

Unripe bael pulp pectin extraction and characterization using the microwave assisted method

Prabhat Kumar Mahour* and Alak Kumar Singh

Harcourt Butler Technical University, Kanpur, Uttar Pradesh, India. *E-mail: prabhatmahour@gmail.com

Abstract

In this study, pectin was extracted from unripe bael using a microwave-assisted extraction technique. Optimization was done based on independent and dependent parameters. Pectin yield was the dependent variable in the study, whereas the independent factors were microwave power, time, and solid solvent ratio. Pectin extraction was carried out using different combinations of microwave power (180, 360, 540 watts), time (1, 2, 3 minutes), and the ratio of solid-solvent (1:20, 1:30, 1:40 g/mL). Using Box-Behnken response surface methodology, 17 experimental runs were performed. A microwave power of 360 watts, a time of 3 minutes, and a solid solvent ratio of 1:20 g/mL were the ideal parameters that achieved the maximum pectin yield of 19.8%. The yield of pectin was analyzed using multiple regression statistical analysis. Qualitative and quantitative analysis of pectin yield was studied, which revealed an equivalent weight of 263.15, a methoxyl content of 9.92 percent, anhydrouronic acid content of 123.2%, and a degree of esterification of 45.71%.

Key words: Microwave-assisted extraction, unripe bael, optimization, pectin, characterization

Introduction

Bael is a native and unexplored fruit of India. Its remarkable nutritional and therapeutic qualities make it different from other fruits. According to Sonawane *et al.* (2020), bael fruit is rich in β -carotene, and its pulp contains highly bioactive components. Various human disorders can be efficiently treated by the tree's parts, such as leaves, fruits, stems, bark, and roots, at various stages of development (Bhattacharjee *et al.*, 2017). Good diuretic, tonic, and mild laxative properties are found in the raw, ripe bael pulp (Pawar *et al.*, 2015). Bael grows in central and south India, as well as in the sub-Himalaya. It is called Bilva in Sanskrit, Vilvam in Tamil, and Bael in Hindi (Rajan *et al.*, 2011).

The naturally occurring source of dietary fibers with prebiotic potential is pectin, a polymer present in the cell walls of all plant species (Surolia *et al.*, 2022). Conventional techniques have been used for years to extract pectin however, more recent techniques like enzyme extraction, supercritical water extraction, microwave-assisted extraction, and ultrasound extraction are becoming more and more popular. Microwave-assisted extraction is characterized by its high purity, low processing time, and huge handling capacity. The conditions for enzymatic extraction are gentle, it uses little energy, and it produces no pollutants (Sandarani *et al.*, 2017). The traditional method exposes the pectin to extended heat treatment during the process, which leads to pectin breakdown and lower pectin extraction quality and quantity (Koh *et al.*, 2014). A significant contribution to the development of a novel class of health-promoting active packaging is made by pectin-based nanoemulsions (Gurev *et al.*, 2023). Because pectin forms gels in acidic conditions, it is useful in biological applications for managing weight. Due to their ability to swell and adhere to the stomach wall prior to digestion, pectin gels cause satiety and reduce appetite when they come into contact with the aqueous environment of gastric juices (Pei *et al.*, 2024).

Microwave Assisted Extraction (MAE) is a highly efficient technique with significant potential, as reported in various studies. Compared to the traditional approach, Microwave-assisted extraction shortens the extraction period and uses less solvent (Tongkham *et al.*, 2017). Using waste food resources, microwave-assisted extraction is a novel and environmentally friendly technique to extract bioactive components (Thirugnanasambandham *et al.*, 2017). Because of its ability to penetrate materials, microwave energy, which is a non-ionizing radiation, can do so. Since it is non-ionizing, it leaves the target material's chemical structure unaltered. The microwaves convert their electromagnetic energy into molecular motion, releasing heat. The Microwave extraction method is the fastest and most effective way of extracting pectin (Karbus *et al.*, 2021). MAE operates at frequencies between 300 MHz and 300 GHz using a non-ionizing wave. Rapid heat buildup from these waves results in enhanced solvent diffusion and cellular damage. The molecule is transferred to the solvent medium as a result (Lasunon *et al.*, 2022). The re-orientation of water molecules inside the plant causes microwaves to generate heat instantaneously, making it more effective than conventional heating. Microwave energy rapidly and efficiently generates the heat necessary for the extraction process (Sarah *et al.*, 2018). MAE causes polar molecules to vibrate more quickly at higher temperatures, thereby improving the capillary-porous plant cell wall components and raising the extraction yield (Spinei *et al.*, 2022). Microwave radiation causes the inactivation of pectin esterase by generating heat rapidly (Rivadeneira *et al.*, 2020). The necessary bioactive components are extracted from raw materials, and thermolabile elements are efficiently protected by MAE (Zakaria *et al.*, 2021). Pectinesterase and pectic substances interact when exposed to microwave energy, which improves the extraction of pectin (Mahmud *et al.*, 2021). A large variety of biologically active compounds can be extracted using the MAE technique, which also produces a homogenous temperature

distribution (Sen *et al.*, 2024). Microwave extraction differs from traditional extraction due to their distinct heat transfer methods, which can lead to non-isothermal temperature distributions (Mao *et al.*, 2023).

The objective of this paper was to optimize microwave-assisted pectin extraction from unripe bael by analyzing the effects of microwave power, time, and solid-solvent ratio on yield, and to evaluate the pectin's qualitative and quantitative properties.

Materials and methods

Raw material: The Kaghzi variety of unripe bael with an average size of 960 g was purchased from Chandra Shekhar Azad University, Kanpur, Uttar Pradesh. The required chemical reagents were available in the Department of Food Technology at Harcourt Butler Technical University Kanpur.

Unripe bael pulp powder preparation for pectin extraction:

The Maskey *et al.* (2018) procedure was followed for extracting the pulp from the unripe bael fruit. Unripe bael fruit was crushed with a hammer and washed with tap water for cleaning. The pulp was then scooped out using a spoon. Using a cabinet tray dryer (Armfield UOP 8 MKII), pulp was dried at 60 °C. Additionally, dried pulp was ground into a powder using a mixer grinder (Prestige Iris Ltd., 750 watts) and stored for later use in a sealed, airtight container.

Microwave-assisted extraction of pectin: Weighed 2 g of unripe bael powder and was mixed with 60 mL of 0.5M HCL (solvent) using a solid solvent ratio. Then, the solution was placed under the microwave. Pectin extraction was carried out utilizing various combinations of microwave power (180W, 360W, 540W), solid solvent ratio (1:20, 1:30, 1:40), and time (1, 2, and 3 minutes). After each experimental run, the solution was kept at room temperature and filtered through muslin fabric. For 20 minutes, centrifugation was carried out at 5000 rpm. After removing the supernatant from the centrifuge tubes, the solution was coagulated with an equal amount of alcohol and allowed to precipitate for an hour. Following precipitation, the muslin fabric was used to separate the coagulated pectin, and ethanol was used to clean it. The wet pectin was then dried at 45°C using a tray dryer until all of the moisture was removed and the weight was constant.

Pectin yield estimation: The mass of the extracted extract, represented as a percentage, indicates the extraction yield. It evaluates the ability of a solvent to extract certain compounds from the original one. The extracted yield is calculated using the acquired extract following filtration (Siddiqui *et al.*, 2021). Following equation was used to estimate the pectin yield.

$$\text{Pectin yield (\%)} = \frac{X}{Y} \times 100$$

where X= extracted dried pectin weight (g). Y= dried powder weight (g)

Equivalent weight: In a 250 mL conical flask, a 0.5 g sample with 5 mL of ethanol was added. Then, 1g of NaCl with 100 mL of distilled water was added, followed by 6 drops of phenol red indicator, and then titrated against 0.1 Sodium hydroxide to sharpen the endpoint further. The appearance of the pink color indicated that the titration was complete (Siddiqui *et al.*, 2021).

$$\text{Equivalent weight} = \frac{\text{Weight of sample} \times 1000}{\text{Volume of Alkali} \times \text{Normality of alkali}}$$

Methoxyl content (Meo): For this estimation, the solution collected after completing the equivalent weight was combined with 25 mL of 0.25 N Sodium hydroxide. After mixing the solution, it was left to cool at room temperature for half an hour. Following this, 25 mL of 0.25 N hydrochloric acid was added to the solution, and the equivalent weight was determined by titrating against 0.1 N sodium hydroxide until the same endpoint as previously determined (Azad *et al.*, 2014). The following equation was used for calculation.

$$\text{Methoxyl content(\%)} = \frac{\text{Volume of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample}}$$

Total anhydrouronic acid content (AUA): The equivalent weight and methoxyl content assessments were used to determine the AUA (Surolia *et al.*, 2022). Equation (4) provides a formula for estimating it. In this case, z = mL (titre) of Sodium hydroxide from equivalent weight determination, W=weight of sample, y = mL (titre) of sodium hydroxide from methoxyl content determination, and the molecular unit of AUA (1 unit) = 176 g.

$$\text{AUA(\%)} = \frac{176 \times 0.1z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{W \times 1000}$$

Degree of esterification: Methoxyl and total anhydrouronic acid content data were used to determine it (Surolia *et al.*, 2022). It was computed using the following equation.

$$\text{Degree of esterification (\%)} = \frac{176 \times \text{Meo(\%)} \times 100}{31 \times \text{AUA(\%)}}$$

Experimental design: In this study, pectin was extracted from unripe bael using the microwave-assisted extraction method. The process parameter was optimized by using the response surface methodology. The variables utilized, which were categorized as high (+1) and low (-1) according to Table 1, were microwave power (A), time (B), and solid solvent ratio (C). The 17 experimental runs were accounted for with three variables, along with five center points each. Table 2 displays the experimental setup utilizing the box Behnken method to optimize the pectin extraction process parameters.

Table 1. Independent Variable's Range with coded levels

Factor	Parameters	LowCoded	HighCoded
		(-1)	(+1)
A	Microwave Power (Watt)	180	540
B	Time (minutes)	1	3
C	Solid solvent Ratio (g/mL)	1:20	1:40

Optimization and validation: Using response surface methodology with Software Design Expert Version 13.0.5.0 (State Ease), optimization was done to filter out the factors influencing pectin extraction from unripe bael pulp through Box-Behnken design with 3-level factorials. A p-value of less than 0.05 suggests that the model is significant. ANOVA was used to measure the significance of the three factors influence. The adequacy of the model was evaluated using the adjusted R² and anticipated R².

For the experimental runs in this study, the quadratic model was recommended. Using multiple regression analysis, the acquired data was used to determine the relationship between the independent and dependent variables. A mathematical equation,

Table 2. A Box Behnken experimental design with coded values using RSM along with pectin yield obtained

Exp. No	Microwave Power (Watt)	Time (minutes)	Solid-solvent ratio (g/mL)	Pectin yield (%)
1	360 (0)	2 (0)	1:30 (0)	16.7
2	360 (0)	2 (0)	1:30 (0)	17.3
3	360 (0)	1 (-1)	1:40 (+1)	13.9
4	360 (0)	2 (0)	1:30 (0)	17.1
5	540 (+1)	2 (0)	1:40 (+1)	14.7
6	180 (-1)	3 (+1)	1:30 (0)	13.9
7	360 (0)	3 (+1)	1:40 (+1)	18.2
8	540 (+1)	3 (+1)	1:30 (0)	15.3
9	360 (0)	3 (+1)	1:20 (-1)	19.8
10	180 (-1)	2 (0)	1:20 (-1)	14.7
11	180 (-1)	1 (-1)	1:30 (0)	8.2
12	180 (-1)	2 (0)	1:40 (+1)	13.5
13	540 (+1)	2 (0)	1:20 (-1)	16.5
14	540 (+1)	1 (-1)	1:30 (0)	10.3
15	360 (0)	2 (0)	1:30 (0)	15.9
16	360 (0)	2 (0)	1:30 (0)	16.4
17	360 (0)	1 (-1)	1:20 (-1)	14.2

which was further expressed as a second-order polynomial equation (6), was used to represent each response used to correlate

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^n \beta_{ij} X_i X_j$$

Where,

Y= Response, $\beta_0, \beta_i, \beta_{ii}$ are the regression coefficient while n represents the number of independent variables. In this model number of variables were 3 so ($n = 3$), whereas X_i and X_j were the independent variables. The ranges of i and j were ($i=1, 2, 3, \dots, n$ and $j= 1,2, 3, \dots, n$)

Results and discussion

Pectin yield analysis: Using the microwave-assisted method, the aim was to optimize pectin process parameters based on yield and examine their effect on pectin yield from unripe bael pulp. As demonstrated in Table 2, the research study employed a Box-Behnken experimental design, with three variables and three levels for each. Various combinations of variables were used for optimization, including microwave power (180W, 360W, 540W), time (1,2,3 minutes), and solid solvent ratio (1:20, 1:30, 1:40). After 17 experimental runs, the combination of 360 W of microwave power, 3 minutes of time, and a 1:20 (g/mL) solid solvent ratio produced the highest pectin yield (19.8%). Pectin yield decreases as the solid solvent ratio rises because the solute particles become more soluble with time, decreasing the extraction solvent's viscosity and hastening the release and dissolution of these compounds. Consequently, pectin yield rises when the solid-solvent ratio falls. When microwave power rises, the yield also increases. However, as power levels fall, yield begins to decline. Prolonged microwave power may cause the extract to deteriorate, warming the mixture of solute and solvent. This means that as the microwave heats the solution more, the amount of product produced increases. Conversely, as the microwave heats it less, the amount of product produced decreases due to the solution deteriorating from prolonged heating.

ANOVA determined the statistical significance of the recommended model for pectin yield, and the findings are shown in Table 3. With a F value of 63.41, the model is considered

significant. An F-value this high will only be due to noise 0.01% of the time. Model terms with P-values less than 0.0500 indicate the significance of the model. When compared to pure error, the lack of fit's F-value of 0.28 indicates that it is not significant. The insignificant lack of fit confirms the model's fitness.

Moreover, the pectin yield's R^2 value was determined to be 0.9879, meaning that 98.79% of the data in the model should be accounted for. The outcome indicates that the expected R^2 of 0.9507 and the adjusted R^2 of 0.9723 confirm that as well, with a sufficient ratio of 32.77 in relation to the model significance. The predicted and adjusted values must be less than 0.2, and adequate precision must be more than 4. The coefficients are shown in Table 4 as coded factors.

The second-order polynomial equation was formulated using the response surface model. Equation (7) illustrates the interaction between the independent and dependent variables for pectin yield. $Y = 16.68 + 0.81 X_1 + 2.58 X_2 - 0.61 X_3 - 0.17 X_1 X_2 - 0.15 X_1 X_3 - 0.32 X_2 X_3 - 3.21 X_1^2 - 1.54 X_2^2 + 1.38 X_3^2$

Table 3. ANOVA (Analysis of Variance) for yield of pectin

Source	Mean square	F- Value	P- Value	Significance
Model	13.68	63.41	<0.0001	significant
A – Microwave Power	5.28	24.47	0.0017	
B –Time	53.05	245.82	<0.0001	
C – Solid Solvent Ratio	3.00	13.91	0.0074	
AB	0.1225	0.5677	0.4758	
AC	0.0900	0.4171	0.5390	
BC	0.4225	1.96	0.2045	
A ²	43.52	201.69	<0.0001	
B ²	9.99	46.28	0.0003	
C ²	8.08	37.43	0.0005	
Residual	0.2158			
Lack of Fit	0.0875	0.2804	0.8378	not significant
Pure Error	0.3120			
Cor Total				
R ²				
Adjusted R ²				
Predicted R ²				
Adeq Precision				
Mean	15.09			
Std. Dev.	0.4645			
C.V %	3.08			

(Source: Design Expert Version13.0.5.0(State Ease) (Significance at $P<0.05$)

Table 4. Coefficients in terms of coded factors

Factor	Coefficient Estimate	df	SE	95% CI Low	95% CI High	VIF
Intercept	16.68	1	0.2077	16.19	17.17	
A-Microwave Power	0.8125	1	0.1642	0.4241	1.20	1
B-Time	2.58	1	0.1642	2.19	2.96	1
C-Solid Solvent Ratio	-0.6125	1	0.1642	-1.00	-0.2241	1
AB	-0.1750	1	0.2323	-0.7242	0.3742	1
AC	-0.1500	1	0.2323	-0.6992	0.3992	1
BC	-0.3250	1	0.2323	-0.8742	0.2242	1
A ²	-3.21	1	0.2264	-3.75	-2.68	1.01
B ²	-1.54	1	0.2264	-2.08	-1.00	1.01
C ²	1.38	1	0.2264	0.8497	1.92	1.01

(Source: Design Expert Version13.0.5.0 (State Ease) (Significant at $P<0.05$)

Pectin characterization analysis: From the experimental runs, the optimum conditions that produced the highest pectin output were selected for pectin characterization.

Equivalent weight: The most significant factor of the unripe bael pulp pectin's qualitative characteristics is its equivalent weight. Under optimal conditions, the equivalent weight was calculated to be 263.15.

Methoxyl content (%): The methoxyl content of pectin influences both the texture and functional qualities of the pectin gel. The methoxyl content of pectin was found to be 9.92%.

Anhydrouronic acid content (%): An AUA content of 123.2% was determined from the pulp of unripe bael fruit. As per the guidelines provided by the Food Chemical Codex, it must not be below 65%.

Degree of esterification: This is an important parameter because it needs to be computed along with the methoxyl and anhydrouronic acid contents. 45.71% of the pectin yielded the maximum degree of esterification.

3-D model graph analysis of pectin yield: As seen in Figures 1, 2, and 3, the three-dimensional response surface can be utilized to graphically depict the relationship between the responses and the experimental factors. The dependent variable was pectin yield, and the independent variables were microwave power, time, and solid solvent ratio to optimize the process parameters.

Relationship between microwave power and time: Figure 1 illustrates how time and microwave power affects pectin yield. High time with increased microwave power showed the maximum pectin yield. Through a sudden increase in internal pressure within the plant cell, microwave time speeds up plant cell rupture and encourages sample surface degradation. This facilitates the release of pectin from plant cells into the solvent and surrounding environment.

Relationship between microwave power and solid solvent ratio: Increasing microwave power caused the pectin yield to rise while the solid solvent ratio decreased (Fig. 2). This is because

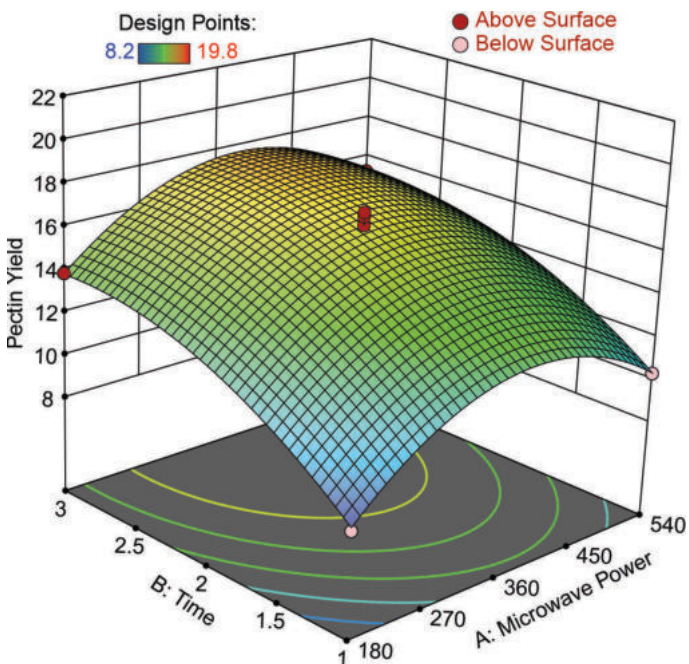


Fig.1. Relationship between Microwave power and time

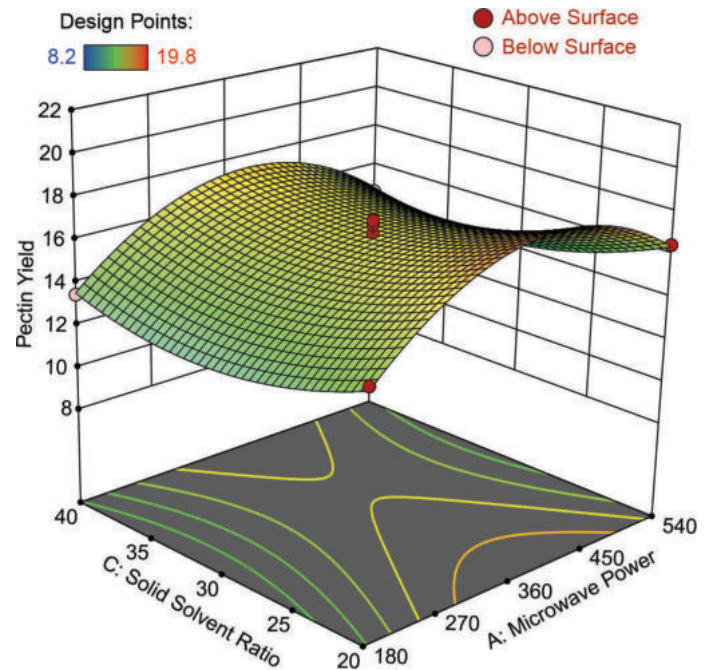


Fig. 2. Relationship between microwave power and solid solvent ratio using a longer microwave could lead to the extract degrading because the solvent and solute mixture would warm up.

Relationship between solid solvent ratio and time: The maximum pectin yield was achieved over a longer period of time with a lower solid solvent ratio, as shown in Fig. 3. This is because the solute particles became more soluble with time,

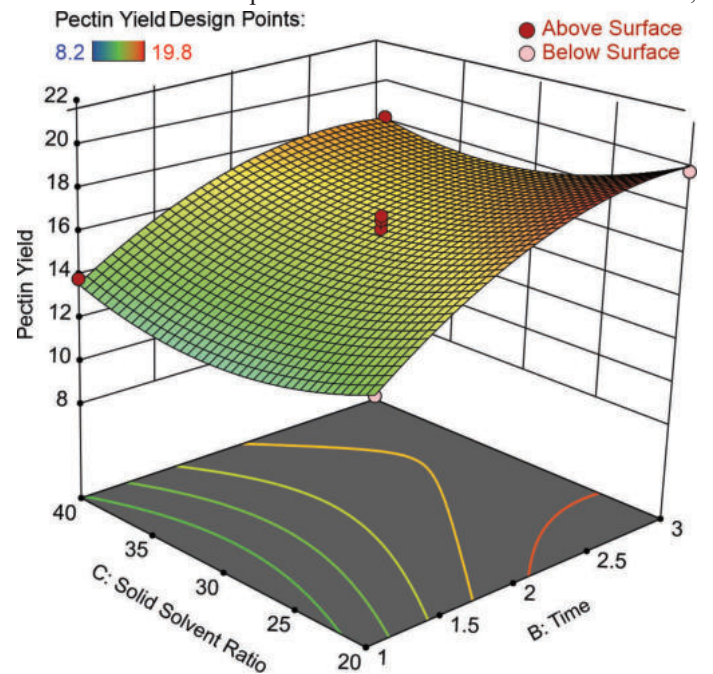


Fig. 3. Relationship between solid solvent ratio and time reducing the extraction solvent's viscosity and accelerating the release and dissolution of the compounds.

Analysis of predicted vs actual values of pectin yield: The analysis of pectin yield focuses on comparing actual measured values of pectin yield against predicted values generated by a model (Fig. 4). This relationship is important for understanding the accuracy and reliability of the predictive model used in pectin production.

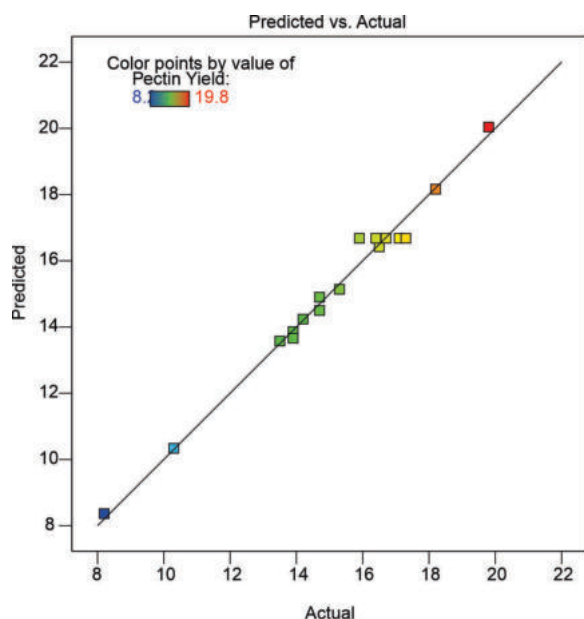


Fig. 4. Analysis of predicted vs actual values of pectin yield

In conclusion, this study successfully optimized the microwave-assisted extraction of pectin from unripe bael using the Box-Behnken response surface methodology. The extraction parameters of microwave power, time, and solid-solvent ratio were crucial in maximizing pectin yield, with the optimal conditions being 360 watts of power, 3 minutes of exposure, and a solid-solvent ratio of 1:20, resulting in a yield of 19.8%. The statistical analysis confirmed the significance of the model, with a high R^2 value of 0.9879, validating the reliability of the optimized conditions. The qualitative analysis further revealed promising characteristics of the extracted pectin, indicating its potential for industrial and health-related applications.

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Conflict of interest: The authors declare that there is no conflict of interest.

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