A COMPARATIVE STUDY BETWEEN MICROWAVE AND ULTRASOUND-ASSISTED PECTIN EXTRACTION FROM UNRIPE BAEL

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Bael is an underappreciated indigenous fruit from the Rutaceae group grown in India and its subcontinents. Its nutritional and therapeutic characteristics set it apart from other fruits and make it easily processed **(Sonawane et.al 2020)**. According to **(Pawar et.al 2020)** all parts of this tree, including the root, leaf, trunk, fruit, and seed, are beneficial for a variety of diseases. Unripe and ripe fruit are considered astringent, digestive, and stomachic and are used for dysentery and diarrhea. The leaf decoction is used to treat asthma, acute bronchitis, inflammation, jaundice, hypoglycemia, and other ailments. Fresh juice from the leaves is consumed with honey as a laxative and febrifuge.

Pectin promotes plant growth, shape change, development, and defense. It's used to lower blood cholesterol and treat gastrointestinal issues. It is claimed that variations in total pectin and alcohol-insoluble solids in bael fruit during growth and ripening did not differentiate between pectin fractions **(Anup et.al 2017)**. Microwave-assisted extraction is a green extraction technology that improves repeatability, simplifies handling, reduces solvent usage, and requires less energy without compromising extraction yield. Microwave-assisted extraction is an alternate method for releasing bioactive components from waste food resources **(Thirugnanasambandham et.al 2017)**. Fruit pulp from Aegle marmelos has a high concentration of antioxidant chemicals in addition to vitamin C, E, and carotene **(Rajan et.al 2011)**.

In microwave-assisted extraction, plant molecules are heated dielectrically by being exposed to microwave radiation **(Sandarani et.al 2017).** Microwave-assisted extraction reduces extraction time, solvent usage, and increases extraction rate compared to conventional methods **(Tongkhamet.al 2017)**. The choice of solvent is critical to the extraction process. Using conventional procedures in combination with new technology, such as ultrasonic-assisted extraction, can increase extraction efficiency **(Thuy et.al 2022)**. Ultrasound-assisted extraction is a secure, effective, and sustainable method that yields a higher-purity finished product within minutes **(Karbuz et.al 2021)**. Ultrasound-assisted extraction is a non-thermal approach that has been researched for extracting plant components **(Yousuf et.al 2018)**. Microwave-assisted extraction utilizes non-ionizing radiation **(Lasunon et.al 2022)**. Ultrasound-assisted extraction (UAE) extracts chemicals from plant matrices using solvents and ultrasound energy. Ultrasounds are mechanical waves with frequencies that exceed the audible

frequency range of human hearing. These waves propagate across solid, liquid, or gaseous mediums, causing the displacement and dislodgement of molecules from where they initially were **(Kumar et.al 2019)**. Microwave heating is more effective than conventional heating because it generates heat instantaneously fromthe reorientation of water molecules inside the plant during the heating process. In order to facilitate the temperature needed for the extraction process, microwave energy produces instantaneous and fast heating **(Sarah et.al 2018)**. Plant cell wall components that are capillary- porous and have a greater capacity to absorb water are enhanced by microwave assisted extraction. This alteration increases the yield at which various plant cell wall analytes, including cellulose, hemicellulose, and pectin, may be extracted **(Spinei et.al 2022)**.

MATERIALS AND METHODS

Raw material

The Kaghzi variety of unripe bael, with an average size of 980 grams, was purchased from ChandraShekhar Azad University, Kanpur.

Methodology

Sample Preparation

The unripe bael pulp was extracted using the procedure described by Maskey et al. (2018). Initially the bael was washed and broken with a hammer, and the pulp was scooped out. The pulp was dried using a cabinet tray dryer (Armfield UOP 8 MKII) at 60 °C. Furthermore, the dry pulp was converted into powder with a mixer grinder (Prestige Iris Ltd., 750 watts) and stored in an airtight container.

Extraction of pectin using Ultrasound Assisted Method

Weighed 2 g of unripe bael powder and mixed it with 60 ml of 0.5 M HCL (solvent) using a solid solvent ratio, as described by Karbuz et al. (2021). The solution should then be placed under an ultrasonicator apparatus (PCi Analytics Model No. PKS-900F, SR. No. 19PKSOCT25, BANDI TECHNOLOGY ISO 9001:2008 Certified) probe. Pectin extraction was performed using various combinations of ultrasound power (60%, 70%, 80%), solid solvent ratio (1:20, 1:30, 1:40), and time (15, 30, 45 minutes) at a constant pH of 1.5 and temperature of 70 °C. After each experimental run, the solution was kept at room temperature and filtered through a muslin cloth. Centrifugation was carried out for 20 minutes 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, coagulated pectin was separated using muslin cloth and washed with ethanol. Wet pectin was then dried at 45°C in a tray dryer until all moisture was removed andthe weight was constant.

Extraction of pectin using Microwave Assisted Method

Weighed 2 g of unripe bael powder and mixed it with 60 ml of 0.5 M HCL (solvent) according tothe solid solvent ratio. The solution was then put in the microwave. Pectin extraction was carried out using different 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 experiment, the solution was maintained at room temperature and filtered through a muslin cloth. Centrifugation took place at 5000 rpm for 20 minutes.

After removing the supernatant from the centrifuge tubes, the solution was coagulated with the same amount of alcohol and left to precipitate for one hour. Following precipitation, the coagulated pectin was separated using muslin fabric and cleaned with ethanol. The wet pectin was then dried at 45 °C in a tray dryer until all of the moisture was removed and the weight remained consistent.

EXPERIMENTAL DESIGN

In the ultrasound-assisted extraction method, the dependent variable is pectin yield, and the independent variables are the ultrasound power intensity (60 to 80 W/cm2), solid solvent ratio (1:20 to 1:40), and sonication time (15 to 45 minutes). The independent variables are classified ashigh (+1) and low (-1). Three variables were used to account for the 17 experimental runs, each containing five center points per block. Each experimental run was repeated three times. The process parameter for microwave-assisted extraction was optimized using a response surface approach. The variables used were classified as high (+1) and low (-1). The independent variablesare microwave power (180 to 540 watts), time (1 to 3 minutes), and solid solvent ratio (1:20 to 1:40 g/ml), and the dependent variable is pectin yield. Three variables were used to account for the 17 experimental runs, with each having five center points. Table 1 shows the experimental setup for both the ultrasound and microwave-assisted methods that use the box Behnken method to optimize the pectin extraction process parameters.

Table 1. Experimental design using RSM for UAE and MAE method

Microwave assisted extraction					**Ultrasound assisted extraction**				
Run	**Independent variables**			**Dependent variable**	**Run**	**Independent variables**			**Dependent variable**
	Microwave power (Watt)	Time (minutes)	Solid solventratio (g/ml)	Pectinyield		Ultrasound Power (W/cm^2)	Solid solvent Ratio (g/ml)	Time (minutes)	Pectin yield
1	360 (0)	2 (0)	1:30 (0)	16.7	1	80 (+1)	1:20 (-1)	30 (0)	6.2
2	360 (0)	2 (0)	1:30 (0)	17.3	2	60 (-1)	1:40 (+1)	30 (0)	5.2
3	360 (0)	1 (-1)	1:40 (+1)	13.9	3	70 (0)	1:30 (0)	30 (0)	10.8
4	360 (0)	2 (0)	1:30 (0)	17.1	4	70 (0)	1:20 (-1)	15 (-1)	11.2
5	540 (+1)	2 (0)	1:40 (+1)	14.7	5	80 (+1)	1:30 (0)	15 (-1)	18.8
6	180 (-1)	3 (+1)	1:30 (0)	13.9	6	60 (-1)	1:20 (-1)	30 (0)	9.2
7	360 (0)	3 (+1)	1:40 (+1)	18.2	7	60 (-1)	1:30 (0)	15 (-1)	11.5
8	540 (+1)	3 (+1)	1:30 (0)	15.3	8	70 (0)	1:40 (+1)	15 (-1)	7.9
9	360 (0)	3 (+1)	1:20 (-1)	19.8	9	70 (0)	1:20 (-1)	45 (+1)	8.1
10	180 (-1)	2 (0)	1:20 (-1)	14.7	10	70 (0)	1:30 (0)	30 (0)	12.5
11	180 (-1)	1 (-1)	1:30 (0)	8.2	11	60 (-1)	1:30 (0)	45 (+1)	14.3

12	180 (-1)	2 (0)	1:40 (+1)	13.5	12	70 (0)	1:30 (0)	30 (0)	11.1
13	540 (+1)	2 (0)	1:20 (-1)	16.5	13	80 (+1)	1:30 (0)	45 (+1)	16.9
14	540 (+1)	1 (-1)	1:30 (0)	10.3	14	70 (0)	1:40 (+1)	45 (+1)	16.8
15	360 (0)	2 (0)	1:30 (0)	15.9	15	70 (0)	1:30 (0)	30 (0)	11.3
16	360 (0)	2 (0)	1:30 (0)	16.4	16	70 (0)	1:30 (0)	30 (0)	10.6
17	360 (0)	1 (-1)	1:20 (-1)	14.2	17	80 (+1)	1:40 (+1)	30 (0)	16.5

ANALYSIS OF PECTIN YIELD

Pectin yield estimation:

The extraction yield was calculated using the mass of the extracted extract as a percentage. It assesses how well a solvent extracts particular chemicals from the original. The extracted yield is determined from the obtained extract after filtering **(Siddiqui et al., 2021)**. Equation (1) was used to estimate pectin yield.

$$\text{Pectin Yield (\%)} = {}^{x}x\frac{100}{Y} \quad \ldots(1)$$

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

Equivalent weight :

A 0.5-gram sample was put into a 250-mL conical flask along with 5 mL of ethanol. Then 1 g of NaCl was mixed with 100 ml of distilled water, followed by 6 drops of phenol red indicator, and finally titrated against 0.1 sodium hydroxide to sharpen the endpoint. The pink color signaled that the titration was complete **(Siddiqui et al., 2021)**. The computation was performed using Equation(2).

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

Methoxyl content:

For this estimation, the solution collected after completing the equivalent weight was combined with 25 ml of 0.25 N NaOH. After mixing, the solution was allowed to cool at room temperature for 30 minutes. Following this, 25 ml of 0.25 N hydrochloric acid was added to the solution, followed by a titration against 0.1 N sodium hydroxide to the same endpoint as before for equivalent weight determination (**Azad et al., 2014**). It can be computed using the formula in equation (3).

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

Total Anhydrouronic Acid content (AUA):

It was determined using the equivalent weight and methoxyl content assessments **(Surolia et al., 2022)**. Equation (4) provides an estimation formula. In this scenario, z = ml (titre) of sodium hydroxide from equivalent weight measurement, y = ml (titre) of sodium hydroxide from methoxyl content determination, and one molecular unit of AUA = 176 g.

$$\text{AUA (\%)} = \frac{176\times 1.1z\times 100w}{100} \times + \frac{176\times 1.1y\times 100}{W\times 1000} \quad \ldots(4)$$

Degree of Esterification:

It was calculated using methoxyl and total anhydrouronic acid content data **(Surolia et al., 2022)**.It was calculated with the specified equation (5).

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

STATISTICAL ANALYSIS

Optimization and Validation:

Using ultrasound and microwave-assisted extraction methods, Software Design Expert Version

13.0.5.0 (State Ease) optimized and filtered out the components influencing pectin extraction fromunripe bael pulp. The Box Behnken design was carried out using a response surface approach. A p-value of less than 0.05 indicates that the model is significant. The model's adequacy was assessedusing the adjusted and predicted R^2.

The quadratic model was proposed for the experimental runs in this comparison of ultrasound and microwave-assisted extraction. The acquired data was analyzed using multiple regression to evaluate the link between the independent and dependent variables. Each response utilized to correlate the response surfaces was represented by a mathematical equation that was then stated asa second-order polynomial equation (6).

$$Y = \beta_0 \sum_{i=1}^{n} \beta i X_i + \sum_{i=1}^{n} \beta_{ii} X^n + \sum_{i=1}^{n} \sum_{j=i=1}^{n} \beta_{ii} X_i \overset{n}{X}_j \quad \ldots(6)$$

Where, Y= Response, β_i, β_i, β_{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 $x_i - x_n$ were the independent variables. The ranges of I and j were *(i*= 1, 2, 3, ...n and j = 1,2, 3 n).

RESULT AND DISCUSSION

Pectin yield analysis using Ultrasound Assisted Extraction method.

Using a box-Behnken experimental design, optimization was performed with various combinations of variables, such as ultrasound power (60%, 70%, 80%), solid solvent ratio (1:20, 1:30, 1:40), time (15, 30, 45 minutes), pH 1.5, and temperature 70 °C. The combination of ultrasound power 80 (W/cm^2), solid solvent ratio 1:30 (g/ml), time 15 minutes, constant pH 1.5, and temperature 70 °C resulted in the highest pectin yield (18.8%) out of 17 experiments. The maximum ultrasound power intensity used during the extraction process may have resulted in the highest pectin yield. When the ultrasound intensity is sufficiently high, the unripe bael pulp's cell wall matrix ruptures, causing the UAE to rise. This improves the material's interactions with the solvent and maximizes the amount of pectin extracted. Pectin's solubility in the solvent medium was increased by increasing the ultrasound power intensity. Table 2 shows the results of the ANOVA test performed to determine the statistical

significance of the proposed model for pectin yield. The response surface model was used to develop the second-order polynomial equation. Equation (7) depicts the interaction of independent and dependent variables on pectin yield.

$$Y = 11.64+2.27X_1 + 1.46X_2+0.83X_3+3.57X_1 X_2-1.17 X_1 X_3+3.00 X_2 X_3+1.00X^2+3.37X^2 + 2.73X^2 \quad ...(1)$$

Pectin yield analysis using Microwave Assisted Extraction method.

The research study used a Box-Behnken experimental design, which had three variables and three levels for each. Several variables were employed 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 experiments, the combination of 360 W of microwave power, 3 minutes of time, and a 1:20 (g/ml) solid solvent ratio resulted in the highest pectin yield (19.8%). Pectin yield drops as the solid solvent ratio increases because the solute particles become more soluble with time, reducing the viscosity of the extraction solvent and hastening the release and dissolution of these compounds. As a result, as the solid-solvent ratio decreases, the yield of pectin increases. The yield rises as microwave power increases. However, as power levels fall, yield starts to decline. Prolonged microwave power may cause the extract to degrade, overheating the solute-solvent solution. This means that when the microwave heats the solution, more product is created. In contrast, as the microwave heats it less, the amount of product generated decreases since the solution deteriorates from extended heating. The Table 3 shows the result of ANOVA to determine the statistical significance of the suggested model for pectin yield. Using the response surface model, the second- order polynomial equation was created. Equation (8) depicts the interaction of independent and dependent variables on pectin yield.

$$Y = 16.68 + 0.81 X_1 + 2.58 X_2 - 0.61 X_3 - 0.17 X_1 X_2 - 0.15 X_1X_3 - 0.32 X_2X_3 - 3.21 X^2 \quad ...(8)$$

Table 2. ANOVA (Analysis of Variance) for yield of pectin using Ultrasound Assisted method

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 3. ANOVA (Analysis of Variance) for yield of pectin using Microwave assisted method

Source	Sum of squares	df	Mean square	F- Value	P- Value	
Model	123.14	9	13.68	63.41	<0.0001	significant
A – Microwave Power	5.28	1	5.28	24.47	0.0017	
B –Time	53.05	1	53.05	245.82	<0.0001	
C – Solid Solvent Ratio	3.00	1	3.00	13.91	0.0074	
AB	0.1225	1	0.1225	0.5677	0.4758	
AC	0.0900	1	0.0900	0.4171	0.5390	
BC	0.4225	1	0.4225	1.96	0.2045	
A^2	43.52	1	43.52	201.69	<0.0001	
B^2	9.99	1	9.99	46.28	0.0003	
C^2	8.08	1	8.08	37.43	0.0005	
Residual	1.51	7	0.2158			
Lack of Fit	0.2625	3	0.0875	0.2804	0.8378	not significant
Pure Error	1.25	4	0.3120			
Cor Total	124.65	16				
R^2	0.9879					
Adjusted R^2	0.9723					
Predicted R^2	0.9507					
Adeq Precision	32.7695					
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)

***Analysis of Pectin's characterization*:**

The optimum parameters that produced the highest pectin yield were selected for pectin characterization based on the experimental runs.

***Equivalent weight*:** This is the most essential aspect in determining the quality of unripe bael pulp pectin. Under optimum conditions, the UAE and MAE methods yielded 238.09 and 263.15, respectively.

Methoxyl content (%): It was found to be 11.16% using the UAE method and 9.92% using the MAE method.

Anhydrouronic acid content (%): The UAE method determined the AUA content of unripe bael fruit pulp to be 137.28%, while the MAE revealed 123.2%.

Degree of esterification (%): It was calculated to be 46.15 and 45.71%, respectively, using the UAE and MAE methods.

3-D model graph Analysis of Pectin Yield using Ultrasound Assisted Extraction method: Figure 1 (a, b, and c) shows how the three-dimensional response surface can be used to graphically represent the relationship between responses and experimental factors. Ultrasound power, solid solvent ratio, and sonication time were the independent variables that were optimized along with temperature and pH to obtain the maximum pectin yield. Figures 1(a), 1(b), and 1(c) show the connections between the solid solvent ratio and ultrasound intensity, the solid solvent ratio and sonication time, and the sonication time and ultrasound power. Figure 1a illustrates the relationship between the ultrasound power intensity and the solid solvent ratio. It demonstrates that at higher ultrasound power and an increased solid solvent ratio, the maximum yield of pectin was achieved. For the relationship between sonication time and solid solvent ratio, as shown in Figure 1b, the yield of pectin increases with increasing sonication time and solid solvent ratio. This is because the solvent increases the surface area of the plant material and increases material swelling, which aids in the solubilization of pectin. The relationship between ultrasonic power and sonication duration, as shown in Figure 1c, indicates that cell wall disintegration and the release of cellular material at high enough ultrasonic power intensities may be responsible for increased pectin yield. The longer the contact time between the extractor and the plant materials, the more mass is transferred from the solid components to the solution.

3-D model graph Analysis of Pectin Yield using Microwave Assisted Extraction method: Figures 2 (a, b, and c) show how the three-dimensional response surface can graphically represent the link between responses and experimental conditions. The dependent variable was pectin yield, whereas the independent variables were microwave power, time, and solid solvent ratio, which were used to optimize process parameters. Figure 2a shows the relationship between microwave power and time in terms of pectin yield. The highest pectin yield is achieved by using a longer time period and higher microwave power. Microwave duration accelerates plant cell rupture and promotes sample surface degradation by causing a fast increase in internal pressure within the cell. This promotes the release of pectin from plant cells into the solvent and surrounding environment. For the relationship between microwave power and the solid solvent ratio, Figure 2b shows how increasing microwave power causes the pectin yield to increase as the solid-solvent ratio falls. Thisis because using a longer microwave could

cause the extract to degrade when the solvent and solute mixture warmed up. Figure 2c shows the relationship between the solid-solvent ratio and time. The maximum pectin yield was obtained over a longer period of time using a lower solid-solvent ratio. The reason for this is that as time went on, the solute particles grew more soluble, which decreased the viscosity of the extraction solvent and sped up the compounds' release and dissolution.

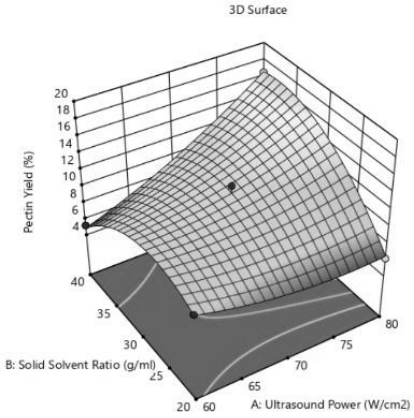

Figure 1(a)

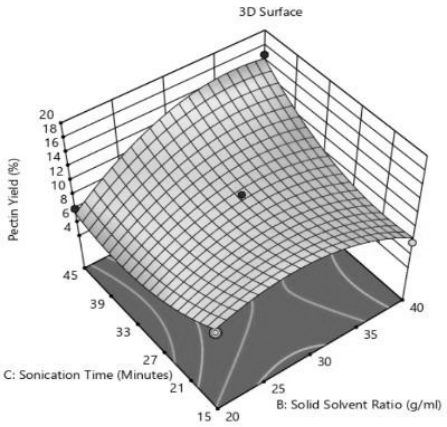

Figure 1(b)

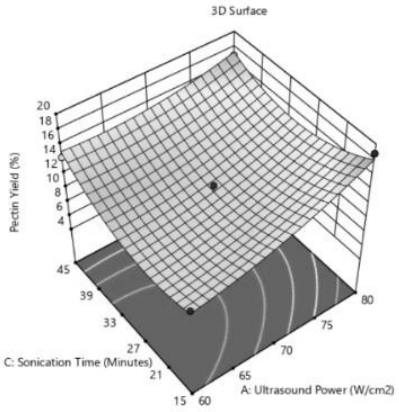

Figure 1(c)

Figure 1 (a) Relationship between Ultrasound power and Solid solvent ratio, (b) Relationship between Solid solvent ratio and Sonication time, (c) Relationship between Ultrasound power and Sonication time

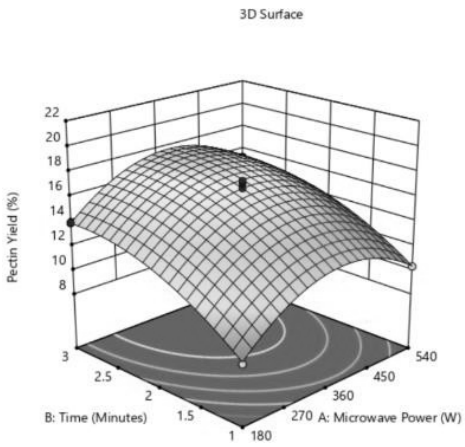

Figure 2(a)

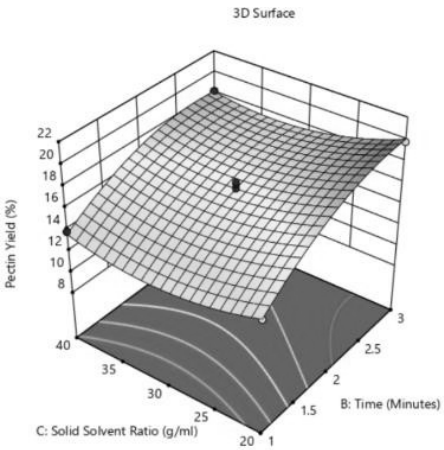

Figure 2(b)

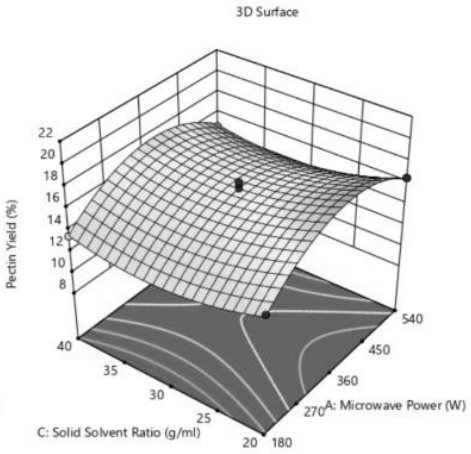

Figure 2(c)

Figure 2 (a) Relationship between Microwave power and time, (b) Relationship between Solid solvent ratio and microwave power, (c) Relationship between Solid solvent ratio and time.

CONCLUSION

It is feasible to use ultrasound and microwave-assisted extraction techniques to extract pectin from unripe bael. The best parameters for generating the highest pectin yield (18.8%) utilizing ultrasound-assisted extraction were an ultrasound power intensity of 80 W/cm^2, a solid solvent ratio of

1:30 g/mL, and a 15-minute time period. The optimal conditions for generating the highest pectin yield (19.8%) from microwave assisted method were a microwave power of 360 watts, an irradiation time of 3 minutes, and a solid solvent ratio of 1:20 g/ml. Both the techniques were a novel approach and superior methods as compare to traditional method for pectin extraction.

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