Optimization of Pectin Extraction from an indigenous variety of Musa acuminata peel from Karnataka using Response Surface Methodology

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Optimization of Pectin Extraction from an indigenous variety of *Musa acuminata* peel from Karnataka using Response Surface Methodology

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Abstract

Purpose The current study aimed to optimise pectin extraction from the non-AIS (crude) sample variety of *Musa acuminata* (Yelakki) peel, which was conducted using Response Surface Methodology (RSM), using a citric acid-assisted extraction method.

Methods The study was conducted with RSM to determine individual, quadratic, and interactive effects of process variables, namely concentration, extraction time, and temperature, on pectin yield were systematically evaluated. It was carried out based on a three-factor design to determine optimal conditions and statistically significant factors.

Results 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 was determined to be 2.025%. Concentration, extraction time, and temperature revealed 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%, respectively.

Conclusion The findings of the work suggest that non-AIS *Musa acuminata* (Yelakki) peel holds potential 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 Yelakki banana, Non-AIS, Citric Acid, CCD, Statistical Modelling

Background

The fruit processing industry produces significant amounts of by-products, particularly peels and seeds, which are used for low-value applications such as animal feed, and 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 [1], [2]. 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 [3]. The amount of galacturonic acid of pectin influences its physicochemical properties [4]. 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 [5].

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 [6]. 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 by 2026, 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 [4].

There are different methods proposed by different scientists for the extraction of pectin and Citric acid, extraction providing an environmentally sustainable and non-corrosive alternative to conventional strong acids, with improved suitability for food and pharmaceutical applications [7]. The influence of extraction parameters on pectin recovery from banana peel has been widely investigated, however, to the best of our understanding, no prior studies have reported the extraction and optimization of pectin yield specifically from *Musa acuminata* (Yelakki) peel using non-AIS crude material using citric acid and Response Surface Methodology (RSM) was employed to model and optimize this process under such conditions. In many reported studies, pectin extraction is performed on Alcohol Insoluble Solids (AIS), which involves pre-treatment of the plant sample with alcohol to remove soluble impurities, and enzymes and enhance purity [8]. 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) is 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 [9], the advantage of RSM is 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 [10]. The level of esterification (DE) greater than 50% classifies pectin as high methoxyl (HM), while a DE below 50% indicates low methoxyl (LM) pectin [11].

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 variables. 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 and quality 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 utilised 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 dried at 50°C for 24 h, ground into a powdered state 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.

Pectin extraction

The method for extracting pectin was carried out following the procedure from Rasco´n-Chu et al. [12] and P. Khamsucharit et al. [5] with slight modification. The process of extracting pectin from powdered dried banana peels was conducted based on the specified parameters of 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). 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 formed white precipitates were 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:

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

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, such as concentration (1.5-8.5%), extraction time (30-90 mins), and temperature (60-105°C), on pectin extraction efficiency (Table 1). 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 [6].

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 lack-of-fit test.

Table 1 Values of Coded Variables

S. No.	Factors	-1	+1	- α	+ α
1	Conc.	1.5	8.5	-0.8862	10.8863
2	Time	30	90	9.5462	110.454
3	Temperature	60	105	44.66	120.34

Determination of Equivalent Weight (EW)

The method outlined by Ranganna [13] 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 colouration, indicative of a pH of 7.5, was observed for 30 s. The neutralized titrate was further used to analyse the Methoxyl Content of pectin. EW was calculated using the equation:

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

Determination of Methoxyl Content (MC)

MC of extracted pectin was assessed using Ranganna's method [13]. 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 the time, 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:

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

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 in the previous publications by Mohamed and Hasan [14] as follows:

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

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. [15].

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

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} [16]. 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 analysed using Hitachi SU6600 FE-SEM. The SEM imaging was performed across a magnification range of 1000 to 35000X, utilizing an accelerating 2 kV voltage [17].

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.

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. 1a) 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. 1b) 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. 1c**)** 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.

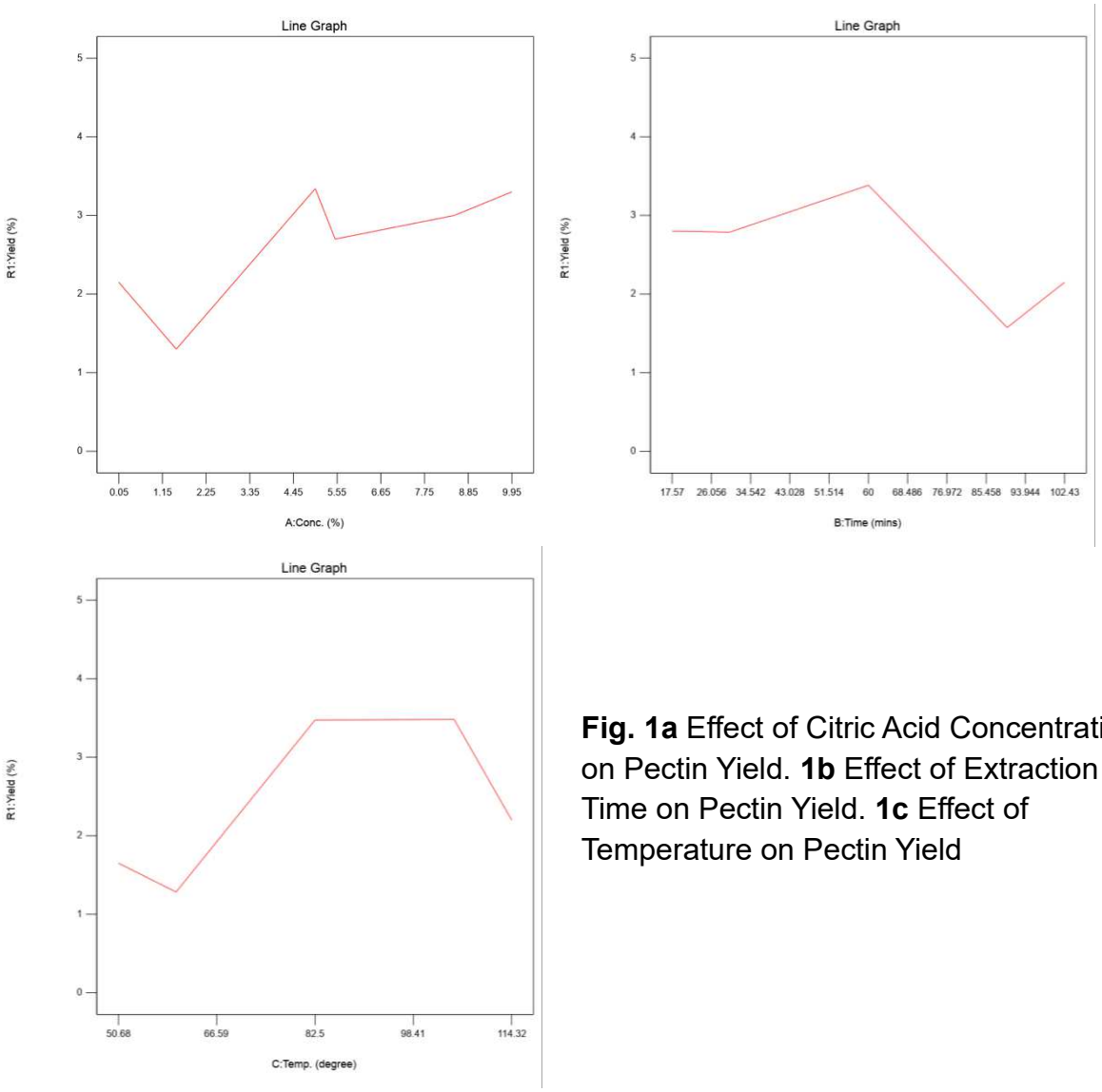
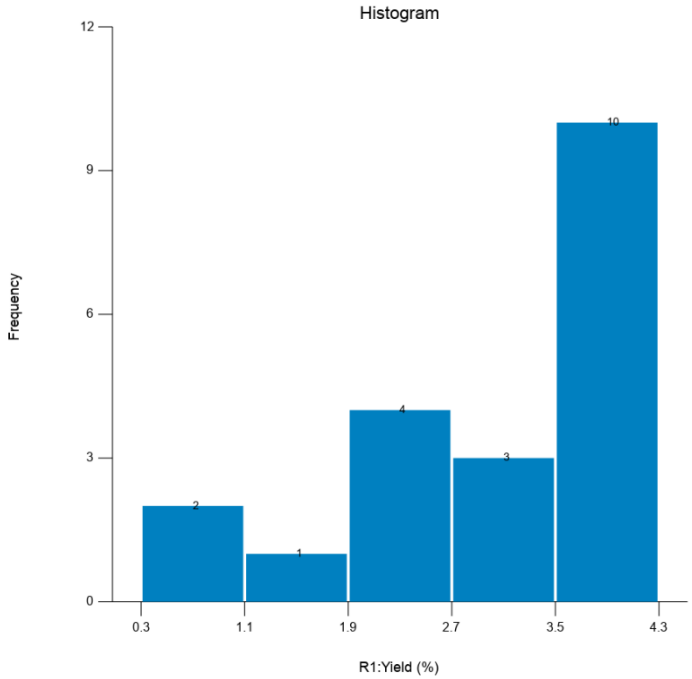


Fig. 1a Effect of Citric Acid Concentration on Pectin Yield. **1b** Effect of Extraction Time on Pectin Yield. **1c** Effect of Temperature on Pectin Yield

A histogram of the pectin yield was constructed to assess the distributional properties of the response variable (Fig. 2). The histogram, comprising a yield range of approx. 0.3% to 4.3% illustrates the frequency of occurrence within defined yield intervals. The observed distribution exhibited a negative skewness, with primary peaks centred around 3.5–4.3% (n=10). A limited yield frequency was observed in the lower yield spectrum, with only two instances falling within the 0.3% to 1.1% range and a single yield between 1.1% and 1.9%, suggesting that higher yields were more predominant, while lower yield outcomes occurred less frequently.

Fig. 2 Frequency distribution of pectin yield (%)



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

Table 2 Central composite design matrix and experimental yields

RSM Model Fitting

The statistical analyses 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 [18], 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.

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 3 ANOVA for the response surface quadratic model of pectin yield

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

Table 4 Regression coefficients of the predicted second-order model

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

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

In this model, positive coefficients indicate synergistic effects, whereas negative coefficients denote antagonistic effects on pectin yield.

The residuals versus predicted plot (Fig. 3a) 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 [19].

The predicted versus actual response plot (Fig. 3b) 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 [4].

Fig. 3a Residuals versus Predicted

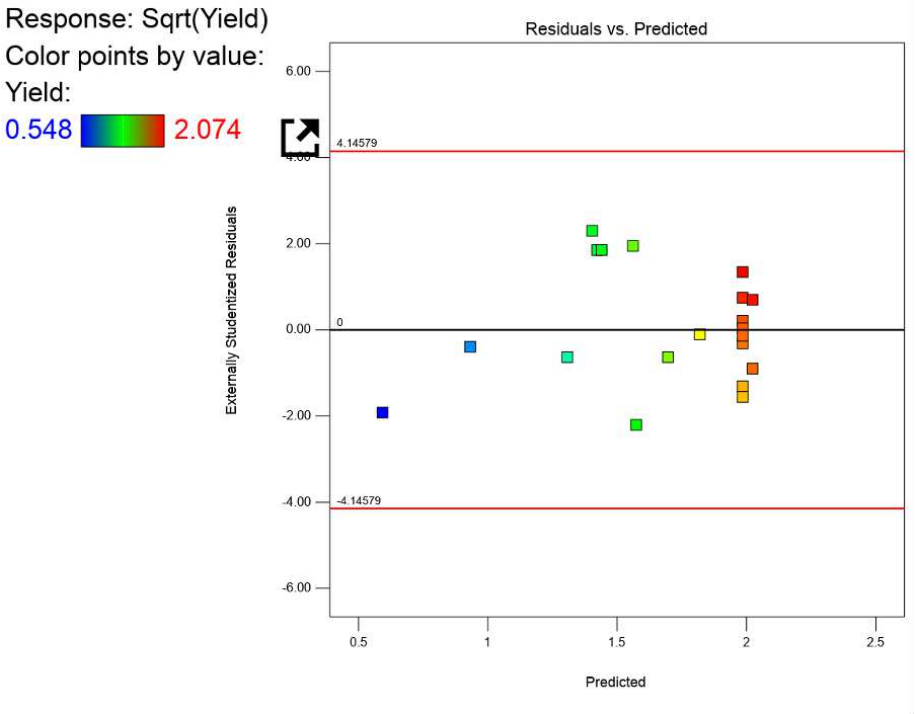
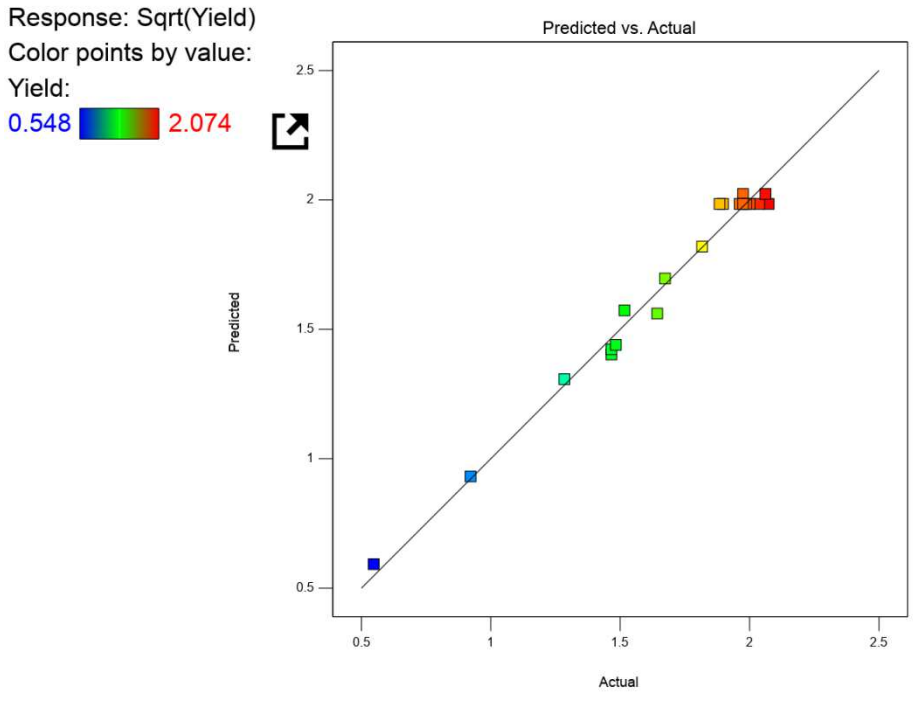


Fig. 3b Predicted versus Actual value



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. 4a and 4b illustrate 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 Fig. 4a 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 Fig. 4b. These results align well with previous studies [7], [20].

Fig. 4a Contour plot

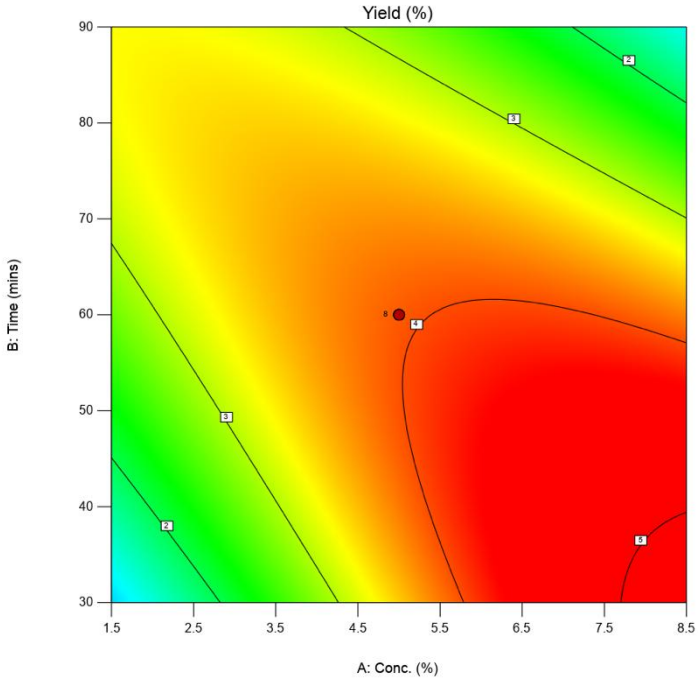
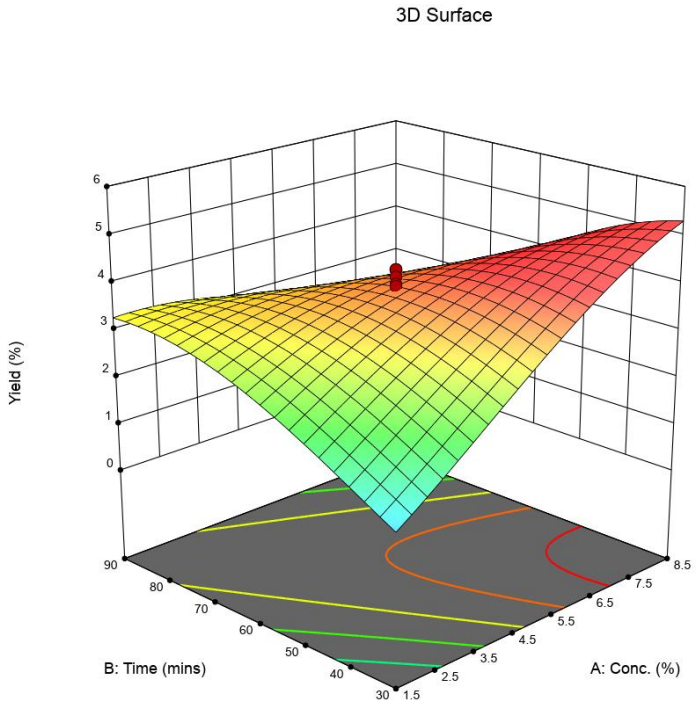


Fig. 4b 3D response surface plot



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. [21] reported pectin yields from banana peel range from 2.4% to 21.7%, depending on the cultivar, pretreatment, and extraction conditions. Similarly, Oliveira et al. [22] 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 [23] observed 7% to 11% of yield under optimized acid extraction conditions.

The current study was conducted on non-AIS crude banana peel powder, which may have contributed to the lower yield. The absence of AIS processing may have allowed endogenous enzymes, such as pectinases [8], to remain active during extraction, leading to partial degradation of pectin and contributing to the lower pectin yield.

Despite the reduced yield, the observed values are consistent with other studies Husnawati et al. [24] 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 [25]. Extraction of pectin from passion fruit peels was also comparable, ranging from 2.25% to 14.60% [26], 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 material of banana peel waste.

Validation of the optimized condition

Pectin extraction from non-AIS banana peel using citric acid was optimised 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, corresponding to 1.5% concentration, 90 mins extraction time, and a temperature of 62 °C, with a higher predicted pectin yield of 2.219%. The result was found to be 2.025% with a small difference between the experimental and the predicted values, indicating strong agreement between them, which confirmed the validity of the model.

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) [2]. 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%) [2], 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 [27], 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% [28], 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 [5], [6]. This limitation highlights the impact of raw material on the purity of the recovered pectin and restricts its application. The degree of esterification classifies pectin as high methoxyl (HM, DE > 50%) and low methoxyl (LM, DE < 50%) [29]. The Degree of Esterification of extracted pectin was found to be 53.49% (Table 5), indicating that the extracted pectins were classified as high-methoxy 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 [5] [30].

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%

Table 5 Physicochemical characterization of extracted pectin

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. 5. 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 [31].

Moisture content in pectin causes a broad absorption band between 2800–3600 cm^{-1}, due to stretching of the hydroxyl groups (O-H) in the pectin. The absorption band at 1725–1760 cm^{-1} was attributed to the stretching vibration of esterified carbonyl groups (C=O), and 1600–1630 cm^{-1} was attributed to carboxyl groups (COO^{-}). Absorption bands in the 1011–1220 cm^{-1} region corresponds to C–O–C stretching vibrations associated with glycosidic rings in the pectin [5], [30]. A weaker carboxyl stretching band and stronger absorption of ester carbonyl bands in apple pomace and citrus peel pectins indicate their high methoxylation. Banana peel pectins exhibited lower intensities for both absorption bands, due to reduced anhydrouronic acid content [5].

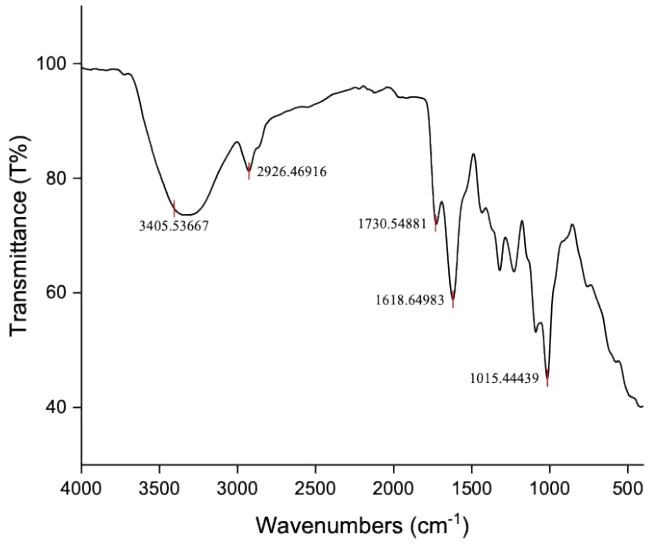


Fig. 5 FTIR analysis

Scanning Electron Microscopy

SEM micrographs Fig. 6a and Fig.6b 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 [30]. Pectin obtained from apple waste by ultrasound-assisted extraction exhibited a compact, flaky, and rigid surface morphology, reported by Mahmoud et al. [32]. Another investigation by Jiang et al. [33] on pectin also reported uneven and abrasive surface morphologies, highlighting the structural heterogeneity of plant-derived pectin. Chemical composition of the pectin was analysed using Energy-Dispersive X-ray spectroscopy (EDX). The spectrum Fig. 6c revealed the presence of elements such as carbon (C), oxygen (O), and minor traces of nitrogen (N), which are typical of polysaccharide structures.

Fig. 6a SEM image of pectin at 35000x magnification (Scale bar-1 µm)

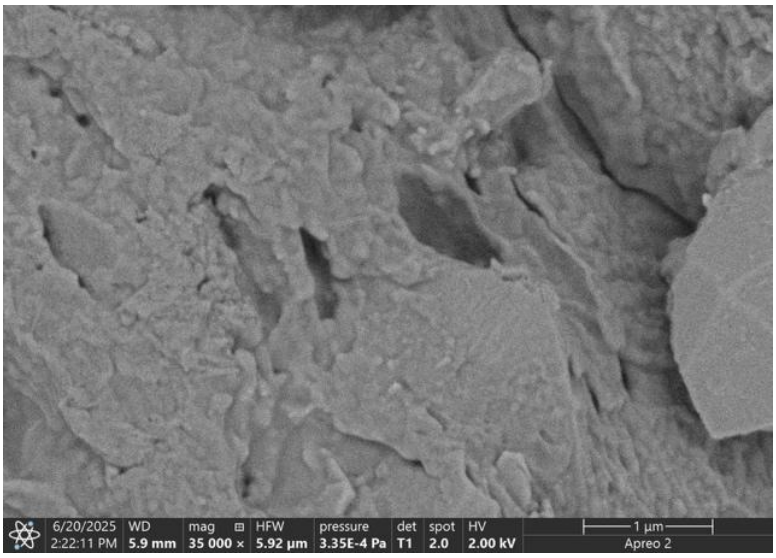


Fig. 6b SEM image of pectin at 25000x magnification (Scale bar-2 µm)

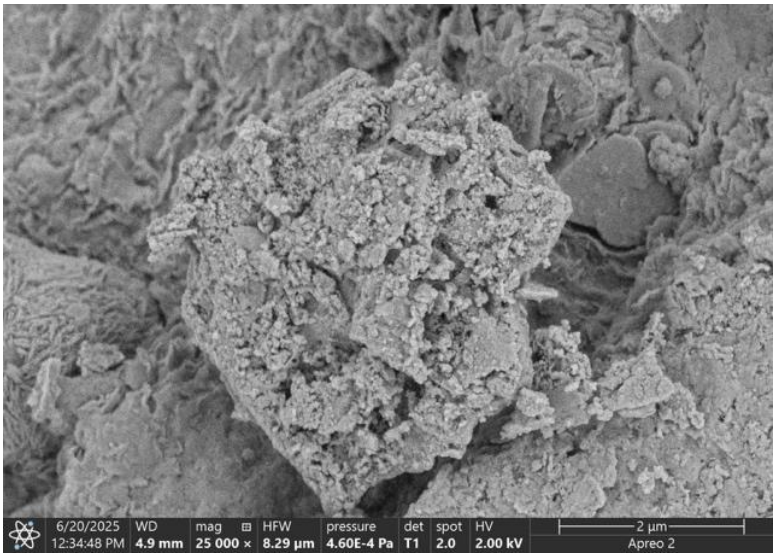
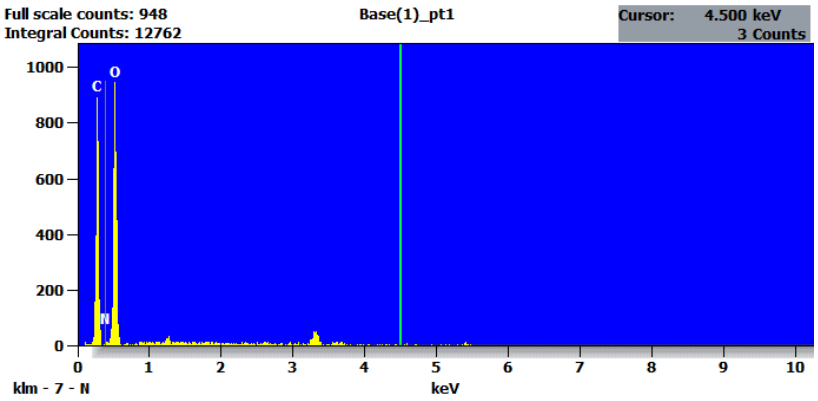


Fig. 6c EDX spectrum



Element	Wt%
C	23.65
N	7.30
O	69.05

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%, 90ins and 62°C, 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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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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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

Reference

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