ORIGINAL ARTICLE

Optimization of the Isolation and Purification Method of Pharmaceutical Grade Pectin from Pomelo Fruit Peels (*Citrus maxima* Merr. [Family Rutaceae])

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ABSTRACT

Background. Pectin is a heteropolysaccharide used in pharmaceutical formulations as a binding agent. Importation of pectin costs billions of Philippine pesos, but the local laboratory-scale production of this excipient from fruit peel wastes is estimated to be cheaper by 80%.

Objective. To address economic and environment concerns associated with pectin production, this study aimed to optimize the isolation and purification of pharmaceutical grade pectin from pomelo (*Citrus maxima* Merr.) fruit peel as basis for commercial-scale production.

Methods. Pectin was extracted from pomelo using different solvents: 6.2% w/w citric acid, 1N acetic acid, 3N hydrochloric acid, 3N nitric acid, and 3N sulfuric acid. Temperatures for extracting pectin were explored at 40°C, 60°C, and 90°C. Obtained pectin samples were characterized based on the following parameters: equivalent weight (EW), methoxyl content (MC), ash content (AC), anhydrouronic acid content (AUA), and degree of esterification (DE).

Results. Highest pectin yield (9.25%) was obtained using 3N nitric acid and 3N sulfuric acid at 90°C.Based from the pharmacopeial standards (MC ≥ 6.7, AUA ≥ 74.0), all the samples did not pass the parameters, except the pectin extracted using 3N sulfuric acid at 90°C (MC = 6.76, AUA = 74.61).

Conclusion. Among the different solvents used for extraction, 3N sulfuric acid produced the highest percent yield of pharmaceutical grade pectin from pomelo fruit peel. Its optimum temperature for extraction was at 90°C. The sample passed the USP standards of MC values not less than 6.76 and AUA values not less than 74. Under the following conditions, pomelo fruit peel have the potential for commercial-scale production of pharmaceutical grade pectin.

Key Words: Citrus maxima, fruit peels, pectin, pharmaceutical grade, pomelo

INTRODUCTION

Pectin is a heteropolysaccharide used as gelling and binding agent in many pharmaceutical tablet formulations.[1] The Philippines has been importing this excipient from Denmark, Belgium, Switzerland, and China, with an average annual cost of PhP 2.2 Billion, or around PhP 27,000 per kilogram.[2] In other countries, commercial pectin is extracted by treating the raw material with hot dilute mineral acid at pH ~2.[3] In an attempt to find cheaper alternative sources of pectin, its local laboratory-scale production was sourced from mango peels by solubilization at 100˚C for 60 minutes with acidified water at pH 2. According to economic analysis, pectin production from mango peels costs Php 5,667.51 per kilogram, which is cheaper as compared to its importation costs.[4]

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Aside from mango, pomelo fruit peel is also used as an alternative source of pectin in other countries.[5] In the Philippines, pomelo production averaged 30 thousand metric tons annually from 2012 to 2014, and its equivalent fruit peel is estimated to be 1,800 tons yearly.[6] There are no extensive researches yet on the pectin production from fruit peels of pomelo. When these waste materials are recycled and upcycled for commercial scale, it can provide low-cost materials, improve the Philippine economy, and at the same time address environmental concerns.

To fill the knowledge gaps in obtaining pharmaceutical grade pectin from pomelo fruit peels as an alternative pectin source in the country, this study aimed to optimize the isolation and purification methods by varying factors for extraction such as temperature, and type of solvent used, with its corresponding pH.

MATERIALS AND METHODS

Collection of fruit peel wastes

The fruit peel of pomelo (*Citrus maxima* L.) were obtained from Divisoria Market, Manila. The peels were washed with running water and subjected to blanching treatment, resulting to enzymatic inactivation. This was done by immersing the peels in water at 97°C for 3 minutes, then transferring to another water bath at room temperature for 15 minutes.[7] Subsequently, the peels were dried in an air-circulated oven at 50°C until a constant weight was obtained. The dried peels were then milled and passed through sieve mesh 60. The resulting fruit peel flour was stored in a polyethylene bag at 4°C until used for analysis.[8]

Optimization of pectin extraction and purification

The optimization of pectin extraction was carried out by preparing 15 flasks, which contained five grams each of the fruit peel flour that was then dissolved in 90 mL of distilled water. As shown in Table 1, varying volumes of weak and strong acids were respectively added to the flasks, then subjected to varying temperatures for 1 hour with continuous stirring. The resulting pH values ranged from 1.2-4.2. The hot acid extract was filtered using Buchner funnel through Whatman No. 1 filter paper. The digestion was repeated for another 30 minutes. The filtrate from the first and second digestion were combined and placed overnight in a refrigerator at 4°C after which, pectin was precipitated by adding 95% ethanol to the cooled filtrate, and the mixture was left undisturbed for 30 minutes. The mixture was filtered and then soaked overnight in 95% ethanol. The isolated pectin was washed with 5 mL portions of acetone to remove any remaining impurities. The purified pectin was oven dried at 35-40°C and the percentage yield was computed. The dried pectin isolate was ground to obtain powdered pectin.

Table 1. Acids added to the flasks and their corresponding temperature for extraction

Flask No.	Volume and type of acid added	Temperature for extraction
1	10 mL, 6.2% (w/w) citric acid	40°C
2	10 mL, 6.2% (w/w) citric acid	60°C
3	10 mL, 6.2% (w/w) citric acid	90°C
4	10 mL, 1N acetic acid	40°C
5	10 mL, 1N acetic acid	60°C
6	10 mL, 1N acetic acid	90°C
7	6 mL, 3N hydrochloric acid	40°C
8	6 mL, 3N hydrochloric acid	60°C
9	6 mL, 3N hydrochloric acid	90°C
10	6 mL, 3N nitric acid	40°C
11	6 mL, 3N nitric acid	60°C
12	6 mL, 3N nitric acid	90°C
13	6 mL, 3N sulfuric acid	40°C
14	6 mL, 3N sulfuric acid	60°C
15	6 mL, 3N sulfuric acid	90°C

Characterization of pectin[9]

As summarized in Table 2, the parameters used to characterize extracted and purified pectin were equivalent weight (EW), methoxyl content (MC), ash content determination (AC), anhydrouronic acid content (AUA),

Table 2. Acids added to the flasks and their corresponding temperature for extraction

Parameters	Chemicals/Variables used	Formula
EW	95% ethanol, NaCl, CO_2-free distilled water, phenol red, 0.1N NaOH	$\text{Equivalent weight} = \frac{\text{wt. of sample (g)} \times 1000}{\text{vol. of alkali (mL)} \times \text{Normality of alkali}}$
MC	0.25N NaOH, 0.25N HCl, 0.1N NaOH, phenolphthalein	$\text{Methoxyl content (\%)} = \frac{\text{vol. of alkali (mL)} \times \text{Normality of alkali} \times 31 \times 100}{\text{wt. of sample (g)} \times 1000}$
AC	0.1N HCl, 0.1N NaOH, phenolphthalein	$\text{Alkalinity (\%)} = \frac{(\text{Blank} - \text{Titre}) \times \text{Normality of alkali} \times 60 \times 100}{\text{wt. of ash (g)} \times 1000}$
AUA	alkali milli-equivalents of EW, MC, & AC	$\text{AUA (\%)} = \frac{176\ (\text{m.e. for free acid} + \text{m.e. for saponification} + \text{m.e. for titratable ash}) \times 100}{\text{wt. of sample (g)} \times 1000}$
DE	MC and AUA values	$\text{Degree of esterification} = \frac{176 \times \text{methoxyl content (\%)} \times 100}{31 \times \text{anhydrouronic acid (\%)}}$

**Procedures were lifted from the study of Kanmani P, Dhivya E, Aravind J, Kumaresan K (2014). EW = Equivalent Weight, MC = Methoxyl Content, AC = Ash Content Determination, AUA = Anhydrouronic Acid Content, DE = Degree of Esterification.*

and degree of esterification (DE). The working standard used for comparison was apple pomace pectin. The purity of the samples was primarily based on MC and AUA content.

Statistical analysis

The data were analyzed using Microsoft Excel 2016 Analysis ToolPak. The 2-way ANOVA was used for the analysis of percent yield in response to the variation of solvent type used and temperature applied. The t-test was used to compare other parameters against the standard.

RESULTS AND DISCUSSION

All measurements were done in triplicates for each of the sample. The characterization of the pectin extracted from pomelo fruit peel under various conditions is summarized in Table 3. Among the samples, 3N nitric acid and 3N sulfuric acid at 90˚C gave the highest pectin yield at 9.25%. The different solvent type (p=0.015) and temperature applied (p=0.006) were found to be statistically significant in optimizing pectin isolation in terms of percent yield as seen on Table 3. Samples with less than 1% yield were not sufficient to conduct the AC, AUA, and DE determination. Apple pomace was used as standard due to its high amount of pectic substances.[10]

The EW of pectin samples indicates its jelly-forming ability, wherein better gelling agents possess higher equivalent weight. Among the samples with greater than 1% yield, pectin extracted using 3N hydrochloric acid at 90˚C had the greatest gelling property (EW = 984.13), followed by 3N sulfuric acid at 60˚C (EW = 968.25). This may be attributed to the strong acid properties of the two solvents responsible for dissolving pectin substances with their respective optimum temperatures for extraction.[11] Using t-test to compare against the standard (EW = 1174.84), the samples were statistically significant (p<0.05) in terms of difference in gelling properties.

As per pharmacopeial standards, pectin yields should not have methoxy groups (-OCH_3) less than 6.7% while the anhydrouronic acid ($C_6H_{10}O_7$) content, should not be less than 74.0% of the dried material.[12] The MC is an important factor in controlling the setting time and gel formation of pectin due to methoxy groups present, while AUA content determines the galacturonic acid content that indicates the purity of pectin since galacturonic acid units constitute the pectin backbone.[13] Among the samples subjected to different extraction solvents at various temperatures, both the standard and pectin extracted using 3N sulfuric acid at 90˚C passed the USP criteria. The t-test suggests that the sample is statistically different from the standard (p<0.05). DE values on the other hand indicated the rate of gel formation,[14] the pectin samples had DE values <72%, therefore classified as slow set.

CONCLUSION

To optimize the isolation and purification of pharmaceutical grade pectin from pomelo peel, different solvents were tested at varying temperatures. It was found that the extraction of pectin obtained the highest yield (9.25%) using 3N nitric acid and 3N sulfuric acid at 90˚C. Based from the USP criteria for pectin characterization (MC ≥ *6.7, AUA ≥ 74.0*), only pectin extracted at 90˚C using 3N sulfuric acid (MC = 6.76, AUA = 74.61) passed the parameters. Other

Table 3. Characteristics of pectin extracted from pomelo fruit peel under different conditions

Acid, Temperature	%Y	EW	MC	AC	AUA	DE
6.2% (w/w) Citric acid						
40°C	0.19	1000	1.55	**	**	**
60°C	0.79	1000	1.55	**	**	**
90°C	3.18	606.06	4.34	49.14	54.03	45.6
1N Acetic acid						
40°C	0.29	2000	1.55	**	**	**
60°C	0.33	2000	1.55	**	**	**
90°C	0.55	2000	1.55	**	**	**
3N Hydrochloric acid						
40°C	0.33	888.89	2.07	**	**	**
60°C	3.04	882.74	2.95	48.9	39.83	41.97
90°C	5.27	984.13	3.46	49.48	5.27	39.69
3N Nitric acid						
40°C	0.79	761.17	3.15	**	**	**
60°C	3.06	674.33	1.96	47.46	38.31	29.1
90°C	9.25	888.78	6.18	3.16	62.15	56.48
3N Sulfuric acid						
40°C	3.35	811.97	3.72	45.06	50.92	41.47
60°C	5.93	968.25	4.24	11.34	42.59	56.47
90°C	9.25	844.38	6.76	7.8	74.61	51.47
Apple pomace (Std)	N/A	1174.84	10.31	2.16	79.01	74.07
USP Acceptance Criteria	N/A	N/A	≥ 6.7	N/A	≥ 74.0	N/A

**%Y = Percent Yield; EW = Equivalent weight; MC = Methoxyl Content; AC = Ash Content; AUA = Anydrouronic Acid; DE = Degree of Esterification*
*** Samples were not sufficient in terms of percent yield to perform the tests (Samples with <1% yield)*

parameters for pectin characterization suggested its good gelling quality. Under the conditions using the optimized method of pectin extraction, pomelo fruit peel could be a potential source of pharmaceutical grade pectin.

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Statement of Authorship

All authors approved the final version submitted.

Author Disclosure

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