

Optimization of pectin extraction from the native fruit of *Garcinia binucao* using response surface methodology

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Abstract. Citrus supply for pectin primers in the world market is continually under threat due to the widespread of uncontrolled citrus greening disease outbreaks. As a consequence, a market squeeze in pectin supply was experienced globally and further resulted to a significant surge in prices of pectin derived products. In an attempt to provide an alternative source of pectin primers to the pectin-making industry, we investigate a novel and promising pectin primer native to the Philippines. In this work, we explored extraction of pectin from fruits of the *Garcinia binucao* tree (GBT). We implement a response surface methodology (RSM) to optimize the extraction of pectin from GBT. Box-behken experimental design was used to obtain the optimal conditions in the extraction process. Temperature (°C), extraction time (min), and pH were found to have significant effects on the pectin yield. Actual values of these independent variables were chosen on the basis of preliminary experimental results. Optimum conditions using ridge analysis were found to be: temperature 72.4°C, extraction time 117.5 min, and pH at 1.68. In conclusion, a high pectin yield obtained from GBT fruits strongly suggests that these fruits are promising alternative primers in pectin production.

1 Introduction

Pectin is a biopolymer that has important applications in food, pharmaceuticals, biotechnology, and other related industries. It is found to naturally occur in plants and is highly concentrated at the middle lamella of a plant cell wall [1]. The chemical structure of pectin basically forms a linear polysaccharide that is mainly composed of D-galacturonic acid units with carboxyl groups [2-3]. With this chemical composition, pectin has been extensively used as a derivative for the formation of thickening and stabilizing agents [2]. Currently, commercial pectins are primarily derived from apple pomace and citrus peels [4]. On a dry matter basis, apple pomace comprises about 10–15% pectin while citrus peels yields higher amounts pectin equivalent to 20–30% of its mass [2, 4]. Other sources of pectin include sugarbeet waste, sunflower heads, and mango peel waste [5].

The suitability of pectin and its derivatives to various industry applications has pushed production of pectin derived products at large volumes to satisfy the growing demand in the global market. This necessity for pectin derivatives has driven a steady annual growth rate of 6% in pectin production volumes [6]. However, a massive outbreak of citrus greening disease has infected 80% of the trees grown by key plantations of the citrus industry [7]. This resulted to a shortage of pectin supply which created a significant upsurge to the global market price of pectin [8]. A market price increase of up to 30% is projected if the gap in the demand and supply in the volume of pectin is not immediately resolved [9]. As a

key precursor chemical, the magnitude of this price increase threatens to disrupt the price stability of basic commodities such as food and beverage.

We contribute to research by exploring the possibility of utilizing *Garcinia binucao* (batuan) fruit as an alternative raw material for pectin production. It has been reported that the physicochemical properties of the batuan fruit are comparable to a variety of citrus fruits [10]. With properties similar to citrus fruits, we hypothesize that the pectin content of the batuan fruit would be equivalent to that of a citrus fruit. In the present work, we test this hypothesis by investigating the extent to which pectin can be effectively extracted from the batuan fruit. To determine the maximum possible pectin recovery, we implemented a box-behken experimental design to optimize the process of extracting pectin from batuan fruit.

2 Materials and methods

2.1 Materials and reagents

Fresh and whole Batuan (*Garcinia binucao*) fruits were kindly provided by a local plantation in Masbate, Masbate City, Philippines. The batuan fruits were chopped into an average particle diameter of 1 cm and sun dried for 3 days. The particle size of the dried batuan was further reduced to 100 mesh using a domestic blender. Sulfuric acid, sodium hydroxide, and ethyl alcohol (95%) were all analytical grade and acquired

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from Belman Laboratories. Distilled water was used in all experiments.

2.2 Pectin extraction from batuan fruit

Extraction procedure of pectin (see **Fig. 1**) from the Batuan fruit was adopted from methodologies in published literature [11-12]. The dried and ground batuan fruit was hydrolysed by sulfuric acid with a solid-liquid ratio of 1:10 (w/v) at predetermined pH (1.5, 2.25, or 3.00), temperature (70, 85, or 100°C), and heating time (30, 75, or 120 min) to establish extraction conditions that will give the optimum pectin yield. The solid-liquid mixture was moderately mixed until the desired heating time was achieved. After heating, the mixture was cooled down to room temperature and was filtered to remove insoluble residues. The pH of the filtrate was carefully adjusted to 4.5 with 1 M NaOH solution. Consequently, 95% ethanol was added to the filtrate at an ethanol to filtrate ratio of 2:1. This solution was left undisturbed overnight to induce precipitation of pectin in solution. Impurities from the fibrous pectin coagulum were removed by washing the precipitate with 95% ethanol three times. The pectin extract was finally oven-dried at 60°C for 12 hours. Pectin recovery was calculated using **Eq. 1**.

$$\% \text{ Pectin Recovery} = \frac{\text{weight of pectin precipitate}}{\text{weight of dried batuan}} \times 100 \% \quad (1)$$

2.3 Experimental design

A box-behnken experimental design (see **Table 1** and **Table 2**) generated using Stat-Ease design expert software was implemented to investigate the influence of independent variables (pH; temperature °C; heating time min) in optimizing the desired response (pectin recovery %). All experimental runs were performed in duplicates.

Table 1. Independent Variables Influencing % Pectin Recovery

Factor	Unit	Factor level	
		Low	High
pH	-	1.5	3.0
Temperature	°C	70	100
Heating Time	min	30	120

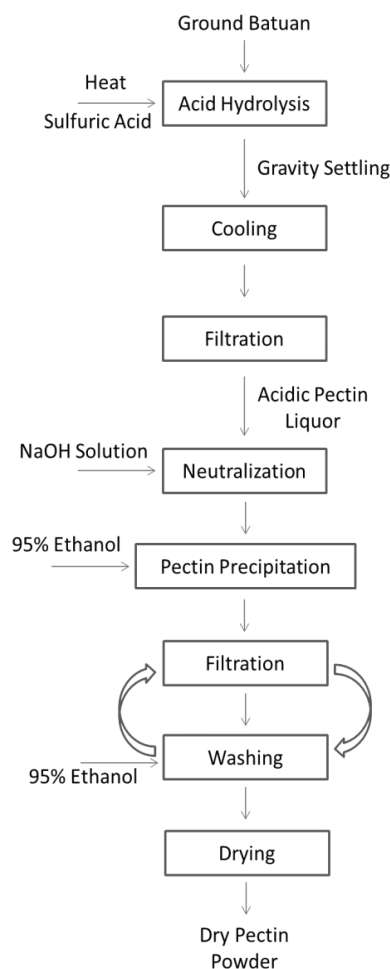


Fig. 1. Pectin extraction procedure implemented in this study.

Table 2. Box-Behnken Design Experimental Runs

Run	Factor 1 A: pH	Factor 2 B: Temperature (°C)	Factor 3 C: Heating Time (min)
1	2.25	100	30
2	2.25	85	75
3	2.25	70	30
4	3	85	30
5	1.5	100	75
6	3	70	75
7	2.25	100	120
8	2.25	70	120
9	3	100	75
10	1.5	85	120
11	1.5	85	30
12	2.25	85	75
13	1.5	70	75
14	3	85	120
15	2.25	85	75
16	2.25	85	75
17	2.25	85	75

3 Results and discussion

3.1 Preliminary experimental tests

A preliminary experiment was first conducted to identify a solvent to solute (solution volume to Batuan mass) solution ratio that would give high yields of pectin. In this regard, A one-factor-at-a-time approach (OFAT) was therefore implemented. The solvent to solute ratio was varied at three levels: 1:10, 1:15, and 1:20. The best ratio was determined by qualitatively assessing the amount of pectin crystals formed in solution. The influence of the moisture content of Batuan to the amount of pectin yield was also assessed. As summarized in **Table 3**, a solvent to dry Batuan mass in a solute ratio of 1:10 were chosen since these factor combinations gave the highest number of pectin crystals in solution.. These results were then used to verify the feasibility of the factor levels chosen in this study.

Table 3. Results of Preliminary Experiments

State of Batuan	Solute to Solvent Ratio		
	1:10	1:15	1:20
Moist Batuan	Low Yield	Low Yield	Low Yield
Dried Batuan	High Yield	Medium Yield	Low Yield

Similarly, factor levels were based on existing extraction procedures in published literature [11-12]. Initial tests were conducted to validate whether pectin can be extracted from Batuan. Factor level combinations were randomly selected based on the experimental run summarized in **Table 2**. As shown in **Fig. 2**, crystals were formed in solution and this indicated that pectin can possibly be extracted from Batuan. These results then established that an extraction procedure can possibly be optimized to maximize the extraction of pectin from Batuan.

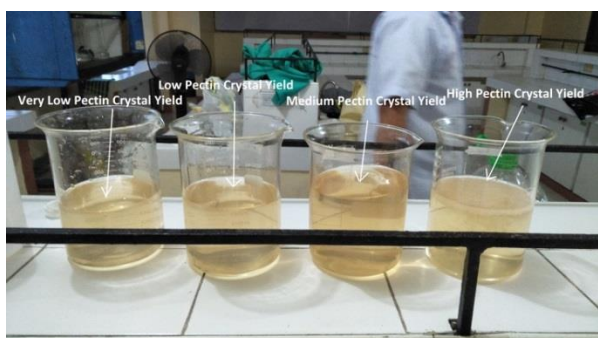


Fig. 2. Formation of pectin crystals in solution.

3.2 Statistical tests and mathematical model building

Box-behnken experimental design with three factor combinations was used to optimize the extraction of pectin from the Batuan fruit. 12 experimental runs were generated by Design Expert 11 Software® (Stat-Ease, USA) to optimize the extraction process. 5 center points were also added to measure the degree of stability and

variability of the response (percent pectin recovery) being maximized. Design Expert 11 Software® (Stat-Ease, USA) was then used to generate a second-order empirical model. Based on the Box-Cox plot given by the software used, a square-root transformation of data was implemented. This was done to reduce the skewness of the response and improve the correlation between the predicted and observed values. The model (p-value < 0.001) with the actual factors is given in **Eq. 2**.

$$\sqrt{\% \text{ Pectin Recovery}} = \text{Intercept} + C_1A + C_2B + C_3C + C_4AB + C_5AC + C_6BC + C_7A^2 + C_8B^2 + C_9C^2 \quad (2)$$

where A corresponds to the factor pH, B is the factor temperature (°C), C is the factor heating time (min), and C₁ to C₉ are coefficients with values summarized in **Table 4**.

Table 4. Values of Coefficients in the Square Root Model

Term	Coefficient Value	p-value	Remarks
Intercept	-44.49	-	-
A	6.90	<0.0001	Significant
B	0.21	0.04379	Significant
C	0.83	<0.0001	Significant
AB	-0.011	0.002687	Significant
AC	-0.077	<0.0001	Significant
BC	-0.0023	<0.0001	Significant
A ²	-0.086	0.6654	Not Significant
B ²	6.45x10 ⁻⁵	0.2503	Not Significant
C ²	-0.00317	<0.0001	Significant

As shown in **Table 5**, the predicted %pectin recovery is in agreement with the experimental values. To test whether the developed empirical model was valid, Design Expert 11 Software® was used to statistically analyze the goodness of fit of the generated model.

Table 5. Observed and predicted values from the Box-Behnken experimental design.

Run	A	B	C	Observed	Predicted	Deviation
1	-1	-1	0	9.09	11.24	-2.15
2	1	-1	0	7.36	7.65	-0.29
3	-1	1	0	20.50	19.85	0.65
4	1	1	0	7.27	5.51	1.76
5	-1	0	-1	9.32	9.24	0.07
6	1	0	-1	10.04	11.77	-1.73
7	-1	0	1	11.26	9.48	1.78
8	1	0	1	0.00	0.00	0.00
9	0	-1	-1	4.21	2.91	1.31
10	0	1	-1	25.59	26.26	-0.67
11	0	-1	1	9.94	9.51	0.43

12	0	1	1	0.00	0.12	-0.12
13	0	0	0	8.71	9.91	-1.20
14	0	0	0	10.20	9.91	0.29
15	0	0	0	9.82	9.91	-0.09
16	0	0	0	9.90	9.91	-0.01
17	0	0	0	11.13	9.91	1.22

As presented in **Table 6**, R^2 values were to unity and strongly suggest that the empirical model has a good fit. Furthermore, the adjusted and predicted R^2 are in good agreement with each other. This highly suggests that there is a strong correlation between the predicted and observed outcomes.

Table 6. Statistical fit of the empirical model.

Fit Statistics			
Std. Dev.	0.3216	R^2	0.9526
Mean	2.85	Adjusted R^2	0.9348
C.V. %	11.28	Predicted R^2	0.8888

3.3 Optimization of pectin recovery

Pectin recovery from Batuan was maximized by using the validated empirical model. The optimum conditions suggested by the software used are shown in **Fig. 3**. The factor combinations with the highest desirability (1.00) were chosen. The percent pectin recovery was estimated to be 26.06%. Furthermore, as presented in **Fig. 4**, the 3D response surface gave also a similar value. To validate these values, confirmatory experimental runs were also done at the suggested factor levels. Based from the results of these runs, an error between 3-8% was noted between the predicted and experimental values. The close agreement between the values obtained from the confirmatory runs further support the validity of the generated empirical model.

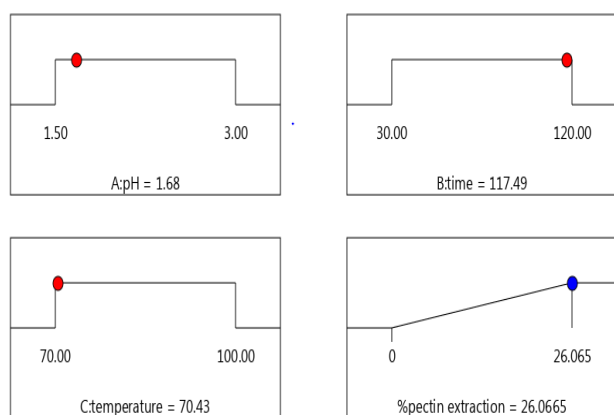


Fig. 3. Factor Level Combination to Maximize Percent Pectin Recovery

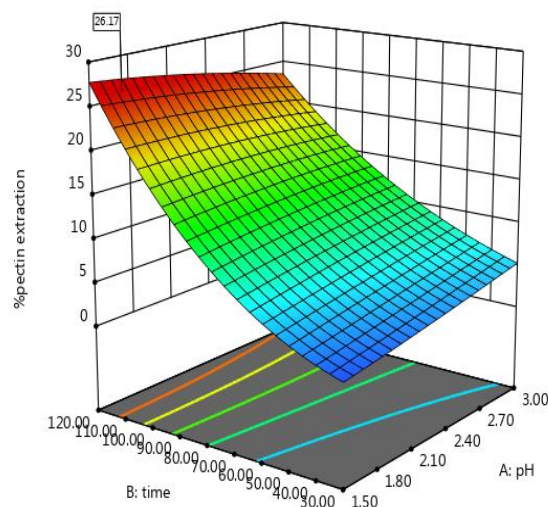


Fig. 4. 3D Response Surface for Percent Pectin Recovery.

4 Conclusion

Pectin has become an invaluable chemical in the past decades. With its shortage being induced by uncontrolled outbreaks of botanical diseases, there is a need to look for other alternative sources of pectin. We contribute to research by investigating Batuan as potential source of this chemical. We maximized the amount of pectin extracted from the Batuan fruit at the following optimum conditions: pH = 1.68; heatime time = 117.50 min; and temperature = 70.4°C. The maximum pectin recovery achieved was found to be 26.26%. In conclusion, this study presented evidences that the Batuan fruit can be an alternative raw material for pectin production.

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