RESEARCH ARTICLE

Optimization of pectin extraction from selected Philippine fruit peel wastes using box-behnken design

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ABSTRACT

Background: Pectin is a pharmaceutically relevant excipient that can be upcycled from selected Philippine fruit peel wastes. Method optimization of pectin extraction leads to maximizing yields from limited resources, while also reducing environmental wastes, and providing local alternative sources.

Objectives: This study aimed to optimize the method of extracting pectin from selected Philippine fruit peel wastes using the Box-Behnken design, by varying the acid extraction solvent, treatment time, and working temperature.

Methodology: The three-level (-1, 0, 1) Box-Behnken design (15 set-ups) was used to optimize the pectin extraction in each of the fruit peel samples (*C. maxima*; *A. heterophyllus*; ripe and unripe *M. indica*; *D. zibethinus*; and *H. undatus*). The three experimental factors were the type of 3N acid used as extracting solvent (HNO_3, H_2SO_4, and HCl); duration of treatment in minutes (60, 90, and 120); and temperature of treatment in °C (60, 75, and 90). The %yield was computed in each set-up, and the projected yields were generated using multiple linear regression. The pectin samples obtained from the optimized conditions were subjected to the physicochemical characterization, with apple pectin as the standard. Degree of esterification (DE), equivalent weight (EW), methoxy content (MC), alkalinity of ash (AA), and anhydrouronic acid content (AUA) were performed.

Results: Maximum yields were extracted from *C. maxima* (28.96%), *A. heterophyllus* (20.12%), ripe *M. indica* (26.23%), and unripe *M. indica* (25.89%), using 3N H_2SO_4, for a treatment duration of 60 minutes, at a working temperature of 90°C. The same acid also produced the highest yield in *D. zibethinus* (7.70%) at 90°C, and *H. undatus* (25.03%) at 60°C, for a treatment duration of 120 minutes.

Conclusion: Optimum conditions were identified to extract pectin in each of the fruit peel samples. The 3N H_2SO_4 produced the highest pectin yields in all of the set-ups, while the treatment time and working temperature vary per fruit peel sample. Pectin extract from *C. maxima*, *A. heterophyllus*, and *M. indica* was comparable to the standard.

Keywords: *pectin, fruit peels, method optimization, Box-Behnken design*

Introduction

Fruit processing industries are expanding simultaneously with other food processing industry. Alongside with the process is the inevitable production of waste in the form of peel, pulp and seeds. Such waste poses problem in disposal and may potentially lead to severe pollution. To reduce waste, the waste by-products are alternatively used to produce economically valuable products such as candied peel, volatile oils, volatile flavoring compounds, microbial biomass, wine, vinegar and pectin. An effort to reduce fruit peel waste is the extraction of pectin, a valuable raw material in both pharmaceutical and food industries.

Pectin is a complex heteropolysaccharide [1] obtained from the primary cell walls of terrestrial plants. It is a polymer of D-galacturonic acid with a variable number of methyl ester groups. The versatile polysaccharide is mainly used as gelling agents, but can also act as thickener, binder, emulsifier, and stabilizer. It may also act as a drug-delivery carrier [2], which may be incorporated in wound healing or biotechnological formulations in the medical field [3]. As a fat replacer, it is used in the pharmaceutical industry for the treatment of diarrhea, while it has also been used as a haemostat agent [4]. Other applications include fat replacers in spreads, salad

dressings, ice cream, and emulsified meat products [5]. The global market consumption for pectin in 2016 reached 34,000 metric tons. Further estimation that by the end of 2026, the global pectin consumption would have soared to 48,735 metric tons [6]. In the Philippines, pharmaceutical industries are currently importing their pectin requirements from Denmark, Belgium, Switzerland, and China [7]. This prevailing situation provides an opportunity to study the isolation of pectin from fruit peel wastes of selected Philippine fruits and come up with a supply of low-cost pectin.

Pectin extraction is usually carried out using chemical methods [8]. Acids are the strongest extracting agents of pectin as they facilitate significant amounts of insoluble pectin that is tightly bound to the cell matrix of the plant material thus resulting in higher yields. Moreover, mineral acids have higher hydrolyzing capacity than organic acids and are expected to cause more depolymerization of pectins [9]. With the method presented, there are still ways to improve the method for a more economical means of extracting pectin from fruit peels as proposed by this research. As part of efforts to provide various sources of pectin and at the same time to reduce fruit peel wastes, this study aimed to optimize the process of pectin extraction from selected Philippine fruit peel wastes, by varying the acid extraction solvent, extraction time and temperature. The method was systematized using the Box-Behnken Experimental Design.

Methodology

Fruit Sample Preparation

Fruits (Table 1) grown from Dizon farm in Davao City that were used in the study were scientifically identified by the Bureau of Plant Industry. The peels were separated from the fruit and were utilized as the starting materials for the extraction of pectin. Briefly, the peels collected were comminuted and individually blanched at a temperature of 97°C for 10 minutes. Afterwards, they were transferred to a clean container and were cooled in a water bath at room temperature for 15 minutes. The peels were filtered, and oven dried at 60°C. The dried peels were milled and passed through a mesh 60 sieve. The resulting fruit peel flour were stored in polyethylene bags at 2-8°C until the extraction of pectin.

Experimental Set-up

A 3-level (-1, 0, 1) Box-Behnken design was used to generate 15 experimental set-ups for each of the 6 fruit peel flour samples. The three (3) significant factors in the extraction of pectin included the time of treatment in minutes (60, 90, and 120); temperature of treatment in °C (60, 75, and 90); and type of 3N acid used as extracting solvent (HNO_3, H_2SO_4, and HCl). The experimental set-ups are summarized in Table 2. All the experiments were conducted at the Institute of Pharmaceutical Sciences Laboratory, University of the Philippines Manila, National Institutes of Health using the appropriate apparatus and glassware (such as the of corrosion-resistant containers for the 3N acids) and required reagents for the procedures.

Extraction of pectin

For every set-up, 5 g of the fruit peel flour was suspended in 90 mL distilled water. Subsequently, 6 mL of acid was added and both the time and temperature were set as indicated in Table 2. The mixture was stirred continuously throughout the course of extraction. It was then filtered and the resulting filtrate was collected, while the residue was re-extracted using the same set-up used. After the second extraction, the residue was discarded and the filtrates from the first and second extraction were pooled. The filtrate was stored at 4°C for 24 hrs. Equal volume of 95% ethanol was added to the filtrate and was allowed to stand for 30 min. The precipitated pectin was collected and was immersed in 95% ethanol for 24 hrs at 4°C. The filtrate was neutralized before discarded to sink. Afterwards, the pectin was collected, washed with acetone, oven-dried at 40°C and

Table 1. *Fruit samples used in the study.*

Common Name	Scientific Name	Family Name
Pomelo	*Citrus maxima*	Rutaceae
Langka	*Artocarpus heterophyllus*	Moraceae
Durian	*Durio zibethinus*	Bombaceae
Mango (Ripe & Unripe)	*Mangifera indica*	Anacardiaceae
Dragonfruit	*Hylocereus undatus*	Cactaceae

Table 2. *Generated Box-Behnken design set-ups using 3 (A, B, C) 3-level factors (-1, 0, 1)*

Set-up	A (Time in minutes)	B (Temperature in °C)	C (Type of acid)
1	-1 (60)	-1 (60)	0 (H_2SO_4)
2	-1 (60)	-1 (60)	-1 (HNO_3)
3	-1 (60)	0 (75)	1 (HCl)
4	-1 (60)	1 (90)	0 (H_2SO_4)
5	-1 (60)	1 (90)	-1 (HNO_3)
6	0 (90)	0 (75)	-1 (HNO_3)
7	0 (90)	-1 (60)	1 (HCl)
8	0 (90)	0 (75)	0 (H_2SO_4)
9	0 (90)	0 (75)	0 (H_2SO_4)
10	0 (90)	0 (75)	0 (H_2SO_4)
11	0 (90)	1 (90)	1 (HCl)
12	1 (120)	-1 (60)	0 (H_2SO_4)
13	1 (120)	-1 (60)	-1 (HNO_3)
14	1 (120)	0 (75)	1 (HCl)
15	1 (120)	1 (90)	0 (H_2SO_4)

milled thereafter. The washings were discarded in non-halogenated containers. The percent yield of pectin was calculated using the formula:

$$Percent\ Yield\ (\%) = \frac{Weight\ of\ powdered\ pectin}{Weight\ of\ fruit\ peel\ flour\ used\ as\ starting\ material} \ x\ 100\ \%$$

Computation for the Projected Yield of Pectin

To compute for the projected %yield per fruit sample, this study followed the general equation: $y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{1,2} AB + \beta_{1,3} AC + \beta_{2,3} BC + \beta_{1,1} AA + \beta_{2,2} BB + \beta_{3,3} CC$, which is the multiple linear regression equation for the 3^3-Box-Behnken design, where:

Term	Meaning
y	Predicted %Yield
β	Coefficients, or the contributing effects of the factors and its interactions
A	Time
B	Temp
C	Type of acid
AB	Time*Temperature interaction
AC	Time*Type of acid interaction
BC	Temperature*Type of acid interaction
AA	Time*Time interaction
BB	Temperature*Temperature interaction
CC	Type of acid*Type of acid interaction

Physicochemical characterization of pectin

The pectin samples obtained from the optimized conditions were subjected to the physicochemical characterization, with apple pectin (Sigma-Aldrich No. 93854) as the standard. Degree of exterification (DE), equivalent weight (EW), methoxy content (MC), alkalinity of ash (AA), and anhydrouronic acid content (AUA) were performed [10].

Results and Discussion

This research utilized a 3^3-Box-Behnken design, therefore generating 15 set-ups for each of the 6 fruit samples. There were 3 factors manipulated, each having three levels (-1, 0, 1): time or duration of treatment in minutes (60, 90, and 120); temperature of treatment in °C (60, 75, and 90); and type of 3N acid used as extracting solvent (HNO_3, H_2SO_4, and HCl). Out of the 27 possible combinations of the 3-level factors, only 13 set-ups were done according to the Box-Behnken design. Two (2) additional set-ups (set-ups 9 and 10) were replications of one set-up (set-up 8) to come up with a total of 15 set-ups. It can be noted in this generated experimental design that the identical set-ups 8, 9, and 10 would already account for the 3 trials, and the projected deviations in values for the other set-ups.

The extraction yield of pectin from fruit samples varies depending on the different factors. Generally, pectin is extracted at condition of high temperature [11]. Several studies have shown that increasing the temperature during pectin extraction resulted to high pectin yields [12-14]. High temperature helps in the solubilization of pectin in the cell walls [15]. Moreover, the protopectin may not be hydrolyzed efficiently by acids at low temperature, thereby resulting to lower pectin yield [16]. Extraction time is also an important factor to be considered. Pectin yield increased significantly as the extraction time was increased in the studies of Vriesmann *et al.* [14] and Canteri-Schemin *et al.* [18]. On the contrary, extraction time has no significant effect on increasing the pectin yield on the study of Kalapathy and Proctor [19]. The differences between studies may be attributed to other factors such as the extracting acids used and their corresponding pH. Acids at low pH are used in pectin extraction such as tartaric, malic, citric, lactic, acetic and phosphoric acid [20]. However, cheaper mineral acids like HNO_3, H_2SO_4, and HCl are more commonly used [18]. The strength of acid has a significant effect on the pectin yield. Strong acid solutions may lead to highly soluble small pectin molecules as a result of the partial hydrolysis, which may not be precipitated further [19], thus dilute acids are preferred. In the study, different diluted acid resulted to different amounts of recovered pectin. This indicates that the nature of extractant affects the pectin extraction [21]. Different acid has different capacity to penetrate into the cells of plant samples which may be attributed to their pH thereby affecting their contact with pectic substances, as well as the conversion of these substances to soluble pectin [22].

Based on the experimental data on %yield (Table 3), it can be deduced that the set-up with the highest %yield per fruit sample utilized H_2SO_4 as the extracting solvent. For *C. maxima* (28.96%), *A. heterophyllus* (20.12%), ripe *M. indica* (26.23%), and unripe *M. indica* (25.89%), the best duration of treatment is 60 minutes at a working temperature of 90°C. For *D. zibethinus* (7.70%) and *H. undatus* (25.03%), the treatment duration of 120 minutes produced the highest yield, but the former should be at 90°C working temperature, while the latter at 60°C. On the other end, the set-up with the

Table 3. *The % yield of the 15 set-ups for each fruit sample*

Set-up	% Yield					
	C. maxima	*D. zibethinus*	*A. heterophyllus*	*Ripe M. indica*	*Unripe M. indica*	*H. undatus*
1	27.29	5.35	17.83	18.01	21.95	2.63
2	0.18	0.11	0.04	0.22	0.33	0.09
3	2.00	0.76	0.53	1.28	1.82	0.33
4	28.96[b]	4.82	20.12[b]	26.23[b]	25.89[b]	5.55
5	0.22	0.07[c]	0.04	0.07	0.54	0.03
6	0.16[c]	0.23	0.08	0.12	0.06[c]	0.04
7	2.05	0.84	0.42	1.08	1.35	0.23
8[a]						
9[a]	20.09	3.19	18.12	20.87	23.36	7.99
10[a]						
11	1.82	0.58	0.71	1.36	1.08	0.66
12	20.64	5.43	15.18	17.54	25.03	25.03[b]
13	0.21	0.30	0.01[c]	0.05[c]	0.25	0.01[c]
14	2.49	0.76	0.49	1.47	1.82	0.64
15	20.34	7.70[b]	14.67	19.95	17.55	4.22

[a] The presented %yield is already the mean for set-ups 8, 9, and 10, where the standard deviation for each fruit sample were based: 0.42 (*C. maxima*), 0.81 (*D. zibethinus*), 13.53 (*A. heterophyllus*), 0.66 (ripe *M. indica*), 0.62 (unripe *M. indica*), and 0.02 (*H. undatus*).
[b] Highest %yield per fruit sample
[c] Lowest %yield per fruit sample

lowest %yield per fruit sample utilized HNO_3. In general, set-ups across all fruit samples produced relatively lower yield with HCl (set-ups 3, 7, 11, and 14, with 0.23-2.49%) and HNO_3 (set-ups 2, 5, 6, and 13, with 0.01-0.22%); while the set-ups produced relatively higher yield with H_2SO_4 (set-ups 1, 4, 8, 9, 10, 12, and 15, with 2.63-28.95%).

Using the multiple linear regression equation for the 3^3-Box-Behnken design, the formula to compute for the predicted %yield was generated per sample (Table 4). All equations had a p-value <0.05, which meant that the lines follow a reliable and significant trend. The greater the coefficient values (±β), the greater the influence of its respective individual variables (A, B, and C), as well as its variable interactions (AB, AC, BC, AA, BB, and CC), to predict the %yield.

Basing from the means (β_0), *C. maxima*, *A. heterophyllus*, ripe and unripe *M. indica* were suggestive to be potential sources of pectin in terms of %yield, using the respective optimum conditions for extraction. Pectin can also be extracted from *D. zibethinus* and *H. undatus*, as long as the optimum conditions for extraction are followed with respect to the sample being used. In terms of the physicochemical characterization (Table 5), the DE of the isolated pectin from the optimized method ranged from 7.05±1.18, to 65.26±2.90. The corresponding values in other tests also suggest that the pectin samples are low methoxyl pectins (LM-pectin, <50%), except for *M. indica* that had a high methoxyl pectin (HM-pectin >50%) [23]. LM-pectin may be included in controlled-release formulations in pellets form, while HM-pectin in tablets form [24]. In terms of AUA, at least 74% is the USP acceptance criteria, however the standard used did not pass this criteria. Based on the data, pectin from *C. maxima*, *A. heterophyllus*, and *M. indica* can be compared to the standard.

Table 4. *Equation for computing the projected % yield per fruit sample.*

Sample	Equation of the line (p-value, R-squared)
C. maxima	y = 20.9858 - 1.7777A - 0.0987B + 1.6462C - 0.6284AB + 0.4250BC + 1.4799AA + 1.1699BB - 21.8669CC *(0.0003, 0.9757)*
D. zibethinus	y = 3.4685 + 0.6724A + 0.4717B + 0.1332C + 0.9243AB - 0.6049AC - 0.6384BC + 1.0989AA + 1.0489BB - 3.9406CC *(0.0010, 0.9796)*
A. heterophyllus	y = 18.1084 - 1.1782A - 0.0682B + 0.4987C - 0.8755AB + 0.3113AC + 0.7263BC - 0.6023AA - 0.5473BB - 17.4948CC *(0.0001, 0.9937)*
Ripe M. indica	y = 20.9022 - 0.8788A + 0.9991B + 0.7605C - 1.0035AB + 0.1651AC + 0.7994BC - 0.1694AA - 0.3244BB - 20.1183CC *(0.0006, 0.9834)*
Unripe M. indica	y = 23.5946 - 1.2551A - 1.0679B + 1.3720C - 2.6176AB + 1.1951AC + 1.1158BC - 0.2803AA - 0.8853BB - 22.8663CC *(0.0000, 0.9991)*
H. undatus	y = 8.7714 + 1.6564A - 2.6456B + 1.1081C - 4.0757AB + 2.1098AC - 9.4145CC *(0.0420, 0.7420)*

Conclusion

Fruit peel wastes can be a source of pectin wherein the yield depends on various extracting conditions, involving temperature, time and solvent used during extraction. Degree of esterification (DE), equivalent weight (EW), methoxy content (MC), alkalinity of ash (AA), and anhydrouronic acid content (AUA) were performed to characterize the qulity of pectin. Based on the study, the peels of *C. maxima*, and *A.*

Table 5. *Physicochemical composition of pectin isolated from fruit peel wastes*

Sample	DE	EW	MC	AA	AUA
C. maxima	23.06±2.7	535.51±28.36	1.74±0.18	41.74±0.58	42.77±0.74
A. heterophyllus	47.05±1.18	575.40±28.06	0.41±0.06	27.87±2.19	33.09±0.99
H. undatus	36.09±0.06	1568.63±138.65	1.09±0.05	54.23±5.81	17.07±0.75
M. indica	65.26±2.90	1429.74±57.77	4.09±0.35	7.27±0.57	35.55±1.50
Apple standard	83.57±1.33	2178.03±133.92	7.26±0.26	1.84±0.43	49.28±0.99

heterophyllus yield high amounts of low methoxyl pectin, while high methoxyl pectin for ripe and unripe *M. indica*, when chemically extracted at 90°C for 60 minutes using sulfuric acid.

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