8 to zero in coded space. The stationary coordinates were obtained at x_1= 0.013, x_2= -0.104, and x_3= 0.102, which correspond in real units to 5.05% citric acid concentration, 56.9 min extraction time, and 84.8°C. Substitution of these values into the

regression equation predicted a pectin yield of 3.82%. Since all quadratic coefficients in the model are negative, the stationary point corresponds to a local maximum within the experimental domain. This mathematical maximum reflects the curvature of the fitted surface and is distinct from the numerically optimized solution obtained through desirability analysis.

The residuals versus predicted plot (Fig. 2) was evaluated to examine the assumptions of homoscedasticity and model adequacy. The externally studentized residuals exhibit a random distribution around the horizontal axis, with no discernible curvature, showing constant variance over the range of predicted values. This randomness supports the assumption of homoscedasticity. Moreover, all the residuals fell well within the critical bounds (±4.14579), confirming the absence of outliers. The result aligns with findings reported in similar studies (Pinheiro *et al.*, 2008).

The predicted versus actual response plot (Fig. 2) for square root-transformed yield values revealed a strong linear relationship, where all the data points were closely distributed along the 45° reference line. This indicates that the developed model is appropriate for accurately predicting pectin yield. The graphical alignment of experimental and predicted values further confirms the predictive reliability model. Similar findings have been reported in previous studies (Sundarraj *et al.*, 2018).

(a) (b)

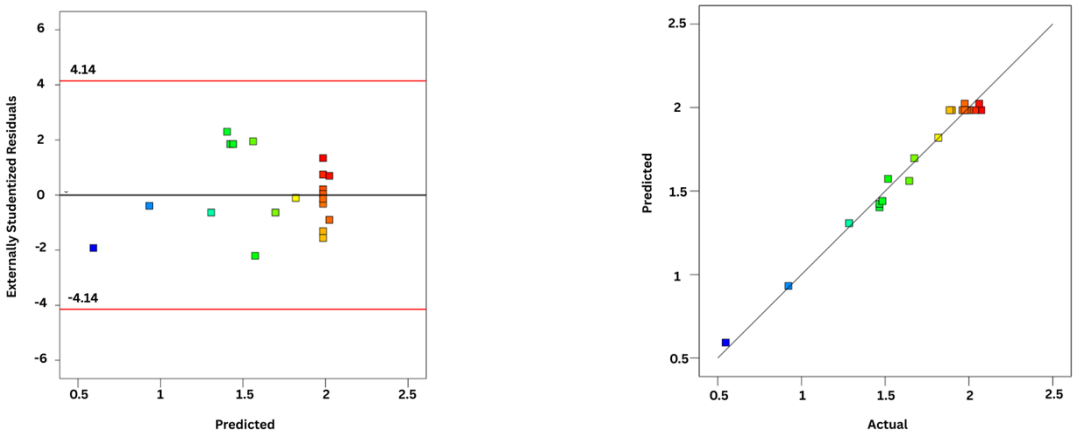


Fig. 2. Residuals versus Predicted (a) and Predicted versus Actual value (b)

Contour and Three-dimensional (3D) Response Surface Plot

To elucidate the effects of extraction parameters on pectin yield, two-dimensional contour and three-dimensional surface plots were constructed using the final regression model, Eqn. (8). Fig. 3 illustrates the contour and 3D surface plots of the effect of concentration and extraction time on pectin yield, with temperature held constant at its center point of 82.5 °C.

The contour plot (a) shows an elliptical pattern, indicating a significant interaction between concentration and time. As the concentration increased, pectin yield was improved consistently, especially at intermediate time durations. This is further supported by the corresponding 3D response surface plots (b). These results align well with previous studies (Pandit, Vijayanand, & Kulkarni, 2015; Vriesmann *et al.*, 2012).

(a) (b)

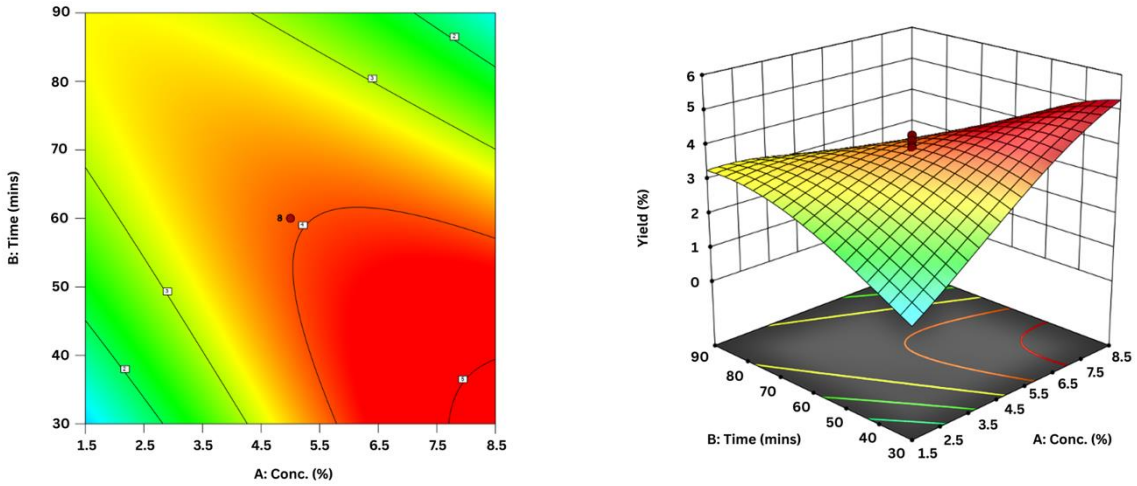


Fig. 3. Contour plot (a) and 3D response surface plot (b)

Analysis of Pectin Yield in Relation to Non-AIS Sample Preparation

The pectin yield obtained in the current research ranged from **0.3% to 4.3%**, which is within the lower to moderate range reported in the previous studies for banana peel and other fruit sources. Happi Emaga et al. (Happi Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008) reported pectin yields from banana peel ranging from 2.4% to 21.7%, depending on the cultivar, pretreatment, and extraction conditions. Similarly, Oliveira et al. (Oliveira *et al.*, 2016) reported pectin yields ranging from 5.2% to 12.2% using citric acid extraction on AIS (Alcohol Insoluble Solids) banana peel samples, and another study (Maneerat, Tangsuphoom, & Nitithamyong, 2017) observed 7% to 11% of yield under optimized acid extraction conditions.

Notably, AIS-based extraction from unripe banana cultivars has demonstrated substantially higher yields. For instance, citric acid extraction of AIS-pretreated unripe banana peels resulted in yields ranging from 10.91% to 24.08%, with the highest yield reported for Kluai Leb Mu Nang (24.08%) (Khamsucharit *et al.*, 2018). Previous studies have indicated that pectin yield decreases with increasing fruit maturation (Azad, Ali, Akter, Rahman, & Ahmed, 2014); Castillo-Israel et al. (Castillo-Israel, Diasanta, Lizardo, Dizon, & Mejico, 2015) reported that unripe Saba banana peel yielded 16.54% pectin, whereas ripe peel yielded 11.87%. In the present study, ripe Yelakki banana peel was employed, which may partially explain the lower recovery efficiency observed relative to AIS unripe materials.

The lower yield in the current investigation can therefore be attributed to two primary factors: the use of non-AIS crude peel powder and the use of ripe fruit material. AIS preparation enriches the alcohol-insoluble cell wall fraction by removing soluble sugars, organic acids, pigments, and low-molecular-weight compounds, thereby increasing the relative concentration of protopectin in the matrix. In contrast, non-AIS material retains these soluble constituents, resulting in a diluted matrix and reduced apparent recovery on a dry weight basis. Furthermore, extraction efficiency is strongly influenced by temperature and acidity; previous studies have demonstrated that increased temperature and lower pH enhance pectin solubilization, although excessively harsh conditions may promote structural degradation. Residual enzymatic activity reported in untreated plant tissues (Broxterman & Schols, 2018) could also contribute to structural modification of pectin, thereby affecting recovery efficiency.

Despite the reduced yield, the observed values are consistent with other studies

Husnawati *et al*. **(**Husnawati, Astutik, & Ambarsari, 2020**)** reported banana peel pectin yields ranging from 1.92% to 3.68%, and *Durio zibethinus* peel has been shown to yield 2.27% - 9.35% pectin (Wai, Alkarkhi, & Easa, 2009). Extraction of pectin from passion fruit peels was also comparable, ranging from 2.25% to 14.60% (Liew, Chin, & Yusof, 2014), depending on acid strength and extraction time. Current findings are in reasonable agreement with published data for the non-AIS sample and demonstrate the feasibility of pectin recovery from crude materials, potentially representing a simplified processing alternative.

Validation of the Optimized Condition

Pectin extraction from non-AIS banana peel using citric acid was optimized using RSM in Design Expert version 22, applying numerical optimization to define the optimal levels of process variables. The optimization was based on statistically defined criteria for each independent variable and the response to identify process conditions that maximize pectin yield. Experimental validation was performed in duplicate under the model-predicted optimal conditions to confirm the statistical design approach.

To verify the stability and predictive accuracy of the model, a numerical optimization module was applied to generate 55 potential solutions based on their higher desirability values. Among these solutions, the condition that had the highest desirability (0.616) was selected as the optimal condition for experimental validation. Although the overall desirability value (0.616) was moderate, it reflects optimization strictly confined within the statistically validated boundaries of the Central Composite Design without extrapolation beyond coded factor limits. Given that only pectin yield was maximized within predefined experimental constraints, the obtained desirability represents the highest model-supported solution permitted by the quadratic response surface rather than an unconstrained theoretical maximum.

The optimal condition corresponding to 1.5% concentration, 90 min extraction time, and a temperature of 62 °C, with a higher predicted pectin yield of 2.219%. The experimental result was found to be 2.025% with a small difference from the predicted value, indicating strong correlation between them, which confirmed the validity of the model. The selected optimum corresponds to the highest composite desirability predicted by the fitted quadratic model within the experimental design space.

Physicochemical Characterization of Pectin

The physicochemical characterization of pectin was conducted under the model-optimized extraction conditions. The equivalent weight of extracted pectin from a non-AIS banana peel sample was determined to be 750.91 g/mol (Table 5), which was higher than the values reported for apple pomace (551g/mol) and citrus peel (577g/mol) but comparatively lower than pectin derived from other varieties of banana peels (943-1456 g/mol) (May, 1990). Methoxyl content of the extracted pectin was found to be 4.75% (Table 5), which is similar to the different varieties of banana peel (3.86%-5.97%) (May, 1990), but lower than citrus peel (9.06%) and apple pomace (7.92%).

The purity of pectin is determined by its total anhydrouronic acid (AUA) content, which, according to Food Chemical Codex (Kamble *et al*., 2017), should be at least 65% to be used as a food additive or for pharmaceutical purposes. In the current study, the Anhydrouronic acid content (AUA) of extracted pectin was found to be 50.45% (Table 5), this value is comparable to those reported for pectin extracted from various banana peel cultivars (34.56–66.67%) and Saba banana peel pectins 39.68 to 57.32% (Castillo-Israel *et al*., 2015), it remains below the threshold required for food or pharmaceutical use, while it is lower than the values observed for citrus peel and apple pomace (Aklilu, 2021; Khamsucharit *et al*., 2018). This limitation highlights the impact of raw material on the purity of the recovered

pectin and restricts its application in the food and pharma industry.

However, although the AUA content does not satisfy Food Chemical Codex specifications for direct food or pharmaceutical additive applications, pectin with moderate purity has been extensively reported for functional material applications where regulatory purity thresholds are not mandatory. Pectin-based biopolymer systems have demonstrated considerable potential in active and biodegradable packaging due to their film-forming capacity, barrier properties, and ability to retard lipid oxidation and microbial proliferation (Huang *et al*., 2021), and utilized in films, paper substitutes, foams, cosmetics and plasticizer formulations (Biswal, 2024; Thakur, Singh, & Handa, 1997). In addition, the presence of a carboxyl functional group enables effective coordination interactions with metal ions, supporting its application as a biosorbent for heavy metal remediation (Martínez-Sabando, Coin, Melillo, Goyanes, & Cerveny, 2023). Furthermore, pectin extracted from agro-industrial residues has been investigated for bioplastic synthesis and composite material development (Listyarini, Susilawati, Nukung, & Yua, 2020). Therefore, despite not fulfilling FCC purity requirements, the extracted pectin remains technically suitable for value-added non-food applications within sustainable biomaterials.

The degree of esterification classifies pectin as high methoxyl (HM, DE > 50%) and low methoxyl (LM, DE < 50%) (Mesbahi, Jamalian, & Farahnaky, 2005). The Degree of Esterification of extracted pectin was found to be 53.49% (Table 5), indicating that the extracted pectins were classified as high-methoxyl pectin. The observed DE value was lower than previously reported for citrus peel (62.83%) but comparable to apple pomace (58.44%), likely as a result of the depolymerization of pectin, which reduces the DE (Khamsucharit *et al.*, 2018) (Kumari, Singh, & Chauhan, 2023).

Table 5- Physicochemical charaterization of extracted pectin

Parameters	Values
Equivalent Weight	750.91 ± 31.76 g/mol
Methoxyl Content	4.75 ± 0.18%
Anhydrouronic Acid	50.45 ± 1.02%
Degree of Esterification	53.49 ± 1.73%

FTIR Analysis of Pectin

The chemical structure of the extracted pectin was characterized by FTIR to confirm its properties and to evaluate the impact of the extraction process on its functional groups, as presented in Fig. 4. The spectral region between 950 and 1200 cm^{-1} is considered as the carbohydrate fingerprint region, allows to identify key functional groups present within the polysaccharides (Fissore, Rojas, & Gerschenson, 2012).

Moisture content in pectin causes a broad absorption band between 3600-3000 cm^{-1}, due to stretching of the hydroxyl groups (O-H) associated with intra- and intermolecular hydrogen bonding within galacturonic acid in the pectin. Absorption bands in the 3000-2900 cm^{-1} region are attributed to aliphatic C-H stretching vibrations (Santos *et al.*, 2020). Although methoxy ($O-CH_3$) stretching within this region may theoretically enable differentiation between low- and high-methoxyl pectins, its diagnostic utility is limited due to overlap with the broad and intense O-H stretching band, which obscures the comparatively weaker methoxyl signals (Gnanasambandam, 2000).

The absorption band at 1760-1725 cm^{-1} was attributed to the stretching vibration of esterified carbonyl groups (C=O), and 1630-1600 cm^{-1} was attributed to carboxyl groups (COO^-). The carbonyl band at 1730cm^{-1} may be contributed from both methyl and acetyl ester groups; however, FTIR does not enable definitive discrimination between these functionalities due to spectral overlap. Hence,

the degree of acetylation was not quantified in the present study. Absorption bands in the 1220-1011 cm^{-1} region correspond to C–O–C stretching vibrations associated with glycosidic rings in the pectin (Khamsucharit *et al*., 2018; Kumari *et al*., 2023). High-degree esterified pectins are characterized by relatively greater intensity of the ester carbonyl (C=O) stretching band near (~1753 cm^{-1}) and comparatively reduced intensity of the carboxylate (COO^-) asymmetric stretching band near (~1640 cm^{-1}), whereas low-degree esterified pectins exhibit the inverse spectral profile (Fellah, Anjukandi, Waterland, & Williams, 2009; Santos *et al*., 2020).

In the present study (Fig. 4), a weak absorption band was observed at ~1730cm^{-1}, corresponding to esterified carbonyl stretching, while a comparatively more intense band was observed at ~1618 cm^{-1}, attributable to the asymmetric stretching of carboxylate groups. Although the carboxylate band appeared dominant, the titrimetric determination yielded a degree of esterification (DE) of 53.49 ± 1.73%, confirming its classification as high-degree esterified (HDE) pectin. This apparent spectral discrepancy may be attributed to the borderline nature of the carboxylate ion compared to the ester carbonyl. Furthermore, the intensity of the ~1618 cm^{-1} region can be influenced by residual moisture (O-H bending) or the ionization state of the pectin backbone (Fellah *et al*., 2009; Manrique & Lajolo, 2002). Consequently, when interpreted alongside chemical quantification, these spectral characteristics remain consistent with an HDE classification.

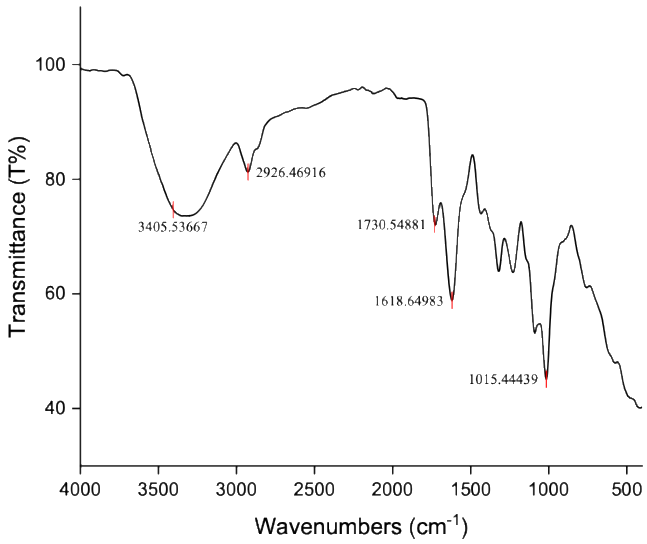
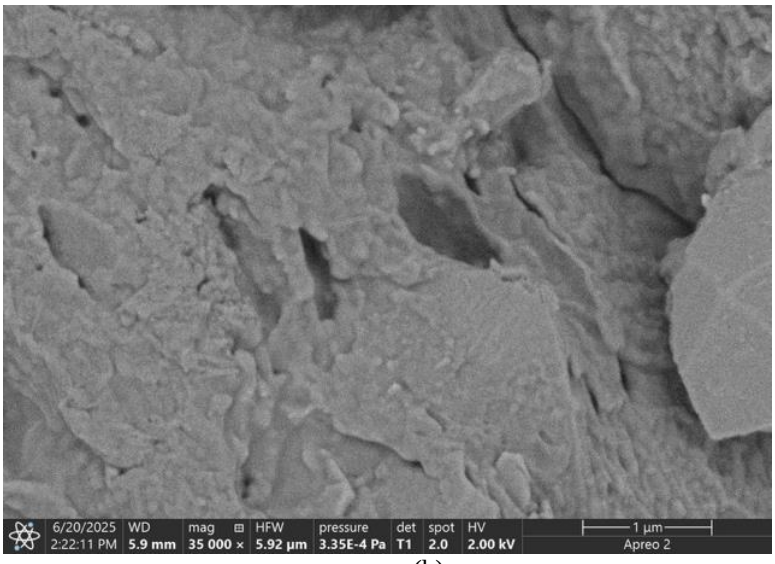
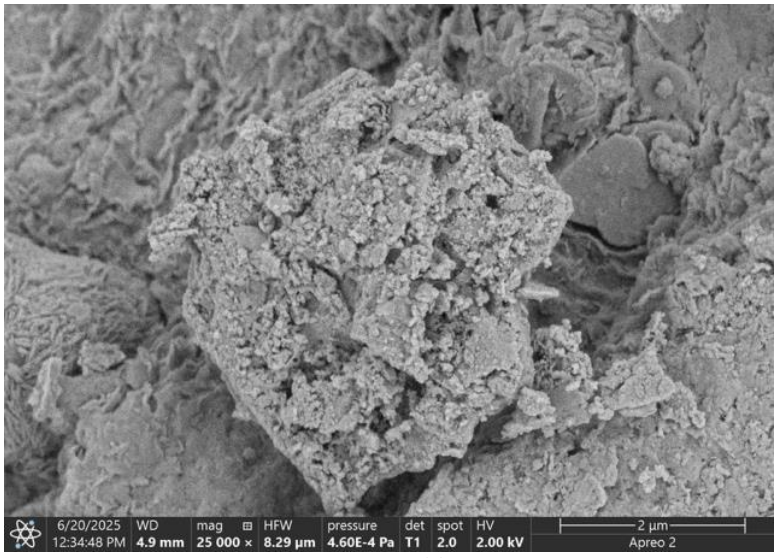


Fig. 4. FTIR analysis

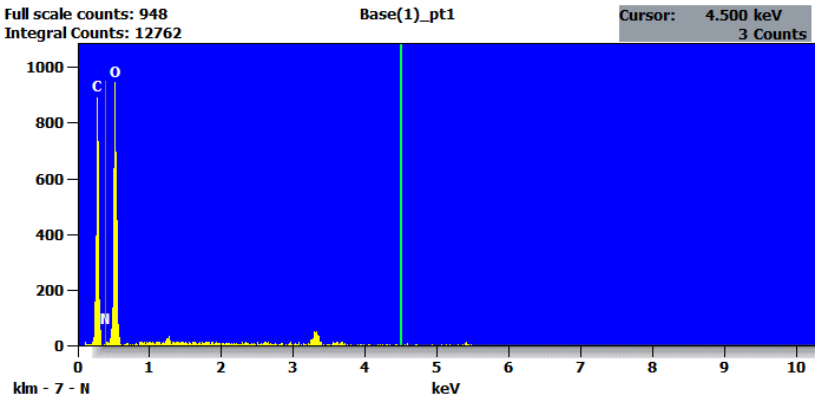
(a)



(b)



(c)



Element	Wt%
C	23.65
N	7.30
O	69.05

Fig. 5. SEM image of pectin at 35000x magnification (Scale bar-1 µm) (a), SEM image of pectin at 25000x magnification (Scale bar-2 µm) (b) and EDX spectrum (c)

Scanning Electron Microscopy

SEM micrographs (Fig. 5) of the pectin revealed uneven, compact, and irregularly aggregated particles with rough surfaces and porous microstructures, which are flaky, and this may be due to the high proportion of neutral sugar side chains in the pectin structure (Kumari *et al.*, 2023). Pectin obtained from apple waste by ultrasound-assisted extraction exhibited a compact, flaky, and rigid surface morphology, reported by Mahmoud et al. (Mahmoud, Abu-Salem, & Azab, 2022). Another investigation by Jiang et al. (Jiang *et al.*, 2012) on pectin also reported uneven and abrasive surface morphologies, highlighting the structural heterogeneity of plant-derived pectin. Chemical composition of the pectin was analyzed using Energy-Dispersive X-ray spectroscopy (EDX). The spectrum (Fig. 5) revealed the presence of elements such as carbon (C), oxygen (O), and minor traces of nitrogen (N), which are typical of polysaccharide structures.

Conclusion

The present study utilized Response Surface Methodology (RSM) to model and optimize the citric acid-assisted extraction of pectin from non-AIS *Musa acuminata* peel (Yelakki) indigenous to Karnataka. The study revealed that linear terms (A and B), their interaction (AB), and the quadratic terms (A^2, B^2, and C^2) significantly influence pectin yield, while other interaction effects were statistically non-significant. The RSM model showed strong predictive performance, which is supported by a high R^2 value, low CV, and adequate precision, indicating good model fit and a sufficient signal-to-noise ratio. The optimum condition of the pectin yield was achieved at conc., extraction time, and temp. of 1.5%, 90miin and 62°C respectively, with the desirability of 0.616 was 2.025%. In accordance with the determined DE value, the extracted pectin was categorized as high methoxyl pectin. The observed value of AUA was lower than the criteria for use as a food and pharmaceutical additive. Future studies may explore advanced extraction techniques such as enzymatic, microwave or ultrasound-assisted methods to enhance the AUA content of pectin extracted from *Musa acuminata* (Yelakki) peel and improve its applications in food and pharmaceutical formulations.

Abbreviations

CAE: Citric Acid-Assisted Extraction; Non-AIS: Non-Alcohol Insoluble Solids; RSM: Response Surface Method; CCD: Central Composite Design; EW: Equivalent Weight; MC: Methoxyl Content; AUA: Anhydrouronic acid; DE: Degree of Esterification; FTIR: Fourier transform infrared spectroscopy; SEM: Scanning Electron Microscope

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Data availability

The datasets obtained during the current study are available from the corresponding author on reasonable request. All the data analysed during this study are included in this research article and supplementary materials.

Declarations

Consent to Publish

Not applicable

Consent to Participate

Not applicable

Ethics declaration

Not applicable

Clinical Trial Registration

This study doesn't involve any clinical trial

Author Contribution

E.J.B. conducted the research, data collection, performed the statistical analysis and wrote the manuscript. **U.S.K.** Experimental design, formulated, and reviewed the manuscript.

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روش‌شناسی سطح پاسخ- بهینه‌سازی مبتنی بر استخراج پکتین از پوست یک موز بومی

ایژیلاراسان جی بلاجی[۱]- اوما شانکار کی[۱*]

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چکیده

مطالعه حاضر با هدف بهینه‌سازی استخراج پکتین از نمونه غیر AIS (خام) پوست موز آکومیناتا (Yelakki) انجام شد که با استفاده از روش استخراج به کمک اسیدسیتریک و با استفاده از روش سطح پاسخ (RSM) انجام شد. این مطالعه با استفاده از RSM انجام شد تا اثرات فردی، درجه دوم و تعاملی متغیرهای فرآیند (غلظت اسیدسیتریک، زمان استخراج و دما) بر بازده پکتین به‌طور سیستماتیک ارزیابی شود. این مطالعه بر اساس یک طرح سه عاملی برای تعیین شرایط بهینه و عوامل آماری معنی‌دار انجام شد. شرایط بهینه برای استخراج، غلظت ۱/۵٪، مدت زمان استخراج ۹۰ دقیقه و دمای ۶۱/۵۹ درجه سانتی‌گراد با مقدار مطلوبیت۰/۶۱۶ بود. در این شرایط، بازده پکتین ۲/۰۲۵٪ در مقایسه با پوست موز (AIS 8-20 ٪) بسیار کمتر بود. غلظت، زمان استخراج و دما اثر آماری معنی‌داری ($p < 0.05$) بر بازده پکتین نشان دادند. پکتین استخراج‌شده به‌عنوان پکتین با متوکسیل بالا طبقه‌بندی شد و DE آن ۵۳/۴۹٪ بود که کمی کمتر از مقادیری است که معمولاً برای منابع متعارف گزارش می‌شود. درجه استری شدن در محدوده قابل قبول برای پکتین HM باقی می‌ماند و حاوی محتوای متوکسیل ۴/۷۵٪ و اسید آنهیدرورونیک ۵۰/۴۵٪ است. یافته‌های ما نشان می‌دهد که پوست موز آکومیناتا (Yelakki) بدون AIS به‌طور بالقوه به‌عنوان یک ماده اولیه کم‌هزینه برای تولید پکتین در نظر گرفته می‌شود. مطالعات آینده ممکن است بر افزایش بازده پکتین با استفاده از روش‌های استخراج جایگزین و اصلاح بیشتر پارامترهای فرآیند از طریق تکنیک‌های بهینه‌سازی با استفاده از RSM، ANN یا سایر مدل‌های آماری تمرکز کنند.

واژه‌های کلیدی: اسید سیتریک، غیر AIS، مدل‌سازی آماری، موز یلاکی، RSM

۱- گروه علوم زیستی، دانشگاه مسیح، بنگلور، هند
(*- نویسنده مسئول: Email: uma.shankar@christuniversity.in)

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