Original Research Article

Extraction and Characterization of Pectin from Ripe Mango (*Mangifera indica*) Peel

ABSTRACT: Pectin is a multifunctional constituent of cell wall of different plants at different concentration with a widespread application. Mangifera indica peel is excessively produced as a by-product of local food processing plants in Cebu. The objective of the study is to characterize pectin based on its presence of amide, ash content, moisture content, equivalent weight, methoxyl content, total anhydrouronic acid content, degree of esterification, organoleptic evaluation, and solubility.
RESULTS: The isolated mango pectin had lower equivalent weight, lower methoxyl content, and lower total anhydrouuronic acid content compared with apple pectin.
CONCLUSIONS: Chemical characterization confirmed the presence of carbohydrates and methoxyl content in pectin. Physical tests for mango pectin yielded an equivalent weight of 568.2, low AUA content (63.4 %), high degree of esterification (51.1), slightly higher ash content (5.53%), and an acidic pH (4.38).

Keywords: Characterization, Extraction, Mango,Pectin

1. INTRODUCTION

According to the Phillipines Statistics Authority et. Al. (2017), mango is the third most important fruit crop of the Philippines based on export volume and value next to banana and pineapple. Major use of mango is the consumption of the sour-sweet pulp being eaten raw or for different kinds of food preparation and processes, the seed and the skin (peel), which comprise 30% to 50% of the weight depending on the variety, are typically treated as waste and immediately disposed without further utilization.Worldwide several millions of tons of mango wastes are reproduced annually from factories thus researchers are now exploring the use of pectin from mango peel so as to avert the undesirable effects of their accumulation on the environment(Shaibu and Dinshiya et.al.2022).

Pectin is a multifunctional constituent of cell wall, it's a high-value functional food ingredient. It is produced commercially as a white to light brown powder, mainly extracted from fruits. Pectin (Fig.1) is a linear chain of α- (1-4)-linked D-galacturonic acid that forms the pectin-backbone. Pectin is a non-starch linear polysaccharide consists of α- 1, 4 D-galacturonic acid. Pectin is found in the primary cell wall and middle lamella of the plant providing consistency and mechanical resistance to it (Hamed and Mustafa, 2018). Pectic substances consist of an associated group of polysaccharides that are extractable with hot water or with aqueous solutions of dilute acids (Fissore, 2012).

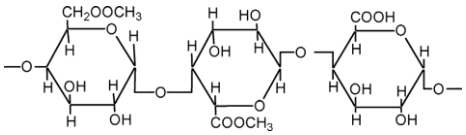

Figure 1. Structure of pectin (Hassan *et al*., 2018)

Mango (Mangifera indica Linn. is known by its common name as mango or mangga and a popular fruit in the tropical regions. It is the national fruit of India and the Philippines, and the national tree of Bangladesh. Mango tree belongs to genus Mangifera of the family Anacardiaceous.(Jimenez and Mendoza et.al.,2024).Mangoescontain high levels of vitamin C,vitamin E, Caretinoid, dietary fiber and pectin(Mubarik et.al.,2020).

In a previous study, pectin was extracted from unripe banana peel powder using hydrochloric acid (HCl) at 90 ± 5˚C in five different time periods at pH 2. Pectin yieldsvaried from 7.5% to 13%. The best extraction condition using HCl were temperature 90°C, pH 2 and extraction time of 4 hours with a pectin yield of 13%. The structure of the product was confirmed by Fourier transform infrared spectroscopy (FTIR) analysis. Extracted pectin was characterized by the estimating parameters moisture, ash content, methoxyl content, anhydrouronic acid content, degree of esterification and equivalent weight (Kamble *et al*., 2017).

Pectin was extracted from banana and mango peels using the acid extraction method. This study investigated the effect of temperature, time and pH on the yield and physicochemical characteristics of pectin extracted from banana and mango peels. The yields of pectin under these optimum conditions were found to be 11.31% and 18.5% for banana and mango peel, respectively. The finding of the study showed that pectin, being used as food and pharmaceutical additives, can be obtained from banana and mango peel (Pranati Srivastava *et al*., 2011).

Comparative extraction and characterization of pectin from three different fruits peels (orange, sweet lime and papaya) were conducted. The result indicated that different extraction conditions such as pH, temperature, and time has an effect on the extraction yield. Extraction using HCl gave the best result at optimum temperature of 85°C and optimum pH at 2.0 for 60 min. Under such conditions, orange peel had 36% yield of pectin which was greater than sweet lime (21%) and papaya (19%).While extraction from orange peel using the citric acid at 85°C and pH 2.0 for 60 min yielded 29% of pectin which was greater than sweet lime (17.3%) and papaya (16.2%). Thus, yield of pectin using HCl for extraction was found to be more than citric acid. Orange peel showed high yield of pectin as compared to sweet lime and papaya (Yadav *et al*., 2015).

Pectin was extracted from pomelo using different solvents: 6.2% w/w citric acid, 1N acetic acid, 3N HCl, 3N nitric acid, and 3N sulfuric acid. Temperatures for extraction of pectin were explored at 40°C, 60°C, and 90°C. Obtained pectin samples were characterized based on the following parameters: equivalent weight, methoxyl content, ash content, anhydrouronic acid content, and degree of esterification (Arollado *et al*., 2018).

The potential of Carabao mango (*Mangifera indica* Linn) peels as a source of pectin was investigated in line with the Philippines' total dependence on imported pectin. A previous work established an extraction process to produce pectin from carabao mango peels which conformed to United States Pharmacopeia (USP) standard. Results showed that dried carabao mango peels yielded 21.65% pharmaceutical grade pectin. The product was characterized as high methoxyl pectin because of its high galacturonic acid content (92.82% - 98.65%). It is applicable for food formulation because of its high degree of esterification (76-79). The total dietary fiber and sugar contents were 77.4% and 4.8%, respectively, indicating usefulness for better digestive functions. Its gelling properties were comparable with the analytical grade pectin (Gragasin *et al*, 2014).

Another study characterized pectin based on their degree of esterification, equivalent weight, ash content, methoxyl content, presence of amide, total anhydrouronic acid content and moisture content. It further states that high methoxyl, or HM-pectins, have a DE of 50% or greater while low-methoxyl (LM-) pectins have a DE of less than 50% (Dixon, 2008).Pectin from papaya peel was extracted using HCl and citric acid at different time, temperature and pH combination. The characterization of the extracted pectin was done by calculating the ash content, moisture content, equivalent weight, methoxyl content, anhydouronic acid content and degree of esterification which varied from 7.3%-9.67%, 4.8-7.2%, 455.1-912.17, 6.2-7.5%, 84.3-72.5% and 49.2-53.4% respectively (Altaf *et al*.,2015)

Characteristics of pectin

Presence of amide. The presence of amide serves as a molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the amidated ester in ratio to all esterified groups. This measure can be a fingerprint for pectin characterization. The reaction is meant to determine if there is presence of aliphatic amide (Dixon, 2008). Acceptance criteria: 1030.92±0.69 (USP, 1980).

Ash content. This refers to the residue remaining after incineration. Ash content determination is a basis for judging the identity and cleanliness of pectin and gives information relative to its adulteration with inorganic matter (Jenkins, 1977). Acceptance criteria: Not more than 10% (FAO, 2009).

Identification of carbohydrates. This test establishes the identity of pectin, a linear polysaccharide. Pectin consists mainly of D-galacturonic acid (GalA) units, joined in chains by means of α - (1-4) glycosidic linkage. These uronic acids have carboxyl groups, some of which are naturally present as

methyl esters and others which are commercially treated with ammonia to produce carboxamide groups (Novosel'skaya et al., 2000).

Moisture content. This parameter is used to measure yield and quality of pectin. The chemical, physical and microbial stability of pectin are affected by water content and thus influences the textual properties of pectin (Kim et al. 2004).

Methoxyl content. This is an important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the methyl ester in ratio to all esterified groups (Dixon, 2008). Acceptance criteria: minimum of 6.7% (USP-NF, 2015).

Equivalent weight. This is used to calculate the anhydrouronic acid content and the degree of esterification (Girma et al., 2016).

Total anhydrouronic acid content. This is essential to determine the purity and degree of esterification, and to evaluate the physical properties (Girma et al., 2016). The importance of anhydoruronic acid content is to determine the galacturonic acid units which constitutes backbone of pectin (Arollado et al., 2018). Acceptance criteria: Not less than (NLT) 74% (USP-NF, 2015).

Degree of Esterification (DE). This is an important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the methyl ester. Degree of esterification is measured through various techniques, but titrimetry is a classical method of DE determination (Girma et al., 2016).

pH of pectin. As the pH is lowered, ionization of the carboxylate groups is suppressed, and this results in a reduction in hydration of the carboxylic acid groups. As a result of reduced ionization, the polysaccharide molecules no longer repel each other over their entire length, and as a result, they can associate and form a gel. However, pectin with increasingly greater degrees of methylation will gel at a higher pH, because they have fewer carboxylate anions at any given pH. In general, maximum stability is found at pH 4, (Sundar Raj et al., 2012).

material and methods

Collection and preparation of plant sample

Mango (*Mangifera indica* Linn) peel, other parts of the plant and fruit samples were submitted to the Department of Agriculture, Field Office Region VII for authentication. Ripe mango peels were obtained as a waste from a fruit processing plant, Profood International Corporation. Peels were carefully washed and dried in an oven for 24 h at 50-55 ºC (Berardini, 2005).

Extraction of pectin from mango peel

One kilogram (Figure 2) of dried fruit peel was weighed, cut into pieces, and powdered using an electric blender. Powdered peel were further passed through sieve # 20 and stored in air tight container until used. A total of 400 g mango peel powder was mixed with 2200 mL distilled water and acidified with hydrochloric acid to meet the designed pH of 2.0. The mixture was then stirred using a stirrer until all the mango peel powder was evenly wetted by acidified water in homogenous form. The pectin extraction procedure was continued by heating the acidified samples at 90 ± 5°C in a stirring hot plate for 4 h. The solution was then cooled and filtered using a two-layer cheesecloth. The filtrate was collected then added with twice its volume of absolute ethanol. Pectin isolate was obtained, and then recovered by centrifuge at 5000 rpm for 10 minutes (Figure 3). The resulted pectin substance was dried in an oven at 65°C until a constant weight was reached (Kamble *et al*., 2017). The percentage yield was computed using the formula:

$$\%yield = \frac{weight\ of\ dried\ pectin}{weight\ of\ dried\ mango\ peel}$$

Figure 2. Preparation and extraction of pectin
A: mango peel samples;
B: weighing of mango peel;
C: drying of mango peel;
D: dried mango peel;
E: extraction of pectin by acidification;
F: heating of acidified extractive;
G: pectin isolates;
H: dried mango pectin

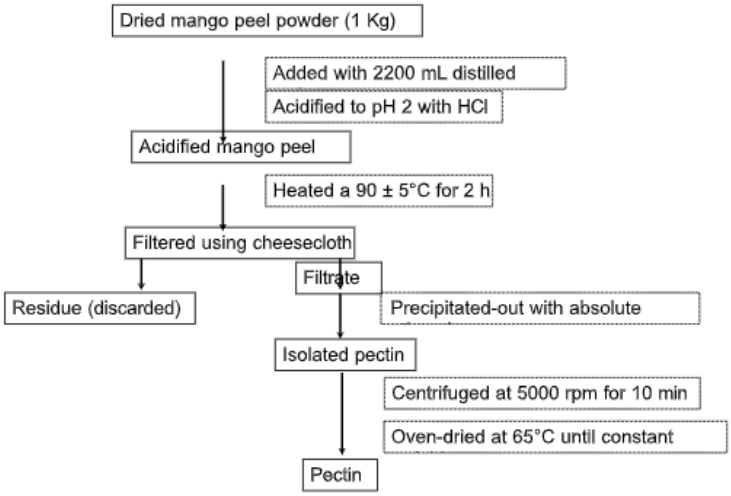

Figure 3. Extraction of pectin from mango peel (Kamble *et al*., 2017)

Characterization method for pectin (Kamble *et al*., 2017)

Organoleptic evaluation of isolated pectin. Isolated pectin was characterized for organoleptic properties such as color, odor, taste, fracture and texture.

Identification tests for carbohydrates. Aqueous extract of pectin (1mL) was mixed with Molisch's reagent followed by addition of sulfuric acid. The violet ring at the junction showed the presence of carbohydrates (Butt *et al*., 2007).

Presence of amide. Sample (1 g) was added to 0.1 N NaOH solution and heated strongly. Smell of NH_3 indicated the formation of amide. Continuous heating was done until no more NH_3 was evolved. Few drops of concentrated HCl were added. Absence of precipitate indicates the presence of aliphatic amide. Acceptable criteria for presence of amide =Not more than 25% of total carboxyl groups of pectin (FAO, 2009).

Ash content. Pectin sample (2 g) was weighed. The sample was ignited slowly, then heated for 3-4 h at 600 ºC. The sample was cooled in the crucible at room temperature in desiccators and weighed properly. The process was continued until constant weight was achieved and final weight was recorded.

$$Ash\ \% = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

Acceptable criteria for % ash content (pectin) = Less than 4% (USP specifications).

Moisture content. Sample pectin (1 g) was weighed and placed into a metal dish. The sample was

dried in an oven for 5 h at 100°C, cooled in desiccators and then weighed. The moisture content was determined using the equation:

$$Moisture\ content = \frac{weight\ of\ residue \times 100}{weight\ of\ sample}$$

Acceptable criteria % for moisture content (Pectin) = Less than 10% (USP Specifications).

Equivalent weight. Sample pectin (1g) was placed in a 250 mL conical flask and 5 mL ethanol added. Sodium chloride (1g) and distilled water (100 mL) were added. Finally, 6 drops of phenol red were added and titrated against 0.1N NaOH. Ttration end point was indicated by pink color. The neutralized solution was stored for determination of methoxyl content and anhydrouronic acid content.

$$Equivalent = \frac{weight\ of\ samle \times 1000}{mL\ of\ alkali \times Nomality\ of\ alkali}$$

Acceptable criteria for % equivalent weight (pectin) = 1030.92 ± 0.69 (USP-NF, 2015).

Methoxyl content. Collection of the neutral solution from determination of equivalent weight, and 25 mL of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 mL of 0.25N HCl was added and titrated against 0.1N NaOH. Methoxyl content was calculated using the following formula:

$$Equivalent = \frac{weight\ of\ samle \times 1000}{mL\ of\ alkali \times Nomality\ of\ alkali}$$

Acceptable criteria for % methoxyl content (pectin) = ≥ 6.7% (USP-NF, 2015).

$$Methoxyl\ content\% = \frac{ml\ of\ alkali \times Nomality\ of\ Alkali \times 3.1}{weight\ of\ sample}$$

Acceptable criteria for % methoxyl content (pectin) = ≥ 6.7% (USP-NF, 2015).

Total anhydrouronic acid content (AUA). Total AUA of pectin was obtained by the following formula.

$$(AUA)\% = \frac{176 \times 0.1Z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{W \times 1000}$$

Molecular unit of AUA (1 unit) = 176g

Where; *z*= mL (titre) of NaOH form equivalent weight determination. *y*= mL (titre) of NaOH from methoxyl content determination *w*= weight of sample.

Acceptable criteria for total anhydrourionic acid %(pectin) = Not less than (NLT) 74% (USP-NF, 2015).

Degree of esterification. This was measured on the basis of methoxyl and AUA content. It was calculated using the following formula:

$$Degree\ of\ esterification\ (DE)\% = \frac{176 \times Me0}{31 \times AUA} \times 100$$

pH of pectin. Sample pectin (1g) was placed into a beaker containing 100 mL of distilled water to make 1%w/v solution. The pH of solution was determined using digital pH meter.

Solubility of dry pectin in cold and hot water. The pectin samples was separately placed in a conical flask with 10 mL of 95% ethanol followed by 50 mL distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 80°C for 15 min (Alamineh, 2018).

3. RESULTS AND DISCUSSION

Characterization of isolated pectin from mango peel

Table 1 presents the percent yield of pectin obtained from mango peels.

Table .1 Percentage yield of pectin from mango peels

	sh peel (g)	d peel (g)	owdered peel (g)	Dried pectin (g)	% yield
Sample 1	3000	1000	400	30	7.50
Sample 2	2000	355	200	20.4	10.2
Sample 3	2000	355	155	12.3	7.94

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Average percent yield	8.54 ± 1.45

Three batches of mango powdered mango peel namely, sample 1, 2 and 3, 400 g, 200 g and 154. 72 g were prepared separately with an average pectin yield of 8.54 ±1.54 %. Also, a translucent gelatinous precipitate was formed in all samples when pectin from mango peel, in solution, was added with twice its volume of absolute (95%) alcohol confirming the presence of pectin (USP 30/NF 25).

The results in Table 2 represents the organoleptic evaluation of pectin in terms of its color, odor, and texture. The produced have similar odor and texture, it only differ in the color which was brownish (Figure 4) while the commercial pectin was yellowish white. This indicates that they have the same characteristics but only varies on color which can be attributed to the color of the dried mango peel.

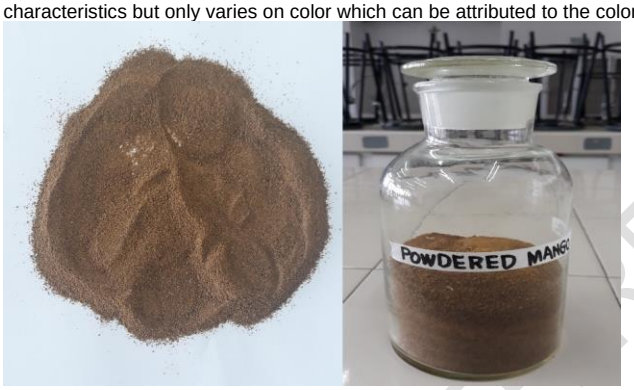

Figure 4. Isolated mango peel pectin

Table 2.Organoleptic evaluation results of Pectin

Sample	**Parameters**		
	Color	*Odor*	*Texture*
Mango pectin (prepared)	Brown	Odorless	Mixture of coarse and fine powder
Commercial pectin	Yellow-white[1]	Odorless	Mixture of coarse and fine powder

[1]Root Gatherers, University of Immaculate Conception.

The prepared pectin was tested for the presence of carbohydrates using Molisch's reagent and also presence of aliphatic amide through amide test. All tests were positive. Molisch's test formed a violet ring while for amide test, a smell of ammonia followed by absence of precipitate.

Table 3 presents other confirmatory tests of pectin (Molisch's test and amide test).

Table 3. Test for Carbohydrates using Molisch's Reagent and amide test

Test	**Reagent**	**Result**	**Image**	**Interpretation**
Molisch's test	□-naphtol in 95% ethanol and sulfuric acid	Formation of violet ring		Presence of violet ring indicated presence of carbohydrate in pectin sample.

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Amide test	0.1N NaOH solution, HCl	Smell of NH_3 and absence of precipitate.		Smell of NH_3 indicated formation of amide. Absence of precipitate confirmed the presence of aliphatic amide.

Table 4 presents the physicochemical characteristics of isolated mango pectin.

Table 4. Physicochemical characteristics of pectin produced from mango peel

Characteristics	**Mango pectin**	**Apple pectin[2]**
Equivalent weight	568	1030.92 ± 0.69
Methoxyl content (%)	5.70	> 6.7 %
Total anhydrouronic acid content	63.4	≥ 74%
Degree of esterification (%)	51.1	Not specified
Ash content (%)	5.53	< 4%
Moisture content (%)	4.67	< 10%
pH of pectin	4.38	1 – 6
Solubility	Insoluble in cold water; Soluble in hot water	-

[2]USP specification of apple pectin from International Journal of Food Properties, 2004.

The isolated mango peel pectin was also tested for its physicochemical characteristics. The characteristics included equivalent weight, methoxyl content, total anhydrouronic acid content, degree of esterification, ash content, moisture content, pH and solubility. The results were compared to those of the pure apple pectin, a commercially form of pectin (Table 4). The isolated mango pectin had lower equivalent weight, lower methoxyl content, and lower total anhydrouuronic acid content compared with apple pectin.

DISCUSSIONS

The mango pectin extracted was brownish, odorless and with a mixture of fine and coarse texture. The produced pectin had similar odor and texture to the apple pectin which is recognized by USP as a commercial pectin. The brownish color of the produced pectin was attributed to the color of the dried mango peel.

Mango peel-derived pectin was chemically characterized using tests such as Molisch and amide tests. Molisch reagent confirmed the presence of carbohydrates. The galacturonic acid present in mango pectin reacts with Molisch's reagent to form a violet ring (Butt *et al*., 2007). The amide test confirmed the presence of aliphatic amide through the absence of precipitate. It was important to remove ammonia from the isolated pectin by heating since ammonia could interfere with the proposed analytical methods.

The methoxyl content of pectin was found to be 5.7%. Methoxyl content is an important factor in determining the gel formation capacity which controls the setting time of pectin and its ability to form gels (Constenla and Lozano, 2003). Spreadability and sugar-binding capacity of pectin increases with an increase in methoxyl content (Madhav and Pushpalatha, 2002). The methoxyl value in this study indicates that mango peel pectin was categorized as low methoxyl pectin which means it can form gels with lower concentrations of sugars (Castillo *et al*., 2015).

Physical tests of the mango peel-derived pectin were conducted. The equivalent weight of pectin was found to be 568.18, which is considered as low equivalent weight. The low equivalent weight could be due to partial degradation of pectin (Azad *et al*., 2014). The total anhydrouronic acid content of pectin was found to be 63.36%. Total anhydrouronic acid content indicates the purity of the extracted pectin and prepared pectin may be classified as low total anhydrouronic acid content type pectin. The low value of

total anhydrouronic acid content means that the extracted pectin might have a high amount of protein, starch, and sugars in the precipitated pectin which means low purity as compared with commercial apple pectin (Ismail *et al.*, 2012). The degree of esterification of pectin was found to be 51.1% which can be categorized as marginally "high methoxyl pectin", where degree of esterification is above 50% (Azad *et al*., 2014).

The ash content of pectin was found to be 5.53% indicating the amount of residue remaining after incineration (Virk and Sogi, 2004). Ash content represents the inorganic salts naturally occurring in the pectin as contaminants thus the prepared pectin's quality is considered low. The prepared pectin has a low moisture content of 4.67%. Low moisture content is necessary for pectin for safe storage as well as to inhibit the growth of microorganisms that can affect the quality of pectin (Muhmadzadeh *et al.*, 2010). The pH of isolated pectin (4.38) was found to be acidic. The results of the physical tests were close to the compendial requirements of an apple pectin. Extraction and preparation methods of mango pectin may have contributed to the unmet physical parameters for standardized pectin. Equivalent weight, methoxyl content, ash content were unmet hence the method of extraction should be improved through the extraction procedure, a more efficient acid extraction should be employed to chelate more Ca^{2+} which contributes to majority of the ash content (Castillo *et al*., 2015).

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4. CONCLUSION

In the present study, the average percent yield of mango peel-derived pectin was 8.54 ±1.54 %. The mango pectin was brown and odorless. Chemical characterization confirmed the presence of a carbohydrate group and methoxyl content in pectin. Physical tests for mango pectin yielded a low equivalent weight, low total anhydrouronic acid content, high degree of esterification, slightly higher ash content, and an acidic pH.

5. CONSENT AND ETHICAL APPROVAL

It not applicable

DISCLAIMER

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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