Microwave Assisted Extraction Of Pectin From Mangosteen (Garcinia Mangostana) Rind

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Abstract: Pectin is a complex mixture of polysaccharides that is present on plants cell wall used mainly in food and beverage industry as thickening and gelling agent. This study used Microwave Assisted Extraction to obtain pectin from fruit waste specifically mangosteen (Garcinia mangostana) rind. Central composite design is used in investigating the effect of microwave extraction condition in the yield of pectin with a total of 34 runs. Effects of extraction conditions such as A- pH of 2 - 3, B- irradiation time of 3 - 9 minutes and C - power level of (336 - 595 watts on the yield of pectin from mangosteen rind were evaluated. The factors that significantly affect the yield are pH and power level and their interactions with value of $p<0.05$ while irradiation time remains insignificant. At a given factors and levels, the yield of pectin ranges from 3.1% to 9.2%. Optimum yield of pectin (9.022%) can be attained at lower limit in ph of 2, irradiation time of 3 minutes and power level of 336 watts. The mathematical model for pectin extraction using this method is expressed in coded equation: Yield = 5.57 – 2.15A – 0.4169C + 0.2250AB + 0.6331AC with an F-value of 1035.21 implies the model is significant. High coefficients of determination was attained with R^2 of 0.9930 against the predicted R^2 of 0.9906, thus confirming adequacy of adjustment of the regression models with the experimental data. The extracted pectin from mangosteen rinds using the optimized conditions have 4.38% ash content, 12.33% moisture, 11.12% methoxyl content, 65.78% anhydrouronic acid and 95.98% degree of esterification. A high-methoxyl and rapid set pectin was attained from the mangosteen rind with the microwave assisted extraction method.

Keywords: microwave assisted extraction, pectin, mangosteen rind, extraction condition

1. Introduction

Mangosteen (Garcinia mangostana) is commonly known as “the queen of fruits” because of having good taste with healthy benefits and healing [1]. It is grown in tropical regions cultivated mostly in warm, moist environments of Southeast Asia just like Thailand, Malaysia, Singapore, Vietnamn, Indonesia and Philippines. The fruit consist of 3 parts, the white edible flesh called the pulp that serves as fresh dessert, the seeds that are extremely bitter and the rind. In the Philippines, mangosteen is used as a raw material for fruit juices and drinks and the leftover such as rind are made into jams, teas and stabilizers. The pectic substances of the rind makes it possible to make emulsifier, stabilizer or commonly commonly known as pectin. Pectin is a polysaccharides with complex structure found in cell wall of plants and fruits. This was composed of methylated ester of polygalacturonic acids [2]. It is primarily used as thickening agents as a gelling and stabilizers in food and beverage industries [3]. These were extracted from fruit peels like apple and citrus which is also a by-product of juice manufacturing [4]. The conventional method of pectin extraction is direct boiling in slightly acidic solution for 2-3 hours. Direct heating is impractical to use due to very long boiling time of around 1-3 hours in which the extracted pectin undergoes thermal degradation. This condition affects its quality, has low production capacity, high amount of solvent and requires high operating temperature. To overcome the limitation of conventional extraction methods, a novel green extraction technique due to its high efficiency and low energy requirement, microwave assisted extraction (MAE) is used. MAE is an extraction process that uses electromagnetic radiation in contact with the solvent containing sample that transmitted into wave to acquire compounds of analytical interest. It is a rapid extraction technique that requires low energy at shorter time that convert electromagnetic into thermal energies [5]. The operating parameters such as pH, rradiation time and microwave power are the important factors that must be considered in the extraction.

2. Objective of the Study

This study aimed to produce pectin from mangosteen rind using microwave assisted extraction. The address the general objective, the following specific objectives must be met:

2.1 To investigate the effect of varying extraction condition such as ph, irradiation time and power level in the yield of pectin.

2.2 To determine the highest yield of pectin at a given extraction condition.

2.3 To suggest regression model for prediction of the yield of pectin from mangosteen rind.

2.4 To determine the properties of pectin attained at best extraction condition.

3. Materials and Methodology

This study used an experimental design with three factors and three levels with replication. Central composite design is used in the evaluation of the effect of extraction condition in the yield of pectin.

3.1 Material Preparation

Mangosteen were gathered from the market of Batangas City and rind were collected. The process was based on the study of passion fruit peel by microwave induced heating [6]. The rind was bathed by running water to remove dirts and lead to enzymatic inactivation. This was submerged in water at 90^{o}C for 3 minutes and transferred to another water bath at room temperature for 15 minutes and dried at no more than 50oC in air-circulated oven until constantly dried.. The dried rind was milled to 80 mesh size particle. The mangosteen fruit rind powder was stored in a freezer at 4^{o}C to maintain freshness.

3.2 Extraction of Pectin

Pectin was extracted using a typical microwave oven. In a 500-liter Pyrex-beaker, 4 grams of powder were added to 200 mL citric acid with varying ph of 2 -3, irradiation time of 3 – 9 minutes and power level of 336W – 595 watts. The solution was cooled and filtered using vacuum filtration apparatus. The filtrate was collected and immersed in absolute ethyl alcohol for at 1:2 ratios for 12 hours to collect the floated pectin. The pectin was collected through filtration dried in an oven at ^{50oC} until constantly weighed. The weight of the dried material obtained is pectin extracted from mangosteen. Replications were done to ensure the accuracy of results. Figure 1 shows the schematic diagram of pectin extraction.

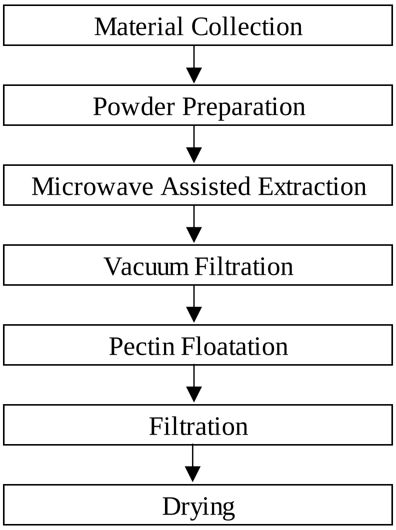

***Figure 1**: Pectin Extraction*

The yield of pectin can be computed using equation 1 :

$$\text{yield} = \frac{\text{weight of pectin}}{\text{weight of dry mangosteen powder}} \text{ x } 100\% \quad (1)$$

3.3 Analysis of Pectin

The moisture content of pectin was obtained by drying the pectin in an oven for five hours at 50°C or until constantly weights. The moisture content was determined using equation 2.

$$\text{Moisture } (\%) = \frac{\text{weight of dried pectin}}{\text{weight of fresh pectin}} \text{ x } 100\% \quad (2)$$

To determine the ash content of the extracted pectin, 2 grams were obtained and ground to pass the 80-mesh screen. This was placed in tared crucible and ignited in a furnace at 600°C for 3-4 hours. The alkalinity of the ash were determined by dissolving in 25 mL of 0.10N HCl. This was gently heated gently up boiling and then cooled. A 0.10N NaOH were used to titrate the sample using phenolphthalein indicator. The ash content was determined using equation 3.

$$\text{ash content } (\%) = \frac{\text{weight of ash}}{\text{weight of pectin}} \text{ x } 100\% \quad (3)$$

Anhydrouronic acid and Degree of Esterification can be determine based on the calculated equivalent weight. Titration A was used as titrant to pH 7.5 using phenol red. Equivalent weight (EW) of pectin was calculated using equation 4.

$$\text{EW} = \frac{\text{weigth of sample (mg)}}{\text{mL of alkali x N of alkali}} \text{ x } 100\% \quad (4)$$

Another important factor in controlling the setting time of pectin are the methoxyl content (MC) and Degree of esterification (DE). A sample of 0.5 grams will be titrated to a neutral solution determine the equivalent weight. A titrant of 0.25N NaOH of 25 mL volume was used to determine the acidity of the substance which were allowed to stand for 30 minutes at room temperature with shaking. A titrant B of HCl of the same volume and concentration were used to totrate 0.10N NaOH to end point(Titration B) [7]. The methoxyl content is calculated using equation (5).

$$\text{MC } (\%) = \frac{\text{vol alkali x concentartion alkali x 3.1}}{\text{weight of sample,g}} \text{ x } 100\% \quad (5)$$

Anhydrouronic acid (AUA) estimation were done not only to to determine the purity of pectin as well as its degree of esterification which is expressed in equation 6.

$$\text{AUA } (\%) = \frac{176}{z} \text{ x } 100\% \quad (6)$$

Here, 176 is the molecular weight AUA

$$Z = \frac{\text{weightof sample,mg}}{\text{meq titration A+meq titration B}} \text{ x } 100\% \quad (7)$$

The degree of esterification can be obtained using equation 8 [8].

$$\text{DE } (\%) = \frac{\text{176 x MC } (\%)}{\text{31 x AUA } (\%)} \text{ x } 100\% \quad (8)$$

3.4 Design of Experiment

Central Composite Design Face Centered is used to evaluate the effect of extraction condition on the yield of pectin from mangosteen rind. Low level values of each factors are the lowest possible and acceptable level in each of the process. Low pH level requires more acid chemicals to be used in the process while short irradiation time speeds up the production rate and low power level setting minimize the energy requirement thus lessen the operating cost. Table 1 shows values of each level per factor.

***Table 1:** Factors and Levels*

Factor	Low level	High level
pH	-1 (2)	1 (3)
Irradiation time (min)	-1 (3)	1 (9)
Power (watt)	-1 (336)	1 (595)

The mid level of pH is 2.5, an irradiation time of 6 minutes and power level of 462 watts. The set up were done in random to minimize the error caused by natural variability. The experiment were conducted in Chemical Laboratory of Batangas State University, Batangas City. Design Expert 11 is the statistical software used to treat the data. Analysis of variance is used to evaluate the significance of each factors at siginificance level of 0.05. For sampling, 34 independent beakers were used, each contain the same amount of mangosteen powder at varying pH.

4. Results and Discussion

The experiment produced 34 responses from various level of the three factors. Table 2 shows the average mean values of each set up.

Table 2: *Mean Values of Yield for Each Set up*

pH	Irradiation time (mins)	Power (watts)	Yield (%)
2	3	336	9.14
2	3	595	6.92
2	6	462	7.85
2	9	336	8.46
2	9	595	6.30
2.5	3	462	5.43
2.5	6	336	5.82
2.5	6	462	5.63
2.5	6	595	5.11
2.5	9	462	5.69
3	3	336	3.16
3	3	595	3.49
3	6	462	3.35
3	9	336	3.28
3	9	595	3.87

The highest yield was obtained at the most acidic region at ph 2, shortest time of extraction at 3 mins and lowest power setting at 336 watts having 9.14% yield while the lowest yield was observed at ph 3, 3 minutes irradiation time and also 336 watts. Even at low setting, the microwave extraction is efficient in which the penetration of extracting agent at high acidity allows the dissolution of the component to be extracted using electromagnetic radiation. Rapid transfer of energy using microwave heating at lower setting happens. Increasing the microwave power would bring a decrease in the extraction of pectin because the extracted pectin swells and break down into tiny particles or cluster which will result to a decrease in the yield of pectin. This case predominates nucleation because of too much penetration of microwave irradiation and time of contact. The splitting of cell and swelling effect that breaks pectin clusters into smaller particles which are not settlelable [6]. At higher pH, the longer irradiation time and power setting is favorable to the yield of pectin. The extracting agent weakens its power at higher pH which require much energy and time to penetrate in the cell wall of the plant. The lower the pH values, the presence of H^+ ions increased hence increasing hydrolysis of extraction of protopectin. Citric acid has 3 hydrogen ions present in which at low pH condition can extract pectin at a higher yield. Low pH due to high acidy requires low energy and irradiation time because the extracting agent itself plays an important role in the extraction. At higher ph 3 which is considered as low acidity medium, it requires higher microwave energy and longer irradiation time of extraction because the extracting power of the solvent decrease caused by the decrease in H^+ ions. At mid pH of 2.5, there is no differences in the yield at all levels of irradiation time and power. Increasing the irradiation time and microwave energy could not influence the yield of pectin. The values ranges from 5.11 to 5.82%. In summary, highest yield of pectin can be attain at low pH. Table 3 shows the analysis of variance of each factors. Analysis of variance is used to determine the significance of each factor with the response. As reflected in the table, both pH and power level affect the yield of pectin while the irradiation does not. The table shows that the assumptions of ANOVA is not violated.

Table 3 *: Analysis of Variance*

Source	Sum of Squares	dF	Mean Square	F-value	p-value	
Model	103.859	4	25.965	1035.213	8E-31	S
A-pH	92.225	1	92.225	3676.979	4.2E-32	
C-power level	3.478	1	3.478	138.650	1.4E-12	
AB	0.810	1	0.810	32.295	3.8E-06	
AC	7.036	1	7.036	280.511	1.9E-16	
Residual	0.727	29	0.025			
Lack of Fit	0.385	10	0.039	2.140	0.0737	NS
Pure Error	0.342	19	0.018			
Cor Total	104.587	33				

Note: S – significant, NS – not significant

The interaction of both the pH and irradiation time and pH and power level are both significant. The model has an F-values of 1035.213 and P-value of 0.0001 implies that the model is significant. The predicted R^2 of 0,9906 is in reasonable agreement with the adjusted R^2 of 0.9921 with an adequate precision of 99.962. The model graphs of yield of pectin from mangosteen were shown in contour plot in Figure 2 and 3D surface in Figure 4 gerenerated from Design Expert 11 software.

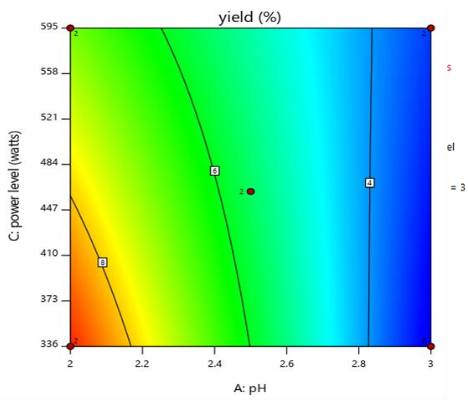

Figure 2*: Contour Plot*

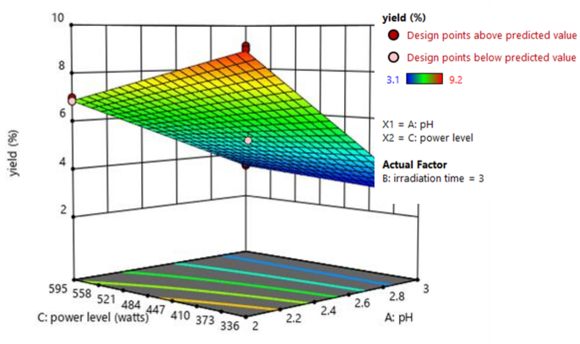

Figure 3*: 3D Surface*

It was clearly shown in the contour plot and 3D surface design that the highest yield of pectin can be attained at low pH and low power level. At lower irradiation time and power level, the use of microwave assisted extraction increase the production rate and lower the energy consumption that decrease the operating cost. However, higher yield was attained at low meaning which would mean an additional use of acids in the extraction process. Based on the findings given above, the quantitative models for pectin yield can be represented by the coded values presented in equation 9.

Coded Values:

$$\% \text{ yield} = 5.57 - 2.15A - -0.4169C + 0.2250AB + 0.6631AC \quad (9)$$

Where A = ph; B = irradiation time; C = power level

Equation 9 can be used to make predictions about a response for a given levels of each factor. The coefficient indicates that the very high level of A and B will have resulted to low yield and can increase by setting it to a very low level. The best condition in the extraction of pectin is at pH 2, 3 minutes irradiation time and 336 watts power level with an expected yield of 9.022% and desirability of 0.993.
The physico-chemical properties of pectin using the best condition has been analyzed. Table 4 shows the physico-chemical properties of pectin.

Table 4: *Physico-Chemical Properties of pectin*

Properties	Pectin	Standard properties	
		High methoxyl	Low methoxyl
Ash content	4.38%		
Moisture	12.33%	<15	<15
Methoxyl content	11.12%	>6.7	<6.7
Anhydrouronic acid	65.78%		
Degree of Esterification	95.98%	>50	<50

Pectin from mangosteen have an ash content of 4.38% which signify a satisfactorily good quality pectin. Low ash content pectin implies to form better gel with a maximum for a good quality gel of 10%. The moisture obtained is 12.33% which is within the standard sopecification. High methoxyl content is the category of pecting from magosteen becausit exceeded 6.7%. High methoxyl content pectin influences the dispersability of pectin in water. In case of anhydrouronic acid content, it has 65.78% which is lower that the commercial pectin of 74.29. Pectin with AUA of <65% have impurities present due to proteins, starch and sugars [9]. The less AUA content of pectin extracted maybe due to presence of sugars in the precipitated pectin. An effective purification method must be used to resolve the problem. The produced pectin conforms with high methoxyl content on the degree of esterification. Pectin is considered as rapid set (DE>72%) and slow set (DE 58-65%) [8]. Thus pectin from mangosteen rind are classified as rapid set and high methoxyl content which causes more rapid setting of gels. Table 5 shows the statistical analysis of the comparison of the properties of pectin produced and standard.

Table 5: *Comparison of Pectin with Standard*

Properties	p-values	computed t-values	Verbal interpretation
Moisture	0.38	1.02	Not significant
Methoxyl content	0.31	-1.22	Not significant
Degree of Esterification	0.34	1.12	Not significant

Based on the table presented, p-value of 0.383 in moisture content, 0.31 in methoxyl content and 0.35 in degree of esterification was obtained using two sample t-test. This values are higher than 0.05 level of confidence which infers no significant difference with standard high methoxyl pectin.

5. Conclusion

The factors that significantly affect the yield of pectin from mangosteen rind is pH and power level. The highest pectin can be attain at extraction condition of pH 2, irradiation time of 3 minutes and power level of 336 watts with a yield of 9.022% and desirability of 0.993. The model equation for the yield of pectin at varying extraction condition is % yield = 5.57 – 2.15A - 0.4169C + 0.2250AB + 0.6631AC where A is the pH, B is the irradiation time and C is the power level. The properties of pectin attained at best extraction condition are 4.38% ash, 12.33% moisture, 11.12% methoxyl, 65.78% anhydrouronic acid and 95.98% degree of esterification which conform to standard properties of high methoxyl pectin.

References

[1] W. Suttirak and S. Manurakchinakorn, "In vitro antioxidant properties of mangosteen peel extract" Journal of Food Science and Technology, vol 51(12), pp.3546–3558, 2014.

[2] J.J. Klemeš, J., P. Yen Liew., W. Shin Ho, J. Shiun Lim, N. Hazirah Hamidon and D. Norulfairuz Abang Zaidel, "Effect of Extraction Conditions on Pectin Yield Extracted from Sweet Potato Peels Residues using Hydrochloric Acid", Chemical Engineering Transactions, vol 56, pp. 979–984, 2017

[3] K. Takamine, J. I. Abe, K. Shimono, "Physicochemical and gelling characterizations of pectin extracted from sweet potato pulp", Journal of Applied Glycoscience vol 54, pp. 211-216, 2007.

[4] R. S. Faravash and F. Z. Ashtiani, "The influence of acid volume, ethanol-to-extract ratio and acid-washing time on the yield of pectic substances extraction from peach pomace" Food Hydrocolloids vol. 22, pp. 196-202, 2008.

[5] S. Pimentel-Moral, I. Borrás-Linares, J. Lozano-Sánchez, D. Arráez-Román, A. Martínez-Férez and A. Segura-Carretero, A," Microwave-assisted extraction for Hibiscus sabdariffa bioactive compounds", Journal of Pharmaceutical and Biomedical Analysis, 156, pp. 313–322, 2018.

[6] F.L. Seixas, D. L. Fukuda, F.R.B. Turbiana, P.S. Garcia, S. Jagadevan and M.L. Gimenes, "Extraction of Pectin from Passion Fruit Peel (Passiflora edulis f. flavicarpa) by Microwave-Induced Heating" Food Hydrocolloids, vol 38, pp. 186-192, 2014.

[7] V.O. Aina, M.M. Barau,O.A. Mamman, A. Zakari, H. Haruna, M.S.U. Hauwa, Y.B. Abba, "Extraction and characterization of pectin from peels of lemon (Citrus limon), grape fruit (Citrus paradise) and sweet orange (Citrus sinensis)", British J. Pharmacol. Toxicol. Vol 3(6), pp. 259-262, 2012.

[8] R.K. Shaha, Y. Nayagi, A. Punichelvana, A. Afandi, "Optimized extraction condition and characterization of pectin from Kaffir lime (Citrus hystrix)". Malaysia: Res. J. Agric.Fores. Sci. vol1(2) pp. 1-11, 2013

[9] P. C. Kho, C. M. Leong, and N.A. Noranizan "Microwave-assisted extraction of pectin from jackfruit rinds using different power levels," vol. 21, no. 5, pp. 2091–2097, 2014.

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