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Downstream Purification of Pectin Precipitated from Mango Peel Extract

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Abstract. Pectin was prepared from industrial waste mango peels through typical hot acidified water extraction and alcohol precipitation. Its downstream purification method was investigated. Purification of mango pectin should include thorough washing with highly pure alcohol to remove small molecular weight impurities. Thorough alcohol washing should include mashing the pectin precipitate with highly pure ethanol and pressing to squeeze out liquids. Incorporation of thorough alcohol washing after alcohol precipitation yielded 8.72±0.20% pectin from alcohol-insoluble mango peel residue. The obtained pure mango pectin has high degree of esterification (71.30±0.96%) and galacturonic acid content (90.30±0.64%), thus it can be a good alternative source of commercial pectin used for jam and jelly preparation.

1. Introduction

Mango (*Mangifera indica* L. Anacardiceae) is among the tropical fruits abundant in the Philippines. It has established local and international markets both as fresh and processed fruit. The mango peels represent about 16-19% (w/w) of the whole fruit [1]. They are generated daily in large quantities in mango processing industry adding up to the existing disposal problems. The recovery of valuable components from these waste by-products is considered to be a reasonable way of utilizing these wastes and adding value to it while addressing both economic and environmental concerns.

Mango peels are rich source of pectin, fiber and polyphenols [2-4]. This work focuses on the extraction of pectin from mango peels. Pectin is a heterogeneous polysaccharide having broad applications in the areas of food and beverage, pharmaceutical, cosmetics and biotechnology because of its thickening, gelling and emulsifying properties [5-6]. Pectin in its native nature consists of homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II regions [5]. The homogalacturonan region consists of sequences of (1→4)-α-linked-D-galacturonic acid units. The carboxyl group of the galacturonic acid units can be esterified and presented as methylester group. The galacturonic acid units may also consist of O-acetyl group at C-3 or C-2 position. The rhamnogalacturonan I region is consists of sequences of (1 → 2)- α -L-rhamnose-(1 → 4)- α -D-galacturonic acid, with ends being glycosidically linked to the homogalacturonan region. The rhamnose units are substituted at C-4 or C-3 with side chains of neutral sugars. The rhamnogalacturonan II region is consists of homogalacturonan backbone with around nine galacturonan units, to which four structurally different polymeric side chains are linked [5]. Commercial pectin contains mostly of homogalacturonan region (≥65% galacturonic acid content) and conventionally categorized as high-methoxyl (HM) or low-methoxyl (LM) pectin [5,7]. The ratio of esterified galacturonic acid groups to total galacturonic acid groups in pectin is termed as degree of esterification (DE). HM-pectin has DE of greater than 50%, and requires a minimum amount of soluble solids and a pH around 3.0 or lower in order to form gels. LM-pectin has DE of less than 50%, and requires the presence of calcium or other divalent cations to form a gel.

Pectin is industrially extracted from fruit wastes by hot acidified water. Extraction conditions are varied, typically pH ranges from 1.5 to 3.0, temperature ranges from 60ºC to 100ºC, and time ranges from 0.5 h to 6 h, to give a material that has the desired gelling capacity and degree of methylation [8]. Pectin is then isolated from the extract more commonly by alcohol precipitation. It is separated as a stringy gelatinous mass which is washed, dried and ground. The yield, quality and suitability for an application of extracted pectin depend on the type of raw material used (variety, conditions of processing) and the extraction method [5]. There are already several studies on extracting pectin from mango peels [3,9-15]. However, these studies are focused on optimizing extraction condition for better yield or finding better extraction process. In such studies, reported downstream purification method of pectin mostly include washing with alcohol [3,10-15]. It may be combined with pressing or further washing with acetone [3,10,12,13,15]. Others reported to directly purify the extract through dialysis with demineralized water [9]. In this current study, the effect of downstream purification method on the quality of pectin in terms of galacturonic acid content was investigated.

2. Materials and Methods

2.1 Materials

Fresh mango peels were collected as by-product from a local mango processing industry. All other chemical reagents used were of analytical grade purchased commercially. The fresh mango peels were washed with water, dried at 60ºC in a solar drying facility, and milled into powder. The mango peel powder (MPP) was extracted with 80% ethanol (1:10 g/mL) at 70ºC for 3 h in an incubator shaker (New Brunswick G25) to remove alcohol-soluble components of peels. The alcohol-insoluble mango peel residue (AIMPR) was separated from the solvent and air-dried overnight to remove excess ethanol.

2.2 Extraction and Purification of Pectin

Three types of pectin were prepared from AIMPR. These were crude pectin, purified pectin and pure pectin. Generally, the AIMPR was mixed with distilled water (1:15 w/v). The pH of the mixture was adjusted to pH 1.5 using dilute aqueous acid, 0.1 N HCl. The mixture was then placed in an incubator shaker (New Brunswick G25) set at 75ºC for 60 min. After extraction, the mixture was filtered to separate solid residues from the extract. The pectin in the extract was precipitated with absolute ethanol at 2:1 v/v ethanol to extract ratio. The mixture was left to stand to allow precipitation of pectin before filtering through a filter cloth. The pectin precipitate (gel-like form) was washed with alcohol, and dried to constant weight.

For crude pectin, the pectin precipitate on the filter cloth was poured or rinsed one time with absolute ethanol (10 mL), and left overnight on the filter cloth to evaporate some of the ethanol. The washed precipitate was still in gel-like form. It was melted over a steaming water bath for 4-5 h and dried in oven at 50ºC to constant weight.

Purified pectin was prepared from crude pectin by applying alcohol post-treatment. Crude pectin was dissolved in distilled water at a concentration of 2 wt.% and re-precipitated with absolute ethanol. The formed precipitate was separated using the filter cloth and pressed to squeeze out liquids. The precipitate was thoroughly washed three times with alcohol by mashing in absolute ethanol, separating using the filter cloth, and pressing to squeeze out liquids. The washed precipitate already looks dry after final washing and was further dried in oven at 50ºC to constant weight.

For pure pectin, the pectin precipitate was separated, thoroughly washed three times with absolute ethanol (10 mL in every washing) and dried as similarly described with purified pectin.

2.3 Quantification and Characterization of Dried Pectin

Pectin yield was determined as gram dried pectin per gram of AIMPR and expressed in percentage. The degree of esterification (DE) and galacturonic acid content (GA) of dried pectin were determined by simple titrimetric method based on literature [13]. Molecular weight distribution profile of dried pectin was determined using high temperature gel permeation chromatograph (HT-GPC, HLC8121GPC/HT, TOSOH). Dried pectin was dissolved in 0.1M $NaNO_3$ solution at a concentration of 0.3 wt.%. An aliquot (3-5 mL) of the prepared pectin solution was placed in the sampling tube for the GPC analysis. The HT-GPC was equipped with TSKgel $G400PW_{XL}$+$G6000PW_{XL}$ column and refractive index detector. A 0.1M $NaNO_3$ solution was used as carrier solvent flowing at 500 µL/min. The pectin solution was analyzed at 80°C for 25 minutes. The molecular weights were determined over a pullulan standard. Mathematica software was used to convert the retention time vs. detector signal graph into molecular weight distribution profile.

Purified pectin was characterization by NMR Spectroscopy. The dried pectin was prepared into 0.5 wt.% solution in D_2O and added with internal standard 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) solution (0.2 mg DSS/mL D_2O) at 0.1 mL/mL-pectin solution. The ^1H NMR spectra of the pectin solution were acquired on a Bruker Avance 600 NMR spectrometer operating at 600.23 MHz and 353.2 K. The spectra were accumulated with a 30° pulse, an acquisition time of 1.36 s, a relaxation delay of 1 s, 1 scan and a spectral width of 12019.230 Hz, resulting in 32K complex data points.

Mango pectin sample was prepared into gel by dissolving the extracted dried pectin (0.05 g) in distilled water (10.00 g), adding with sucrose (15 g), dissolving through stirring and heating, adding citric acid (0.05 g), continuously stirring and heating for 1 minute and letting cool and set at room temperature.

3. Results and Discussions

3.1 Crude Mango Pectin

The crude and pure pectin extracted from the mango peels are both classified as HM-pectin having degree of esterification of greater than 50% as presented in Table 1. High amount of pectin can be obtained from the crude preparation, however its galacturonic acid content is very low (Table 1). The pectin yield value for the crude pectin is more than twice of what has been reported by others [4,10]. This high pectin yield value and low galacturonic acid content of crude pectin indicates presence of components other than pectin.

Table 1 Yield, Degree of Esterification and Galacturonic Acid Content of Mango Pectin

Sample Code	[%] Yield	[%] DE	[%] GA
Crude	63.40±8.62	67.42±2.66	27.11±3.50
Pure	8.72±0.20	71.30±0.96	90.30±0.64

**values are based on the average±stdev of three separate mango pectin preparation*

3.2 Purification of Mango Pectin

Downstream treatment of extracted pectin, that includes alcohol washing, affects pectin quality. Crude preparation of pectin from mango peels does not include thorough alcohol washing of the precipitated pectin from the extract solution. The dried crude pectin has dark brown color and hard brittle structure like a dry solid caramel, as shown in Fig. 1. The molecular weight distribution profile of extracted crude mango pectin, as illustrated in Fig. 2, includes a high molecular weight fraction ($\geq 10^4$ Da) and low molecular weight fraction ($<10^4$ Da). The high molecular weight fraction represents the pectin molecule while the low molecular weight fraction represents the impurities. Existence of

impurities in mango pectin has been reported by others. Reference [12] ascribed the small molecules in mango pectin, having molecular weight (Mw) of ≤650 to ash, while in [16] the small molecules (Mw<250) were ascribed to salts and/or mono- and disaccharides being mainly of sucrose. Reference [3] reported the co-extraction and existence of sugar and polyphenols on mango pectin. Polyphenols from mango peels have molecular weight of around 1000 Da or less as reported by others [17].

Fig. 1. Photographic appearance of crude and purified pectin

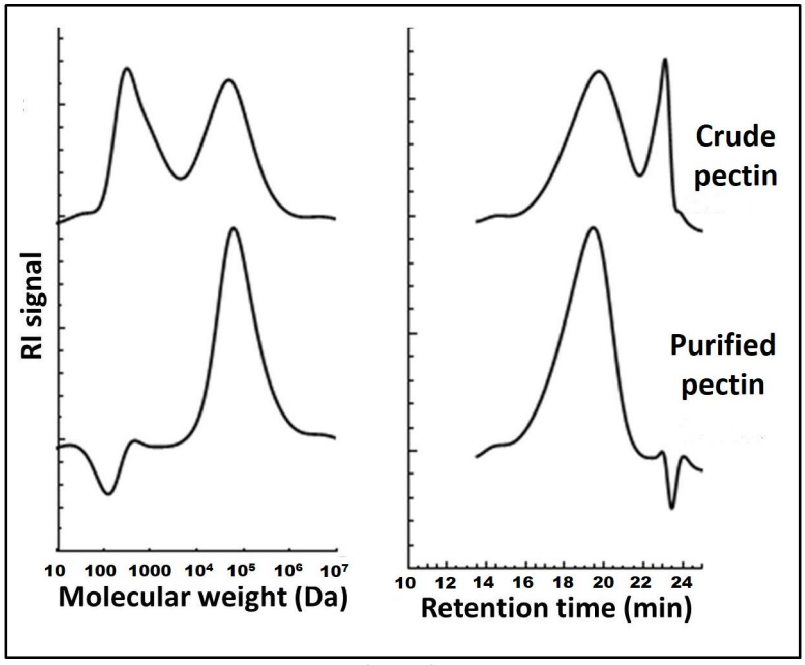

Fig. 2. Gel permeation chromatograms (right) and molecular weight distributions (left)

The impurities or low molecular weight fraction in crude mango pectin were removed by thorough washing with absolute ethanol as illustrated by the reduction of its peak signal in the gel permeation chromatogram and molecular weight distribution profile of the purified pectin (Fig. 2). Proper alcohol washing of the extracted mango pectin that includes mashing (i.e. breaking up lumps) in absolute ethanol and pressing to squeeze out solvent is necessary to remove the impurities. The dried purified pectin had lighter brown color and fluffy cottony structure as shown in Fig. 1. Mango peels contain

soluble sugar and other small molecular weight components being co-extracted with pectin [3,12,18]. Pectin extracted from mango peels are HM-pectin which gels in the presence of soluble solids and acid [3,9-13]. The pectin extract solution of the mango peel waste has the components necessary for an HM-pectin to gel. Thus, the pectin precipitate in gel-like form still contains water and co-extracted solutes. This co-extracted solute promotes pectin gelation by bonding with water [19]. Pectin are insoluble with alcohol and soluble with water. Washing the precipitate with absolute ethanol dehydrates the gel, breaks the gel network and easily washed off the co-extracted solutes. Mashing the precipitate in ethanol is also very important to break the pectin gel that easily lumps or hardens with highly pure alcohol. Thus, the pectin being thoroughly washed with highly pure alcohol has cottony texture and almost look dry right after alcohol washing.

The ^{1}H NMR spectrum of the purified pectin, as illustrated in Fig. 3, shows signals for the five protons, H-1, H-2, H-3, H-4 and H-5, of galacturonic acid unit being assigned according to spectral data reported by others [20-21]. The spectrum represents a homogalacturonan structure containing only of galacturonic acid as similarly described in [21]. The NMR result shows that the purified pectin contains high amount of galacturonic acid. Downstream purification of pectin extracted from industrial mango peel waste should include mashing with highly pure alcohol to obtain mango pectin of high galacturonic acid content.

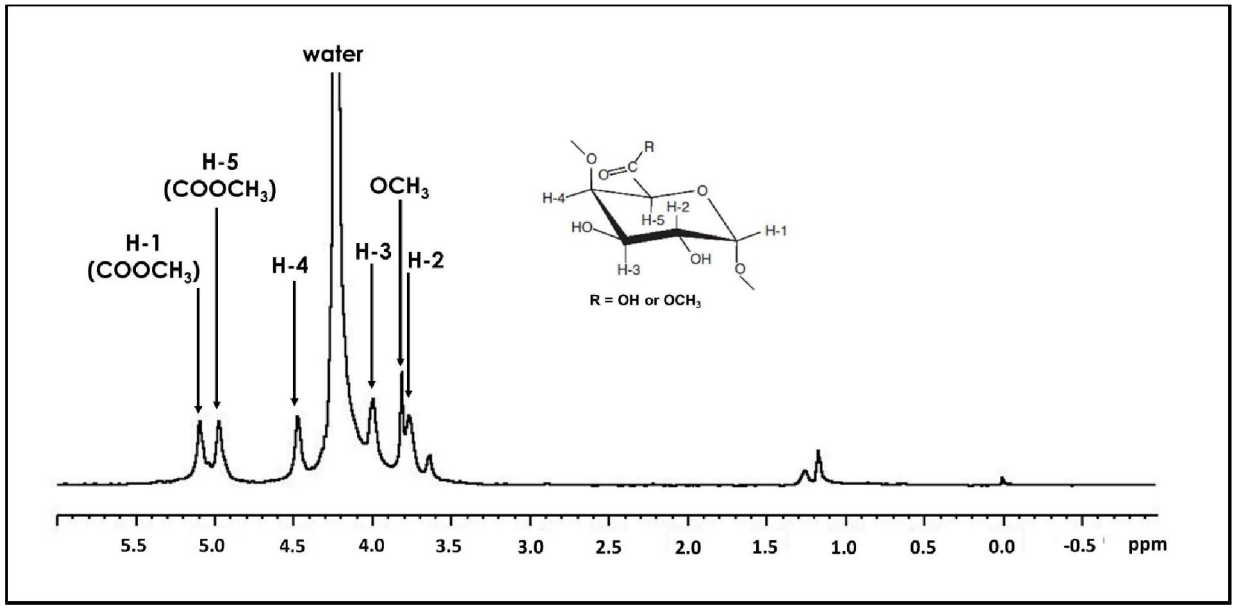

Fig. 3. ^{1}H NMR spectrum of mango pectin in D_2O at 80^{o}C

3.3 Pure Mango Pectin

Highly pure pectin can be obtained from industrial mango peel waste by incorporating thorough alcohol washing on its production process. The yield is lower compared to crude pectin but its galacturonic acid content is much higher as presented in Table 1. This pure pectin was able to form a gel in the presence of sugar and acid at gel composition of 0.2 wt.% mango pectin, 0.2 wt.% citric acid, 60 wt.% sucrose and the rest being water as shown in Fig. 4. These results indicate that mango pectin can be a good alternative source for commercial pectin such as those used for jam or jelly preparation.

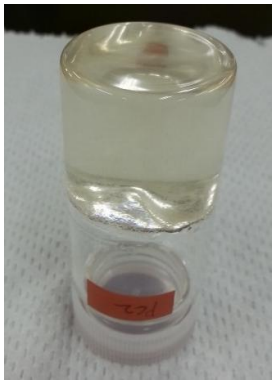

Fig. 4. Gel formed from pure mango pectin

4. Conclusion

HM-pectin can be obtained from industrial waste mango peels through typical hot acidified water extraction and alcohol precipitation. Even without employing additional process in removing co-extracted solutes prior to alcohol precipitation, pectin of high galacturonic acid content was obtained through incorporation of thorough alcohol washing in the downstream purification process. Thorough alcohol washing should include mashing the pectin precipitate with highly pure ethanol and pressing to squeeze out liquids.

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