
Ultrasound Assisted Extraction of Pectin from Unripe Bael Using Box-Behnken Design Based Optimization of a Process Parameters

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Abstract: - The present study was conducted for Pectin extraction from unripe bael using Ultrasound Assisted Extraction technique. Optimization was done on the basis of independent and dependent parameters. The independent factors in the study were ultrasound power intensity, solid solvent ratio, and sonication time, while the dependent variable was pectin yield. Pectin extraction was carried out using different combination of Ultrasound Power (60%,70%,80%), the ratio of solid-solvent (1:20, 1:30,1:40) and Time (15, 30, 45 minutes) with constant pH 1.5 and Temperature 70°C. Using Box Behnken response surface methodology 17 experimental runs were performed. The optimum condition at which highest pectin yield 18.8% obtained was ultrasound power intensity 80 (W/cm²), Solid solvent ratio 1:30 (g/ml) and sonication time 15 minutes. The yield of pectin was analyzed using multiple regression statistical analysis. Qualitative and quantitative analysis of pectin yield was studied which reveal the equivalent weight 238.09, Methoxyl content 11.16 percent, Anhydrouronic acid content 137.28% and degree of esterification 46.15%.

Keywords: - Ultrasound assisted extraction, Unripe Bael, Optimization, Pectin, Ultrasound power.

1. INTRODUCTION

Bael, having the scientific name (*Aegle marmelos*), is a medicinally important fruit tree that grows on plains and in dry forests throughout India, Malaysia, Sri Lanka, Fiji, Pakistan, Myanmar, Vietnam, Thailand, and the Philippines [1]. It is native to India and is utilized in traditional treatments. The practitioners of Ayurvedic medicine utilize nearly all of its parts, but the fruits have the most medicinal potential [2].

Pectin is a polysaccharide that is widely used in the form of gel, thickener, emulsifier, stabilizing agent, and edible coating in the food industry. High methoxy and low methoxy are two subcategories of pectin related to the degree of methylation. Pectin's recovery is an essential unit of operation in the food industry in order to meet rising demand. At the commercial level, by hydrolyzing protopectin into pectin, the extraction of pectin is done at high temperatures, although there are also innovative approaches to pectin production [3].

For the removal of burning sensations in the soles, the unripe fruit's sesame oil plays an effective role. The leaf decoction is used to cure various ailments. Bael pulp is used to treat diarrhea and problems related to pregnancy [4]. According to Thuy et al. [5], extraction efficiency can be improved by combining

traditional procedures with new technologies, such as ultrasonic-assisted extraction. In terms of commercial economy, ultrasonic-assisted extraction performs effectively.

Ultrasound-assisted extraction is an efficient and environmentally friendly process that may be done in minutes, resulting in a higher-purity outcome.

Using cavitation bubbles induced by ultrasonic waves, the UAE extracts important components from the source. Ultrasound ensures the desired bioactive component's recovery with limited degradation and increases the rate of mass transfer [6].

Among various extraction techniques Ultrasound is the one that involves waves of sound higher than 20 kHz. Due to the shorter extraction duration, higher yield, and less use of energy, the UAE has been used for the extraction of bioactive chemicals [7].

UAE is usually carried out through an ultrasonic bath and probe. As a source of ultrasound power, both the ultrasonic probe and bath are based on the principle of a piezoelectric transducer. Although ultrasonic water baths are less expensive and simpler to use, their repeatability limits their utility in the extraction process. Because of the higher ultrasonic intensity, probe-based systems are frequently chosen over bath-based systems and used as an effective tool for bioactive compound extraction [8].

2. MATERIALS AND METHODOLOGY

2.1. Raw Material

Unripe bael was purchased from Chandra Shekhar Azad University, Kanpur, Uttar Pradesh. The Kaghzi variety of Bael with an average size of 960 g was used during this method. Hydrochloric acid and ethanol were used as chemical reagents, which were available in the food technology laboratory at Harcourt Butler Technical University, Kanpur.

2.2. Methodology

2.2.1 Unripe bael pulp powder preparation for pectin extraction

Pulp was extracted from unripe bael according to the procedure recommended by Maskey et al. [9]. For the cleanup of dirt, the fruit was washed with tap water and further cracked with a hammer, and the pulp was scooped out using a spoon. Then drying of the pulp was done using a cabinet tray dryer (Armfield UOP 8 MKII) at 60 °C prior to a constant weight achieved. Further, by using a mixer grinder (Prestige Iris Ltd., 750 watt), dried pulp was converted into powder and kept in an airtight, sealed container for later use.

2.2.2 Ultrasound Assisted Extraction of Pectin

Weighed 2 g of unripe bael powder and mixed it with 60 ml of 0.5M HCL (solvent) using a solid solvent ratio. Then place the solution under an Ultrasonicator apparatus (PCi Analytics Model No. PKS-900F, SR. No. 19PKSOCT25, BANDI TECHNOLOGY ISO 9001:2008 Certified Company) probe. Pectin extraction was conducted using different combination of Ultrasound Power (60%,70%,80%), Solid solvent ratio (1:20, 1:30,1:40) and Time (15, 30, 45 minutes) with constant pH 1.5 and Temperature 70°C. After every experimental run, the solution was maintained at ambient temperature and filtered using muslin cloth. Centrifugation was done at 5000 rpm for 20 minutes. Afterward, from the centrifugation tubes supernatant was separated and the solution was coagulated with the equivalent proportion of alcohol followed by the precipitation for one hour. After precipitation, coagulated pectin was separated using muslin cloth and cleaned using ethanol. Then, using a tray dryer, wet pectin was dried at 45°C until the moisture was eliminated and the weight was consistent.

3. PECTIN'S QUALITATIVE AND QUANTITATIVE ANALYSIS

3.1. Estimation of yield of pectin

The yield that was extracted was expressed as the mass of the extracted extract in percentage. It assesses a solvent's capacity to remove certain substances from the original one. After filtration, the extract that was obtained is used for the calculation of the extracted yield [10]. Pectin yield was estimated according to the equation (1).

$$Pectin\ Yield\ (\%) = \frac{X}{Y} \times 100 \quad (1)$$

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

3.2. Equivalent weight

It is essential to characterizing Pectin's behavior. A 0.5 gram sample was added to a 250 ml conical flask., followed by ethanol of around 5 ml. To sharpen the endpoint further, add 1g of NaCl with 100 ml of distilled water, followed by six drops of phenol red indicator, and lastly, titration against 0.1 NaOH. The completion of the titration was indicated as the pink color appeared (Siddiqui et.al., 2021). Calculation was carried out by formula given in equation (2).

$$\frac{Equivalent\ weight = Weight\ of\ sample \times 1000}{Volume\ of\ Alkali \times Normality\ of\ alkali} \quad (2)$$

3.3. Methoxyl Content (Meo)

For this estimation, the solution that was collected after the completion of the equivalent weight was further added to 25 ml of 0.25 N NaOH. The solution was then mixed and allowed to cool for 30 minutes at room temperature. After this in the solution, the addition of 25 ml of 0.25 N hydrochloric acid was done, followed by the titration against 0.1 N sodium hydroxide until the same endpoint as done before for equivalent weight determination[11]. It is possible to compute it using the formula in equation (3)

$$\frac{Methoxyl\ content(\%) = Volume\ of\ alkali \times Normality\ of\ alkali \times 3.1}{Weight\ of\ sample} \quad (3)$$

3.4. Total Anhydrouronic acid content (AUA)

The AUA was identified using the equivalent weight and methoxyl content evaluations [12]. It can be estimated by given formula in equation (4). Where, molecular unit of AUA (1 unit) = 176 g, z = ml (titre) of NaoH from equivalent weight determination, y = ml (titre) of NaoH from methoxyl content determination

$$AUA(\%) = \frac{176 \times 0.1z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{W \times 1000} \quad (4)$$

3.5. Degree of Esterification

It was determined through Methoxyl content and total Anhydrouronic acid content values [12]. It was calculated by given equation (5).

$$\text{Degree of Esterification (\%)} = \frac{176 \times \text{Meo(\%)} \times 100}{31 \times \text{AUA(\%)}} \quad (5)$$

4. EXPERIMENTAL DESIGN

The present study was conducted for pectin extraction from unripe bael using ultrasound assisted

extraction method. A process parameter was optimized using the response surface approach. As shown in **Table 1**, the variables used were ultrasound power intensity (A), solid solvent ratio (B), and sonication time (C), which were coded as high (+1) and low (-1). Three variables account for the 17 experimental runs with five center points per block. Each experimental run was performed in triplicate. The Experimental design using box Behnken for optimizing the process parameters for pectin extraction is shown in **Table 2**.

Table 1. Independent Variable's Range with coded levels

Factor	Parameters	Low Coded	High Coded
		(-1)	(+1)
A	Ultrasound Power Intensity (W/cm ²)	60	80
B	Solid solvent Ratio (g/ml)	1:20	1:40
C	Time (minutes)	15	45

Table 2: A Box Behnken experimental design using RSM for optimizing the pectin extraction with pectin yield obtained (Result)

Exp. No	Ultrasound Power Intensity	Solid-solvent Ratio	Sonication Time	Pectin yield (%)
	(X ₁) (W/cm ²)	(X ₂) (g/ml)	(X ₃) (minutes)	
1	80	1:20	30	6.2
2	60	1:40	30	5.2
3	70	1:30	30	10.8
4	70	1:20	15	11.2
5	80	1:30	15	18.8
6	60	1:20	30	9.2
7	60	1:30	15	11.5
8	70	1:40	15	7.9
9	70	1:20	45	8.1
10	70	1:30	30	12.5
11	60	1:30	45	14.3
12	70	1:30	30	11.1
13	80	1:30	45	16.9
14	70	1:40	45	16.8
15	70	1:30	30	11.3
16	70	1:30	30	10.6
17	80	1:40	30	16.5

5. STATISTICAL ANALYSIS

5.1. Optimization and Validation

Software Design Expert Version 13.0.5.0 (State Ease) implemented a Box Behnken design using response surface methodology utilizing 3-level factorials (-1, 0, 1) to optimize and filter out the factors that influence pectin extraction from unripe bael pulp. The existence of a p value less than 0.05 indicates that the model is significant. The influence of the three factors was significantly determined using an analysis of variance (ANOVA). The predicted R² and adjusted R² were used to assess the model's adequacy.

The quadratic model was recommended for experimental runs in this investigation. The acquired data was used to identify the link between both independent and dependent variables with respect to multiple regression analysis.

Each response utilized to correlate the response surfaces was indicated by a mathematical equation, which was further written as a second-order polynomial equation (6).

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^n \beta_{ij} X_i X_j \quad (6)$$

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 $j = 1, 2, 3, \dots, n$)

6. RESULT AND DISCUSSION

6.1. Analysis of Pectin Yield:

The intent of this research was to investigate the impact of process variables on pectin yield from unripe bael pulp using an ultrasound-assisted method and to optimize process parameters for pectin based on yield. As shown in **Table 2**.

The research study used a Box-Behnken experimental design with 3 variables and 3 levels for each variable. Optimization was carried out using different combination of variables such as Ultrasound

Power (60%,70%,80%), Solid solvent ratio (1:20, 1:30,1:40) and Sonication Time (15, 30, 45 minutes) with constant pH 1.5 and Temperature 70°C. Out of 17 experimental runs, the optimum yield of pectin (18.8%) was obtained with the combination of ultrasound power 80 (W/cm²). Solid Solvent ratio 1:30 (g/ml) at time 15 minutes with constant pH 1.5 and Temperature 70°C.

The highest yield of pectin could be attributed to the maximum amount of ultrasound power intensity used during the extraction process. At sufficiently high intensity of ultrasound, UAE raises due to rupture of the cell wall matrix of unripe bael pulp, which results in an improvement in interactions between solvent and extracted material and hence achieving greater pectin yield. Increased ultrasound power intensity improved pectin solubility in the solvent medium.

The statistical significance of the recommended model for pectin yield was determined using ANOVA, and the findings are shown in **Table 3**. The model's F value is 30.53, indicating that it is significant. Only 0.01% of the time will an F-value this high be caused by noise. P-values less than 0.0500 for model terms demonstrate that the model is significant. The F-value of 1.76 for lack of fit suggests it is insignificant when compared to pure error.

The insignificance of the lack of fit confirms the fitness of the model. Furthermore, the value of R² for the yield of pectin was found to be 0.9752, which indicates that the model's data ought to account for 97.52%. The predicted and adjusted values need to be less than 0.2, and adequate precision must be greater than 4, as the result shows that the predicted R² of 0.7571 and the adjusted R² of 0.9432 confirm that too, with an adequate ratio of 18.42 with respect to the model significance. **Table 4** presents the coefficients in terms of coded factors.

The response surface model was used to formulate the second-order polynomial equation. The interaction between independent and dependent variables for the yield of pectin is shown in equation (7)

$$Y = 11.64 + 2.27 X_1 + 1.46 X_2 + 0.83 X_3 + 3.57 X_1 X_2 - 1.17 X_1 X_3 + 3.00 X_2 X_3 + 1.00 X_1^2 - 3.37 X_2^2 + 2.73 X_3^2 \quad (7)$$

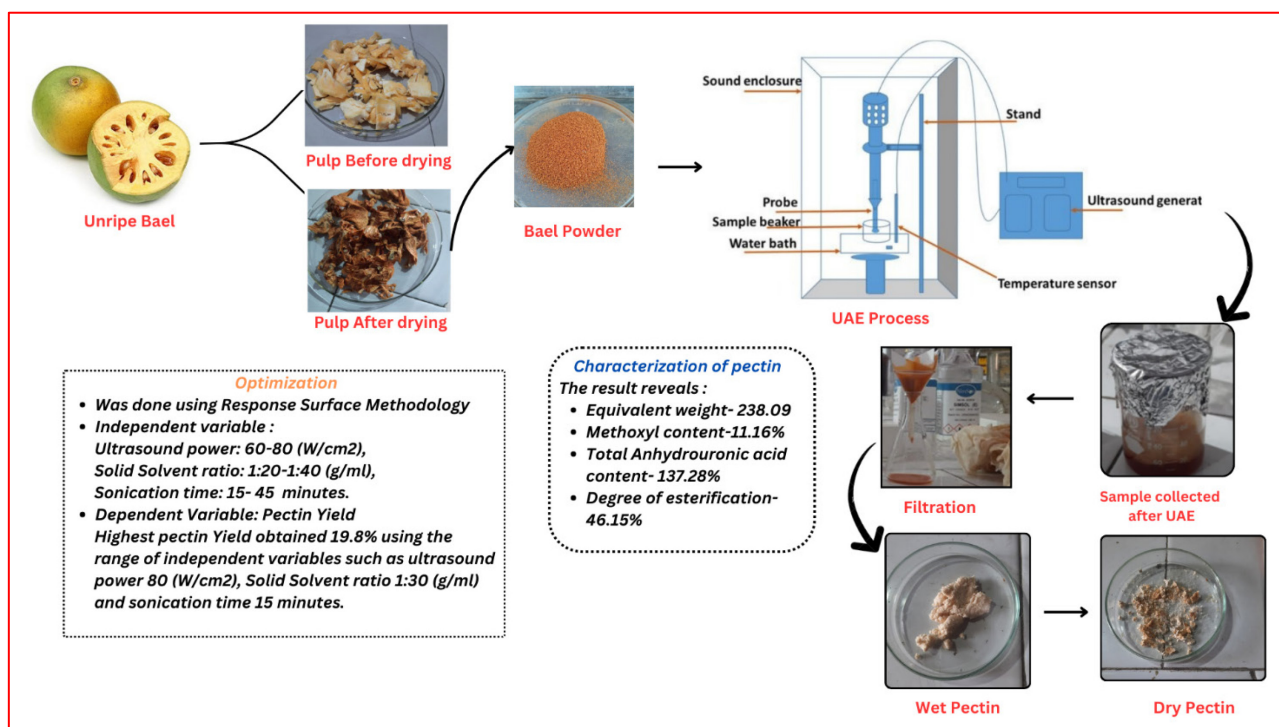


Figure 1. Graphical Abstract

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

Source	Sum of squares	df	Mean square	F- Value	P- Value	
Model	236.08	9	26.23	30.53	<0.0001	significant
A – Ultrasound Power	41.41	1	41.41	48.19	0.0002	
B – Solid Solvent Ratio	17.11	1	17.11	19.91	0.0029	
C – Sonication Time	5.61	1	5.61	6.53	0.0378	
AB	51.12	1	51.12	59.50	0.0001	
AC	5.52	1	5.52	6.43	0.0389	
BC	36.00	1	36.00	41.90	0.0003	
A ²	4.25	1	4.25	4.95	0.0614	
B ²	47.82	1	47.82	55.65	0.0001	
C ²	31.38	1	31.38	36.52	0.0005	
Residual	6.01	7	0.8592			
Lack of Fit	3.42	3	1.14	1.76	0.2933	not significant
Pure Error	2.59	4	0.6480			
Cor Total	242.10	16				
R ²	0.9752					
Adjusted R ²	0.9432					
Predicted R ²	0.7571					
Adeq Precision	18.4266					
Mean	11.81					
Std. Dev.	0.9269					
C.V %	7.85					

(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	Standard Error	95% CI Low	95% CI High	VIF
Intercept	11.64	1	0.4145	10.66	12.62	
A-Ultrasound Power	2.28	1	0.3277	1.5	3.05	1
B-Solid Solvent Ratio	1.46	1	0.3277	0.6876	2.24	1
C-Sonication Time	0.8375	1	0.3277	0.0626	1.61	1
AB	3.57	1	0.4635	2.48	4.67	1
AC	-1.18	1	0.4635	-2.27	-0.0791	1
BC	3	1	0.4635	1.9	4.1	1
A ²	1.01	1	0.4517	-0.0632	2.07	1.01
B ²	-3.37	1	0.4517	-4.44	-2.3	1.01
C ²	2.73	1	0.4517	1.66	3.8	1.01

(Source: Design Expert Version 13.0.5.0 (State Ease) (Significance at $P < 0.05$)

6.2 Analysis of Pectin's characterization:

For pectin's characterization, from the experimental runs, the optimum conditions at which the highest pectin yield was obtained were chosen.

Equivalent weight: It is the major factor in determining the qualitative characteristics of unripe bael pulp pectin. The equivalent weight was determined to be 238.09 at optimal conditions.

Methoxyl Content (%): Pectin's methoxyl content determines its functional properties in addition to the texture of the pectin gel. Pectin's methoxyl content was revealed to be 11.16%.

Anhydrouronic Acid content (%): The AUA content derived from the unripe bael fruit pulp was found to be 137.28%. It should not be less than 65% according to the Food Chemical Codex (1996).

Degree of Esterification: The degree of esterification holds a vital role, as it requires the value of methoxyl content as well as anhydrouronic acid content to be calculated. The pectin with the highest yield had a degree of esterification of 46.15%.

6.3. 3-D model graph analysis of pectin yield:

The three-dimensional response surface can be used to graphically illustrate the relationship between the responses and the experimental variables, as shown in Figures 2, 3, and 4. To optimize the process parameters, the independent variables were ultrasound power, solid solvent ratio, and sonication time, while pectin yield was the dependent variable by keeping temperature and pH constant.

6.3.1 Relationship between Ultrasound Power intensity and Solid Solvent Ratio

In ultrasound assisted extraction method, ultrasound power plays a vital role as one of the

important effective factors. The effect of ultrasound power and solid solvent ratio on pectin yield is shown in Figure 2. At higher ultrasound power with increased solid solvent ratio, the maximum yield of pectin was observed.

6.3.2 Relationship between Solid Solvent Ratio and Sonication time

The increment is seen in the yield of pectin as the sonication time and solid solvent ratio increase, as seen in Fig. 3. This is due to increasing the plant material's surface area with the solvent and enhancing material swelling, which supports the pectin's solubilization.

6.3.3 Relationship between Ultrasound Power and Sonication time

As demonstrated in Figure 4, the connection between ultrasound power and sonication duration suggests that enhanced pectin yield may be attributed to cell wall collapse and the release of cellular material at sufficiently high ultrasonic power intensity. The greater the contact duration between the extractor and the plant materials, the greater the amount of mass transfer from the solid components into the solution.

6.4 Analysis of predicted vs Actual values of pectin yield

The figure 5. reveals the value of pectin yield obtained at optimum condition on the basis of predicted and actual.

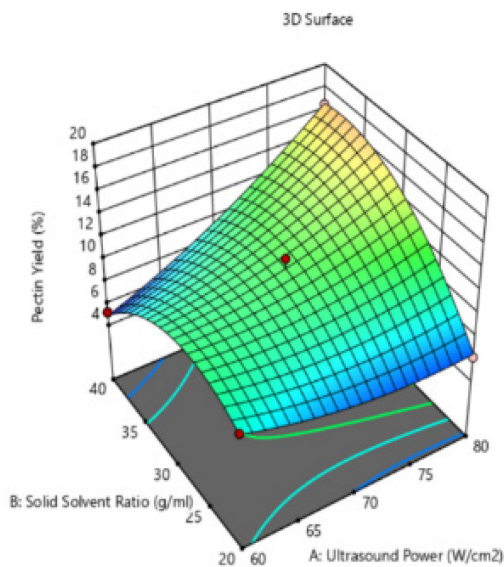


Figure 2. Relationship between Ultrasound power and Solid solvent ratio on pectin yield

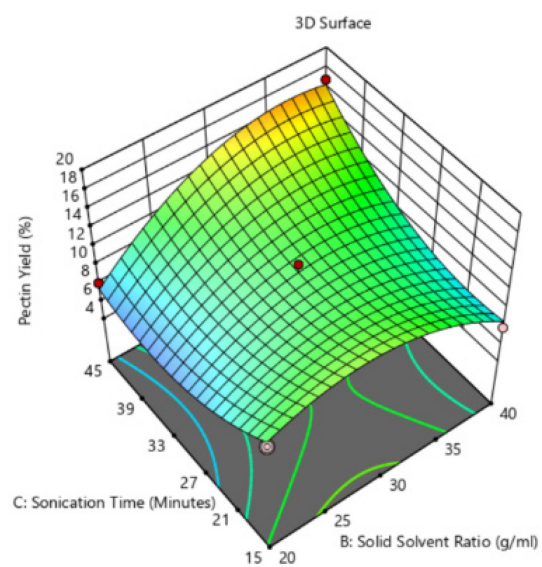


Figure 3. Relationship between Solid solvent ratio and Sonication time on pectin yield

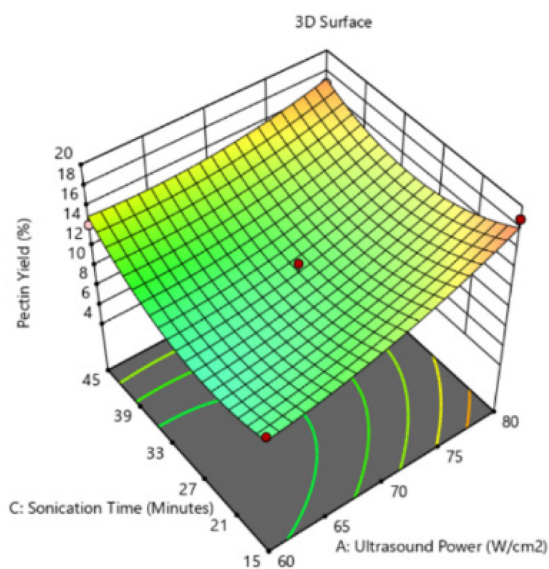


Figure 4. Relationship between Ultrasound power and Sonication time on pectin yield

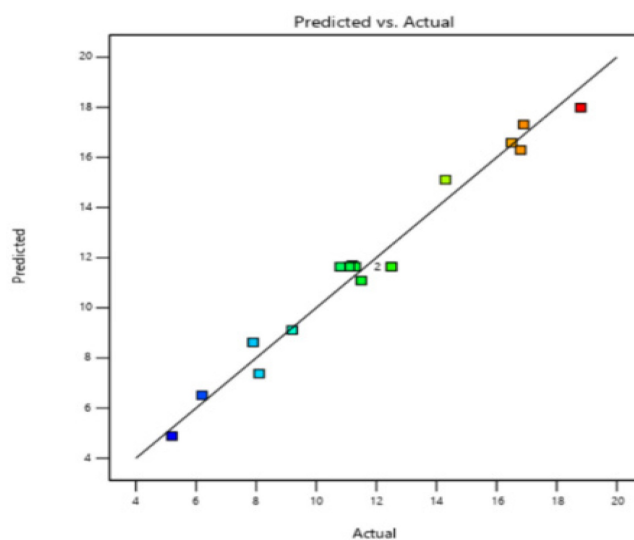


Figure 5. Predicted vs Actual value of pectin yield

7. CONCLUSION

It is feasible to extract pectin from unripe bael using the ultrasound-assisted extraction method. The optimal conditions for obtaining the maximum pectin yield (18.8%) were an ultrasound power intensity of 80 W/cm², a solid solvent ratio of 1:30 g/mL, and a sonication time of 15 minutes. Under these conditions, pectin was characterized, and the equivalent weight, methoxyl content, total anhydrouronic acid content, and degree of esterification were determined to be 238.09, 11.16%, 137.28%, and 46.15%, respectively. For pectin extraction, ultrasound-assisted extraction was used and it was verified that it is the superior method as

compared to conventional method as a high pectin yield was obtained in the shortest amount of time with the least amount of solvent needed. Due to this, the UAE method is considered to be one of the novel techniques for pectin extraction.

ACKNOWLEDGMENTS

It is with deep sense of gratitude and reverences that I express my sincere thanks to all the Professors of food technology department for playing a pivotal role for my guidance, encouragement, help and useful suggestion throughout. Their untiring and painstaking efforts, methodological approach and individual help made it possible to complete this work in time.

I express my thanks to all staff members and friends for all the help and co-ordination extended in bringing out the work successfully in time.

I will be failing in duty if I do not acknowledge with grateful thanks to the authors of the references.

Last but not the least, I am very much thankful to my parents who guided me in every step which I took.

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