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Contributed Paper

Extraction and Physicochemical Characteristics of Acid-Soluble Pectin from Raw Papaya *(Carica papaya)* Peel

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ABSTRACT

Extraction of pectin from raw papaya peel, a waste from restaurants and food processing industries, was carried out using ethanol and aluminium chloride precipitations. The optimized procedures yielding 2.23% and 5.84% on the papaya fresh weight basis, respectively, were presented. Even though aluminium precipitated pectin had higher ash and moisture contents (22.19 and 14.86% compared to 5.22 and 9.72% of ethanol precipitated pectin), it contained much higher galacturonic acid (72.43% compared to 48.39%). Papaya pectins from ethanol and aluminium chloride precipitation have degrees of esterification of 46 and 51%, respectively, as accessed by FT-IR. The viscosities of 1% pectin samples were affected by pH and heating.

Keywords: *Carica papaya*, characteristic, extraction, papaya, pectin.

1. INTRODUCTION

Pectin, a polysaccharide composing of D-galacturonic acids linked with β-1,4 glycosidic bonds, is found in middle lamella of plant cell membrane. Some of its galacturonide units are esterified as methyl galacturonate, whose extent is reported as degree of esterification (DE) or methyl esterification degree (MED) [1]. One of the most important properties of pectin is gelling. The high-methoxyl pectin (DE > 50%) can form gel in a condition having acid and sugar. While the low-methoxyl pectin (DE < 50%) can form gel as a complex with calcium ion. Its gelling property makes pectin be of use in food industries e.g. jam, jelly, emulsion stabilizer and thickener in sauces. Pectin can also be used in medicine as a fiber source, a supplement to lower cholesterol and increase immunity or as dentistry adhesive [1, 2].

Pectin can be extracted from fruit using acid (HCl or HNO_3) or chelating agents (EDTA, ammonium oxalate or sodium hexametaphosphate). Acid extraction seems to be the most widely used method. Commercial pectin is usually isolated from citrus peel or apple pomace. During Jan – Mar 2005, pectin substances were imported to Thailand for a CIF value of 28.7 million Baht [http://www.customs.go.th/Statistic/]. It will be of economical and environmental values if pectin can be produced locally from un-used part of vegetables or fruits. Papaya *(Carica papaya*) peel is one of the best choices since it is enormous waste from restaurants and food processing industries in Thailand. There are many procedures reported for extracting pectin papaya. But they are either not widely distributed or out-of-dated references [3, 4] or complicated protocols [5, 6]. Here we reported the two easy-to-follow methods to productively isolate pectin from unripe papaya peel and some physicochemical properties.

2. MATERIALS AND METHODS

2.1 Materials

Raw papaya peel was obtained as a waste from local restaurants selling papaya salad. Commercial citrus pectin was purchased from Sigma (USA.) in order to compare its physicochemical properties with papaya pectins.

2.2 Extraction of Pectin (modified from [3])

Raw papaya peel was cut into pieces and then minced in a blender. The extraction of pectin was done in boiling water bath using different concentrations of HCl : minced papaya peel at 2 : 1 volume ratio once, followed by 1.5 : 1 volume ratio twice for 30 min. each. The obtained solutions were centrifuged at 4,500xg for 30 min. The experiments were carried out in triplicates.

2.3 Precipitation of Pectin

2.3.1 Ethanol Precipitation (modified from [7])

The pectin solutions previously adjusted to pH 4 –4.5 with 10 M NaOH were precipitated with an equal volume of either 95% ethanol or 95% ethanol in 0.01 M HCl at various time lengths. After filtration, the ethanol insoluble pectin was washed with 70% ethanol and oven-dried at 37°C.

2.3.2 Aluminium chloride precipitation (modified from [3])

Alternatively, the pectin solutions previously adjusted to a specified pH were precipitated with varying concentrations of aluminium chloride, pH 4.0 for different time lengths. After filtration, the aluminium chloride insoluble pectin was washed with 70% ethanol and oven-dried at 37°C.

2.4 Physicochemical Analysis of Pectin

Papaya peel pectin from the most efficient extraction conditions of the two methods and commercial citrus pectin were physicochemically characterized as follows:

2.4.1 Moisture

A moisture content was determined by drying a pectin sample at 100°C to constant weight.

2.4.2 Ash

An ash content was estimated by incinerating a pectin sample in a muffle furnace at 600°C for 4 hr.

2.4.3 Galacturonic Acid Analysis

Total galacturonic acid released after acid hydrolysis of a pectin sample was determined colorimetrically using a modified *m*-hydroxydiphenyl sulfuric acid method [8]. The colored forming product was measured by spectrophotometer 4001/4 using D-galacturonic acid (Fluka, Slovakia) as a standard.

2.4.4 Degree of Esterification

Degree of esterification was accessed with FT-IR spectrophotometry (Nicolet FT-IR 510) using polygalacturonic acid (DE = 0 %), pectin with DE of 30, 45 and 60% as standards [6]. Polygalacturonic acid and pectin standards were purchased from Sigma (USA.), except the one with 45% which was prepared by mixing appropriate quantity of pectins of known DE. Degree of esterification of pectin samples were estimated from standard curve between %DE and ratio between absorbance intensity of 1740 cm^{-1} and the sum of those corresponding to 1740 and 1630 cm^{-1}.

2.4.5 Viscosity

Viscosity of pectin solution was measured by viscometer (Brookfield DV-III Rheometer) with spindle number 21, speed number 25 at shear rate 25 -225 s^{-1}. Pectin samples (1% w/v) were prepared in 0.5 M sodium citrate buffer, pH 1-5 and 0.5 M sodium phosphate buffer, pH 6-7 and were subsequently hydrated at 4°C overnight before measuring viscosity (mPa.s) at 25°C. The viscosities were also taken on another set of pectin samples which had been heated at 100°C for 2 min [9, 10].

3. Results and Discussion

3.1 Optimization for Pectin Extraction

Many variables in pectin extraction steps were optimized. Acid extraction of raw papaya peel with 2 volumes (in the first extraction) and 1.5 volumes (in latter two extractions) of hot hydrochloric acid of different concentrations of 0.02, 0.06 and 0.1 M for three times, followed by either precipitation with 95% ethanol or 0.8% aluminium chloride, pH 4.0, for 1 hr yielded pectin varying from 1.94-3.75 % on fresh weight of papaya peel basis (Table 1). The values of each replicate especially in ethanol precipitation case were rather deviated due to differences in papaya peel sources. But pectin yields compared among different HCl concentrations of the same replicate still followed the same trend. The highest yields (2.46 and 3.75%) of pectin from the acid extraction followed by both precipitation methods were obtained when the papaya peel was treated with 0.06 M HCl.

Table 1. Pectin yields from ethanol and aluminium chloride precipitation methods at various concentrations of hydrochloric acid.

Concentration of HCl (M)	Pectin yield (% of fresh peel weight)							
	Ethanol precipitate [a]				$AlCl_3$ precipitate [b]			
	1	2	3	Avg.	1	2	3	Avg.
0.02	1.1940	1.5749	3.0546	1.9412	2.9230	2.8173	2.7788	2.8397
0.06	2.0986	1.8537	3.4256	2.4593	3.7040	3.7692	3.7817	3.7516
0.10	2.1279	1.7735	3.0323	2.3112	3.4320	3.3942	3.2822	3.3694

[a] pH-unadjusted solutions were precipitated with 95% ethanol for 1 hr.
[b] Solutions adjusted to pH 3.5 were precipitated with 0.8% aluminium chloride for 1 hr.

3.2 Optimization for Pectin Precipitation

Pectin solution extracted by hydrochloric acid was then proceeded into two routes: for ethanol precipitation and aluminium precipitation (Figure 1). For Route 1, pectin solution adjusted to pH 4 -4.5 (since unadjusted solution of which pH was 1.58 had lower yield) was precipitated with 95% ethanol in the presence or absence of HCl in a final concentration of 0.01 M. The presence of 0.01 M HCl resulted in lower yield (1.47% compared to 1.94%). In previous reports both conditions were done. Some used ethanol alone [11, 12], while some used ethanol containing 0.01 M HCl [7]. The precipitation time had an influence on pectin yield in that two hours of ethanol precipitation resulted in the maximum yield compared to 0, 1 and 3 hours (Table 2).

For Route 2, aluminium precipitation of pectin solutions adjusted to pH 2.5, 3.0, 3.5, 4.0 and 4.5 precipitated with 0.4, 0.8, 1.2, 1.6 and 2.0% aluminium chloride, pH 4.0 for 0, 1, 2 and 3 hr (Table 2) yielded pectin ranging from 3.11 - 5.84 %. The highest yield (5.84%) of pectin by aluminium chloride precipitation was obtained when pectin solution previously adjusted to pH 3.5 was precipitated with 1.2% aluminium chloride, pH 4.0 for 2 hr. The pectin yield (0.45-0.71%) extracted by similar methods from whole raw papaya previously reported was lower than our data presented here [3], indicating that higher pectin presented in peel than in flesh.

The final extraction procedures selected on the basis of pectin yield were shown in Figure 1. These outlined procedures gave pectin yield of 2.23% and 5.84% from ethanol and aluminium chloride precipitation methods, respectively.

3.3 Characteristics of Acid-soluble Pectin

The obtained pectins from both methods were characterized as shown in Figure 2. It was found that ash content of pectin from aluminium precipitation was notably higher than that from ethanol precipitation method. Form of pectin precipitated from aluminium

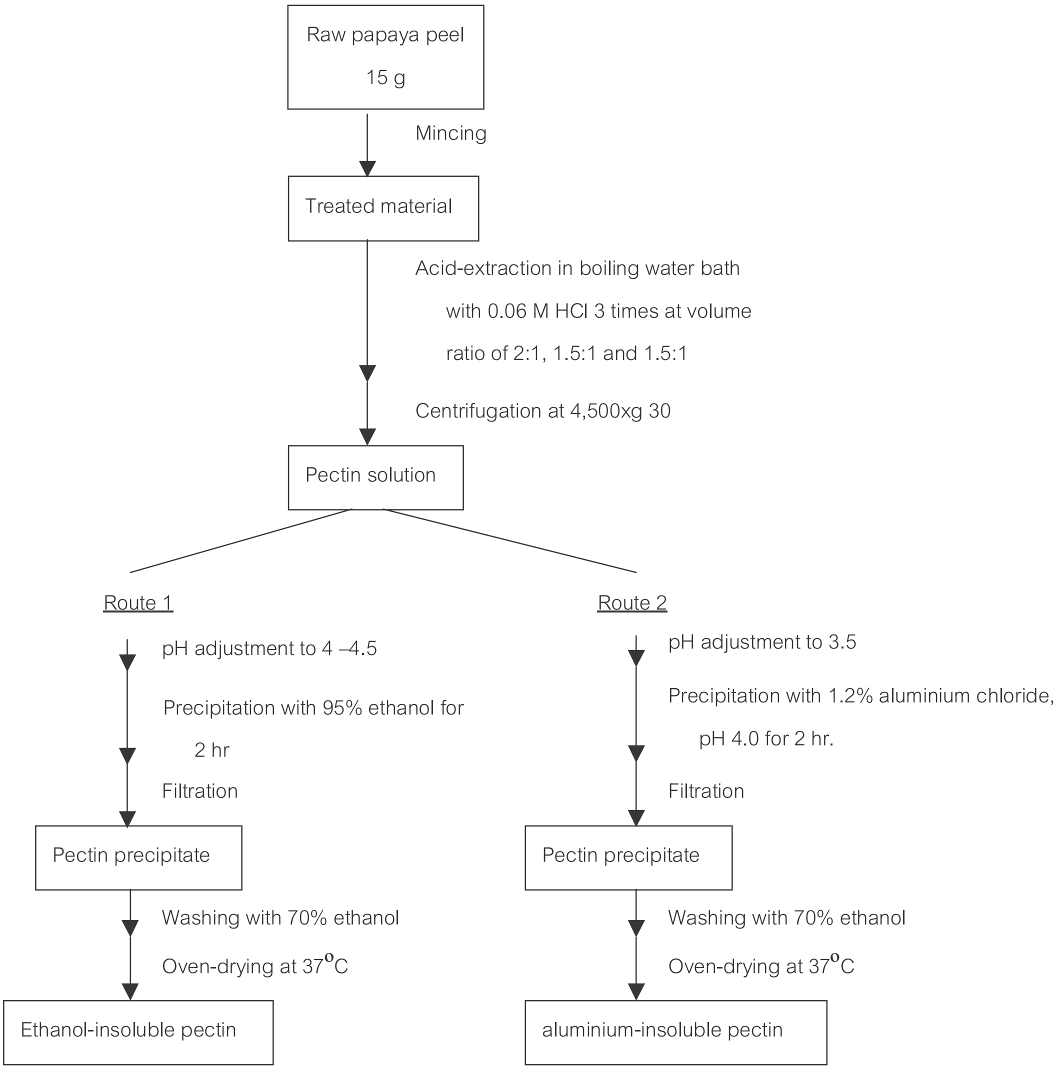

Figure 1. Optimized papaya peel pectin preparation steps by ethanol (Route 1) and aluminium chloride precipitation (Route 2) methods.

Table 2. Pectin yields from ethanol and aluminium chloride precipitation methods at various precipitation time lengths.

Precipitation time (hr.)	Pectin yield (% of fresh peel weight)							
	Ethanol precipitate [a]				$AlCl_3$ precipitate [b]			
	1	2	3	Avg.	1	2	3	Avg.
0 [c]	0.9167	1.0700	1.0000	0.9956	5.2500	6.2310	5.0980	5.5263
1	1.2885	0.9936	0.9167	1.0663	5.5997	4.8449	6.2370	5.5605
2	1.9295	2.1667	2.0705	2.0556	5.1696	5.9296	6.4320	5.8437
3	1.7051	1.8397	1.7692	1.7713	4.6245	4.9270	6.9050	5.5005

[a] pH-unadjusted solutions were precipitated with 95% ethanol.
[b] Solutions adjusted to pH 3.5 were precipitated with 1.2% aluminium chloride.
[c] The solutions were immediately filtered after addition of precipitants.

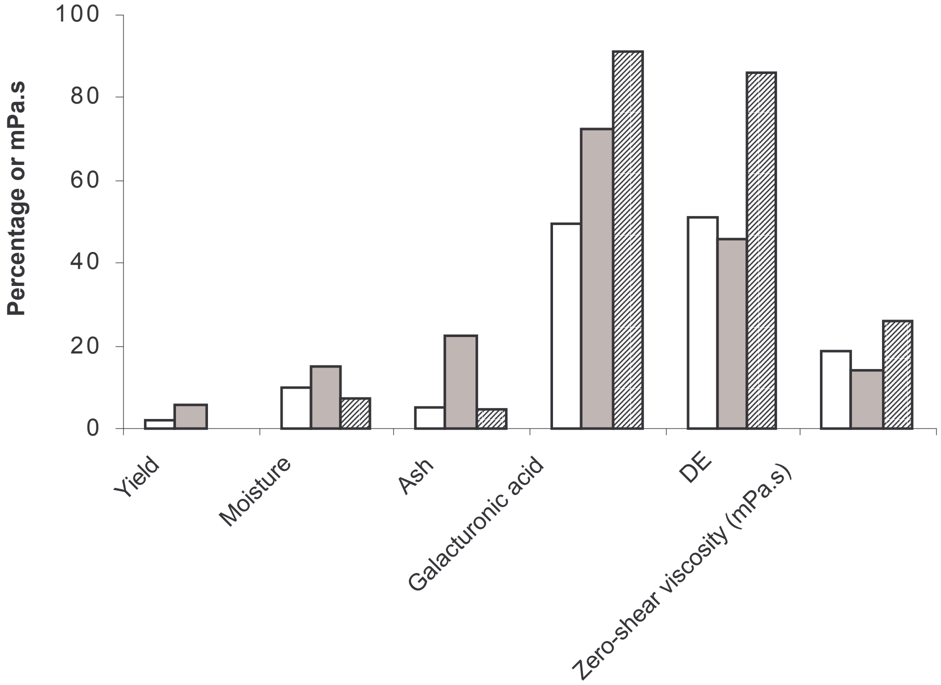

Figure 2. Properties of pectin from papaya peel prepared with ethanol (white bars) and aluminium chloride (gray bars) precipitation methods and commercial pectin from citrus (stripped bars).

precipitation method is as aluminium pectinate, while that from ethanol precipitation method has hydrogen as a counter ion. Since low ash content is more favorable for gel formation, ash content should be reduced, for example by acidified alcohol [3, 9]. Alternatively, dialysis step can be added before drying the pectin precipitate. Even though pectin from Route 2 had higher ash and moisture contents, it contained much higher galacturonic acid constituents (72.43%) than the pectin from Route 1 (48.39%). It was reported that galacturonic acid content is maximum at green stage of papaya fruits [13]. Biswas and Rao [4] reported that unripe papaya fruit contained 55.3% D-galacturonic acid.

Pectin samples and standard pectins of known methoxylation degrees were subjected to FT-IR analysis (an example of spectrums shown in Figure 3). While commercial citrus pectin is high-methoxyl pectin with a DE of 86%, papaya pectins from both methods have %DE value in a boarder line (51 and 46%) between low- and high-methoxyl pectins, similar to the 47-50% value reported previously for water soluble pectin at green stage of papaya whole fruit [6] and 50% value reported for water soluble pectin of ripe papapa fruit pulp [5]. Degree of esterification of papaya pectin can be different depending on ripeness, part of fruit, botanical origin and isolation method.

Viscosities of 1% pectin samples dissolved in buffer of various pHs were measured as a function of shear rate (data not shown). In all cases, viscosity was almost independent of shear rate similar to the result reported for apple pectin [10]. Zero-shear viscosities were then extrapolated from the flow curves. Such numbers of pectin solutions at pH 1 are shown graphically in Figure 2. Papaya pectins prepared by both methods had lower viscosities than citrus pectin. pH had a significant effect on viscosity of papaya pectins in that their viscosities decreased as pH increased from 1 to 5. Heating, however, had

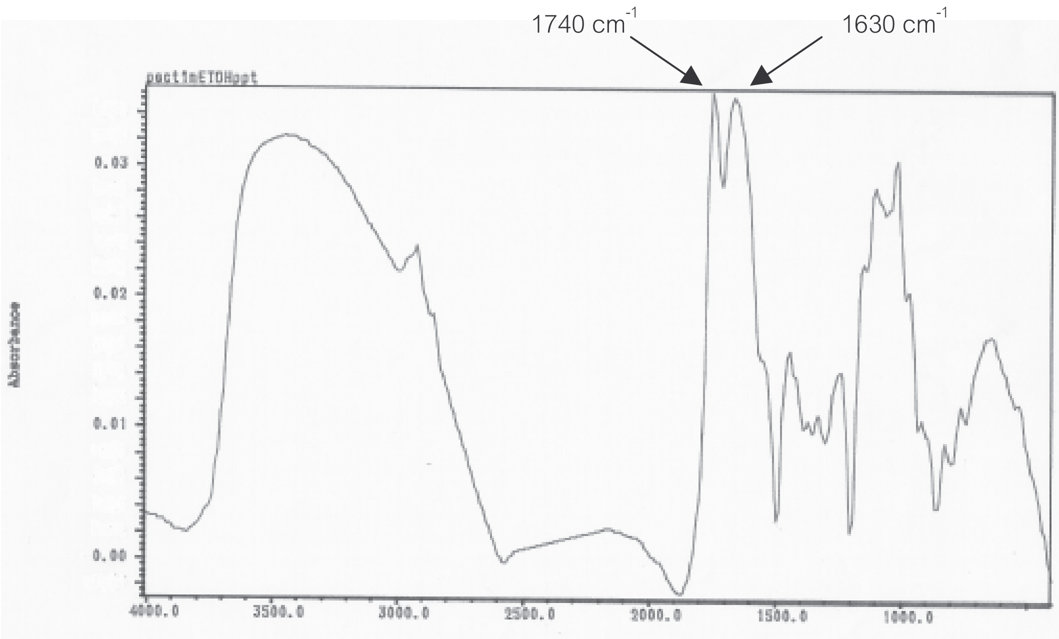

Figure 3. FT-IR spectrum of ethanol precipitated pectin from raw papaya peel.

different effects on pectins. At pH range tested, viscosities of papaya pectin from aluminium precipitation method and citrus pectin increased upon heating, while that of papaya ethanol precipitated pectin either decreased or relatively unchanged. An example of such result at pH 1 is shown in Figure 4. Miyamoto and Chang [9] reported that heating slightly increased viscosity of sunflower pectin. Papaya pectin having viscosities varying with pH and temperature can be used as a gelling agent in appropriate food products.

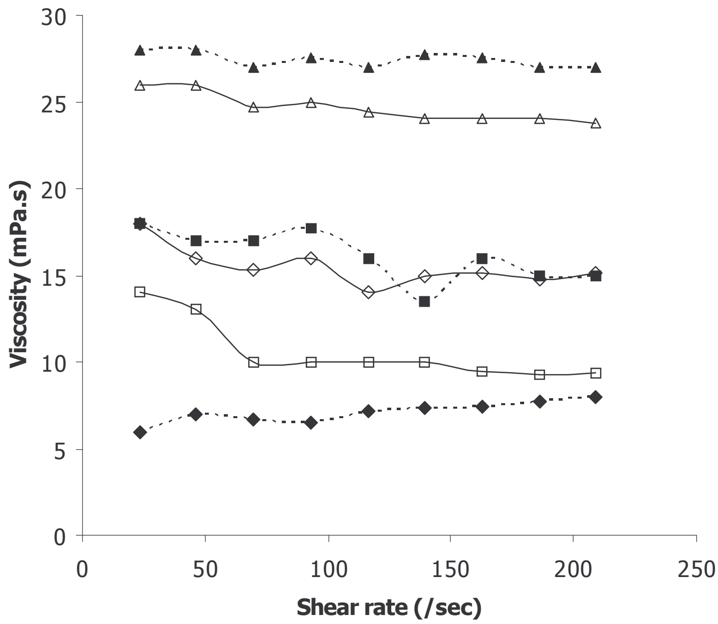

Figure 4. Viscosity of 1% w/v ethanol precipitated pectin (◇,◆), aluminium precipitated pectin (□,■) and commercial citrus pectin (△,▲) at pH 1.0 with (solid symbols, dashed lines) and without (open symbols, solid lines) heat treatment.

4. Conclusions

Pectin could be prepared from raw papaya peel by ethanol and aluminium chloride precipitation methods with yields of 2.23 and 5.84% on a basis of peel fresh weight, respectively. The papaya pectins had 51 and 46% methoxyl ester contents and also contained high amounts of galacturonoic acids, especially aluminium precipitated pectin. The pectin had a highest viscosity at pH 1. Pectin from papaya peel, waste from restaurants and food processing factories, has a high potential for commercial food applications. Thus waste disposal problem can be alleviated.

Acknowledgements

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