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Ultrasound Assisted Extraction of Pectin from Orange Peel

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ABSTRACT
Orange peel is the substantial pectin source which is considered as the primary component of plant tissues. Pectin is utilized as a gelling, emulsifying and thickening agent in numerous food products. At present, industrial pectin extraction is done by utilizing hot water at low pH for a long duration of time which is a time consuming process. However, it is verified that Ultrasonication lessens the extraction time of pectin and maximize yield as compared to the traditional method in use. Identifying the capability of Ultrasound assistance in extraction, this research emphasizes on pectin extraction using orange peels as raw material. Orange peel converted into the powdered form was utilized for extraction of pectin. Pectin was extracted with different combinations of Ultrasound Power (60, 80 and 100%), citric acid concentrations (pH: 1, 1.5 and 2) and extraction time (10, 20 and 30 minutes).As a result, the highest pectin yield of 20.92% was attained at the Ultrasound power of 100 %, Ultrasound Time of 30 Minutes and pH of 1.5of citric acid solution. The research confirms that utilizing Ultrasound and citric acid for pH, enhanced yield and standard of synthesized pectin; and also saves sufficient amount of time and energy.
Key words: *Pectin, Orange peel, Ultrasound assisted extraction, RSM.*

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INTRODUCTION

Citrus fruits are an important crop and orange being the major fruit of all the citrus fruits produced globally [7]. Citrus fruits are chiefly used as either fresh juice or packed drinks and are consumed all round the world in that form. Peel is the key byproduct from the orange processing industry and makes up approximately half of an orange's mass. This orange peel is considered as arich and important pectin source [3].

Pectin is a composite combination of polysaccharides in cell walls of plants, which is mainly composed of d-galacturonic acid [1]. The bulk pectin structure is composed of backbone of (1 → 4) linked d-galacturonic acid units [18].. Pectin is an essential part of the food industries as it is utilized as a stabilizer, gelling agent, emulsifier and thickener for the production of jellies, jams and other such products [6]. Most basic source for marketable pectin production is sugar beet pulp, apple pomace and citrus peels [11]. Traditional pectin extraction is done using hot acidified water; however, this method consumes higher solvent and longer time, is thermally unsafe, energy intensive and offers low efficiencies [15]. The need for replacement of this method of extraction with better methods could be a possible means of minimizing these adverse effects. The newer and better non-conventional methods like Microwave assisted and Ultrasound assisted extraction have developed to curtail the above stated limitations. Ultrasound assisted extraction (UAE) is a simple, economical, efficient and non-thermal method that has been studied in various extraction methods of plant components, for example, extraction of pectin from sisal waste [9], tomato wastes [5], pomegranate peel [12], grapefruit peel[17].The pectin extracted from these studies showed better yield and was also found effective in saving time and energy. Recognizing the capability of Ultrasound, this research emphasizes on extraction of pectin from orange peel through this technology and to obtain conditions for maximizing the pectin yield.

MATERIAL AND METHODS

The chief goal of this research is to standardize and optimize the operating parameters (showed in **Table 2** and **Table 3**) for pectin extraction from orange peel on the basis of the pectin yield. Materials used and procedure adopted in this research is described as follows:

Materials and reagents

Orange peels were bought from Pantnagar local market. Analytical grade chemicals were utilized in the study. Citric acid was mixed in different proportions with deionized water in preparing the acidic medium of different pH for pectin extraction. Different equipments were used to carry out experimental work which includes Sonicator, Magnetic Stirrer, refrigerator, weighing balance, solar drier, hammer mill, centrifuge, tray drier etc.

Experimental design

Response Surface Methodology (RSM) is an assemblage of statistical and mathematical means that can be utilized to define the relations between the independent and response variables. It has a substantial usage in the design, improvement and formulation of novel commodity. It describes the effect of the independent variables or in combination, on the procedures [2]. Box-Behnken design is utilized to prepare experimental design in this research work. In Box–Behnken design the experimental points are situated on a hyper sphere equidistant from the central point [4]. For 3 factors at three levels BBD had 17 experiments with 5 experimental runs at central points. The main factors that affect pectin extraction are Ultrasound Power, time and pH and the three levels and the range of the level of every factor are determined in the experiment design **Table 1**.

Table 1: Experimental design table

Exp no.	Ultrasound Power (A) (%)	Ultrasound Time(B)(min)	PH of Citric Acid
1	90	20	1.5
2	90	30	1
3	90	10	2
4	90	20	1.5
5	100	10	1.5
6	90	20	1.5
7	100	30	1.5
8	100	20	2
9	80	30	1.5
10	80	20	2
11	100	20	1
12	80	10	1.5
13	90	20	1.5
14	90	20	1.5
15	90	30	2
16	90	10	1
17	80	20	1

Pretreatment of raw material

Orange peels were cleaned and washed to eliminate any foreign material if present. Washed and cleaned orange peels were dried in a solar drier at 45°C followed by milling in hammer mill to 1 mm size powdered orange peel, making it convenient for pectin extraction. The powder was packed in a polyethylene bag and stored in the refrigerator until required for the extraction process.

Table 2. Values of Levels of Independent Parameters

S No.	Independent parameter	Coded value	Value of levels		
1	Ultrasound Power (%)	A	80	90	100
2	Ultrasound Time (min)	B	10	20	30
3	PH of Citric acid solution	C	1	1.5	2

Table 3. Values of Constant Parameters

S No	Parameter	Value
1	Solvent volume	200 ml
2	Sample particle Size	1 mm
3	Sample weight	10 g

Pectin extraction

Pectin was extracted with different combinations of Ultrasound Power (80, 90,100 %), citric acid concentrations (pH: 1, 1.5, 2) and extraction time (10, 20, 30 minute). In every experimental run, Orange peel powder (10 g) was mixed with fixed quantity of Citric acid solution (200ml) of pH 1, 1.5 and 2. It was then sonicated for 10, 20, and 30 minutes at the power 80, 90 and 100 %. After Ultrasound treatment, the mixture was kept at the room temperature for a while and filtered. The filtrate (containing pectin) was cooled down and then centrifuged at 6000 rpm for 30 min. Propanol was utilized to precipitate the supernatant and left still for an hour with the intention to let pectin flotation. The floating pectin was then separated by filtration. This wet pectin was dried in Tray drier at 45°C. The resulting dried pectin was milled to powdered pectin and stored for analysis. The yield of pectin was calculated according to equation (2).

Pectin Yield (wt %) = (X / Y) × 100…………….. (2)

Where, X is the quantity of dried pectin extracted from the sample in gram (g) and Y is the quantity of Orange peel powder sample taken up for extraction in gram (g).

Data analysis

The model development was accomplished utilizing response surface methodology with the assistance of Design expert software. Complete second order model as given in Equation was fitted to the data and the model adequacy was tested using R^2 (coefficient of multiple determination) and fisher's F-test. The parametric effect on several responses was done through the analysis of developed models. Regarding three independent variables a second order response function has the following general form

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2$$

Where, β0, βi, βij are constants Xi, Xj are variables (coded).

Multiple regression analysis was utilized to investigate the experimental data so as to develop response functions and obtain variable parameters optimized corresponding to finest results. The estimates of P-valueand model coefficients and connected statistics in relations of lack of fit were acquired through the program. The value of P implies the probability of significance. A high F-value is an indication of the point that the model had a significant lack of fit and hence is insufficient. A model with lower values of P is considered better. The models having P-value lower than 0.1 (indicating the lack of fit is unimportant at 90% confidence level) were acknowledged.

Adequacy of model

Equation is explained through a statistical method called technique of least square which is a multiple regression method utilized to fit a mathematical model to a collection of experiment data producing the lowest residual possible. The results of regression analysis are obtained in terms of ANOVA, regression coefficient and associated statistics, standard deviation, coefficient of determination (R^2), lack of fit, etc. These determine adequacy of predictive model and effect of independent parameters on the response.

RESULTS AND DISCUSSION

This research was undertaken with an objective to interpret the outcome of process factors on yield of pectin from Orange peel using Ultrasound assistance and the optimization of process parameters for pectin on the basis of its yield. The experimental design used for the study was Box-Benhken design having three variables and each variable having three levels. The variables with levels were Ultrasound Power(80, 90,100 %),Ultrasound Time(10, 20 and 30 minutes) and pH of citric acid solution(1, 1.5 and 2). Response surface methodology was utilized to enhance the process parameters. A complete second order mathematical model was fitted in the response. The competence of the model was confirmed using coefficient of determination (R^2) and Fisher's test. The results obtained on numerous characteristics of the study are discussed in detail.

3.1. Effect of Independent Parameters on Pectin Yield

Pectin from orange peel powder was extracted using Ultrasound assistance. To enhance the extraction process, parameters studied were Ultrasound power (80, 90, and 100 %), Ultrasound time (10, 20 and 30 minutes) and pH of citric acid solution (1,1.5 and 2). The quantity of pectin yield for all the 17 experiments is showed in Table 4. The yield of pectin differed from 17.54 % to 20.92 % from sample

amount of 10 g of sample over entire experimental conditions. The maximum pectin yield of 20.92 % was attained when the orange peel was extracted at pH 1.5 for 30 min and at Ultrasound power of 100%.
The experimental data was studied to note the remarkable consequences of numerous process variables on pectin yield. The outcomes of variance for pectin yield are given in Table 5.F-value was used to observe the significance of linear, quadratic and interactive terms. F_{cal} value for model was superior to F_{tab}, which infers that model was significant ($p < 0.01$). The outcome of independent variables on pectin yield at linear level was observed to be highly significant at 1% level of significance ($F_{cal} > F_{tab}$), for quadratic and interactive terms it was also observed significant.
Multiple regression analysis was carried out to fit the response parameter, pectin yield. The regression coefficients for the second order model polynomial equations for the linear, interaction and quadratic terms are shown in Table 5. The statistical analysis exhibits that the projected model was sufficient, having significant fit and with very reasonable values of R^2 for drying time. The R^2 value for the pectin yieldwas 99 %, which infers that the model could account for 99.00% data. Closer the value of R^2 to unity, the better the empirical model matches the actual data. Insignificant value of lack of fit exhibited that the model developed is valid. The second order polynomial equation was developed which represents response, pectin yield as functions of Ultrasound Power, pH of citric acid solution and Extraction Time. An empirical relationship among the responses and input variables in coded form can be explained by the following equation:

$$Y = 20.80 + 0.43A + 0.98B - 0.15C + 0.045AB + 0.22AC + 0.11BC - 0.57A^2 - 1.25B^2 - 0.78C^2 \quad \ldots (4)$$

Where, Y is pectin yield, A, B and C are the coded values of of Ultrasound Power, Extraction Time and PH of citric acid solution respectively.

Table 4. Pectin Yield

Std	Run	Ultrasound Power (%)	Ultrasound Time (min)	PH of Citric acid solution	Pectin Yield (%)
17	1	90	20	1.5	20.42
10	2	90	30	1	19.77
11	3	90	10	2	17.54
14	4	90	20	1.5	20.32
2	5	100	10	1.5	18.38
13	6	90	20	1.5	20.02
4	7	100	30	1.5	20.92
8	8	100	20	2	19.92
3	9	80	30	1.5	19.47
7	10	80	20	2	18.63
6	11	100	20	1	19.82
1	12	80	10	1.5	17.61
16	13	90	20	1.5	20.11
15	14	90	20	1.5	20.22
12	15	90	30	2	19.71
9	16	90	10	1	18.03
5	17	80	20	1	19.39

Table 5. ANOVA Table for pectin Yield

Source	Sum of Squares	DF	Mean Square	F-Value	p-value
Model	21.16	9	2.35	1860.43	< 0.0001
A-Ultrasound power	1.48	1	1.48	1170.65	< 0.0001
B-Ultrasound Time	7.62	1	7.62	6034.10	< 0.0001
C-pH	0.18	1	0.18	144.84	< 0.0001
AB	8.100E-003	1	8.100E-003	6.41	0.0391
AC	0.18	1	0.18	146.33	< 0.0001
BC	0.046	1	0.046	36.58	0.0005
A^2	1.39	1	1.39	1098.85	< 0.0001
B^2	6.60	1	6.60	5221.22	< 0.0001
C^2	2.57	1	2.57	2036.44	< 0.0001
Residual	8.845E-003	7	1.264E-003		
Lack of Fit	1.525E-003	3	5.083E-004	0.28	0.8395
Pure Error	7.320E-003	4	1.830E-003		
Total	21.17	16			
R^2			0.9901		
Adj R^2			0.9773		
Pred R^2			0.9299		

Graphical Analysis

The connection amongst the responses and the experimental variables can be shown graphically by plotting three-dimensional response surface plots (Fig.1, 2, 3) and their interactive effects are explained accordingly. The effect of Ultrasonic power and time on the pectin yield is shown in Figure 1**.** When pH (C) was set at 0 level, both ultrasound Power (A) and Ultrasound Time (B) presented significant result on the pectin yield. It is observed that the peak yield of pectin was 20.92% at the Ultrasound power of 100% and the minimum pectin content was 17.54 % at the ultrasound power of 90 %. Therefore, the pectin yield was highest at the maximum Ultrasound power. In sonication, mass transfer is boosted through micro-streaming which is caused due to breakdown of cavitational bubbles. This leads to the enhancement of mass transfer. This phenomenon destroys the cell wall, allowing transfer and interaction between plant materials and solvents. In this study, sonication leads to the breakdown of outer cell wall of sample deprived of any involvement of heating [14]**.** The pectin yield first augmented with the enhancement of power intensity but declined after reaching a peak. This decline might be because of the reason of the degradation effects of pectin triggered by high intensity of ultrasound [20]**.**

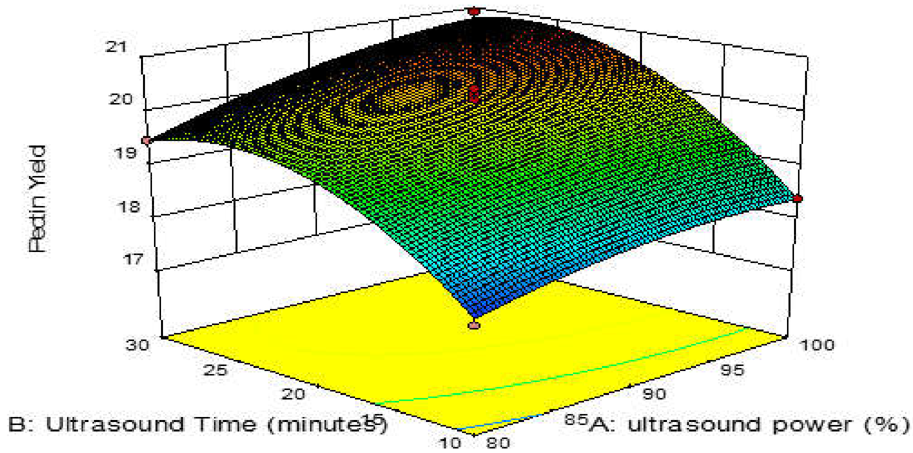

Fig 1. Effect of Ultrasound power (%) and Ultrasound Time on Pectin yield

The effects of Ultrasound time (B) on the pectin yield could be found in **Figure 2**, when Ultrasound power (X2) was fixed at 0 level. The interactive effects of pH solution and Ultrasound time are shown in **Figure 2**. Interactive effects of pH value and extraction time had significantly effect on the pectin extraction. According to [16]**,** the interactive effects of extraction time and pH had significant influence on the pectin extraction from durian rind. Increase in pH solution from 1 to 1.5, and subsequent rise in extraction time from 20 to 30 minutes had significant increased the extraction yields. However, further increment in pH value from 1.5 to 2 reduced the extraction yields. According to [13]**,** increase in extraction time can significant enhanced the extraction yields. The pectin yield augmented evidently with rise in sonication time (X3) from 10 to 20 min; but beyond 30 min, the yield increases slowly with time. The peak pectin content was 20.92 % at 30 minutes and the minimum pectin content was 17.54 % at 10 minutes.

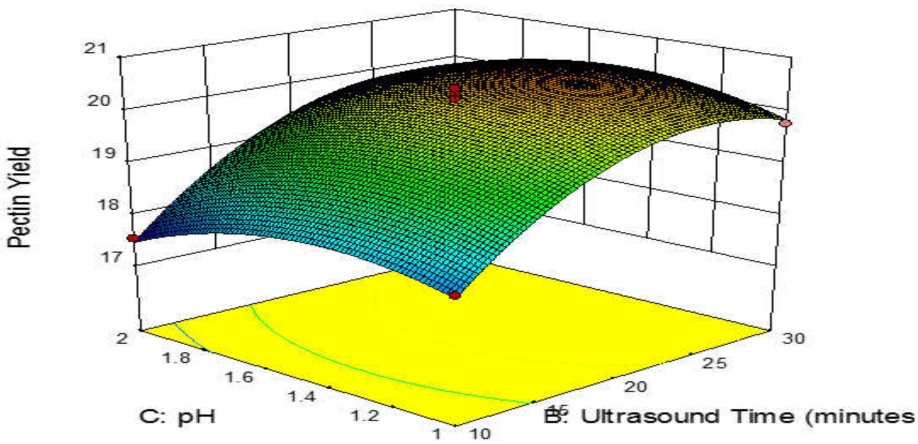

Fig 2. Effect of Ultrasound Time (minutes) and pH on Pectin yield

As shown in **Figure 3**, as the pH increases, the pectin yield decline. The peak yield was 20.92% at pH 1.5 and the lowest yield was 17.54% at pH 2. An appropriate pH is crucial to guarantee good pectin extraction as pH has remarkable effect on pectin extraction. Current study exhibited that the pectin yield was augmented from 17.54 % to 20.92 % as pH declined from 2 to 1.5.As the pH values decreases, the presence of H+ ions increases which enhances the hydrolysis of protopectin. It is also observed that the high acidified solvent has the potential to enhance the hydrolysis of the insoluble pectin components into soluble one, thus intensifying the extraction process from plant materials [8, 9]. The current study shows that maximum pectin could be extracted at pH of 1.5 and this finding is in agreement with the results reported by Zhang *et al*., [20].

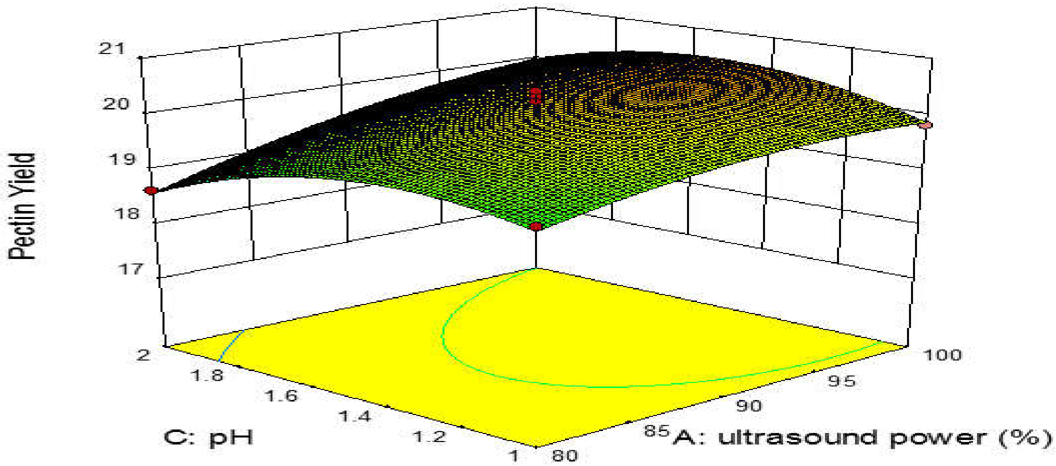

Fig 3. Effect of pH and Ultrasound power (%) on Pectin yield

CONCLUSIONS

Pectin is an important naturally occurring food additive that is widely utilized in the food industry. In this research, varied extraction conditions were studied for pectin extraction from orange peel using Ultrasound assistance. The Box–Behnken design was utilized to correlate the effect of Ultrasound time, pH and microwave power on the pectin yield. The outcome presents that the peak yield of pectin extracted at the ideal conditions of Ultrasound time of 30 min, Ultrasound power of 100 % and pH of 1.5 was 20.92%.

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