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Extraction and characterization of pectin from selected indigenous fruits for further commercial application in food industries

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Abstract - The aims of this study were to extract pectin from selected indigenous fruits, including breadfruit, papaya and santol as well as investigate the chemical and physical properties of extracted pectin to address the potential of these fruits as an alternative source of commercial pectin. Different parts of fruits were used to extract pectin under the acid condition method**.** Pectin yield ranged from 8.52±0.05 to 49.96±0.89% and breadfruit was the highest pectin source. The extracted pectin from breadfruit was categorized as high methoxyl pectin with the degree of esterification between 93.57±0.22 to 96.70±0.17%, which was almost 2-time higher than the commercial pectin (53.10±1.25%). The galacturonic acid contents found in the extracted pectin varied widely from 41.41±0.09 to 73.19±0.59%. The significantly highest galacturonic acid content was found in the pectin extracted from the peel of breadfruit, which strongly indicated that pectin extracted from the peel of breadfruit was good quality. The FT-IR spectra also confirmed that the pectin obtained in this study was overall similar to the commercial pectin. Therefore, the breadfruit peel was highly recommended as a potential alternative source of pectin.

Keywords: Pectin, breadfruit (*Artocarpus altilis* L.), papaya (*Carica papaya* L.), santol (*Sandoricum indicum* Cav.), indigenous fruit, acid extraction

1. Introduction

Pectin is a complex polysaccharide that is found in the cell walls of fruits and vegetables. The chemical structure of pectin is composed of a linear chain of galacturonic acid units that are linked together by α-1, 4 glycosidic bonds (Mesbahi *et al.*, 2005). These galacturonic acid residues are esterified with methanol groups, giving pectin its unique properties. It is widely used as a stabilizing, thickening, and gelling agent in food and beverage applications, as well as in pharmaceuticals and cosmetics because of its capacity to form an aqueous gel (Kozioł *et al.*, 2022). Pectin has many benefits, including its ability to improve the texture and stability of food products, and its ability to act as a fat substitute. It also has prebiotic properties, which means it can help promote the growth of beneficial bacteria in the gut. Additionally, pectin has been shown to have cholesterol-lowering properties (Kalapathy & Proctor, 2001).

The global demand for pectin is expected to grow, driven by the increase in demand for functional food and natural ingredients. The commercial sources of pectin are apple pomaces, lemons and oranges (Kozioł *et al.*, 2022). In this regard, many researchers have investigated to explore new pectin sources due to the high demand for pectin in the global market. The alternative pectin sources that can be used such as banana (Castillo-Israel *et al.*, 2015) (Khamsucharit *et al.*, 2018), dragon fruit (Ismail *et al.*, 2012), jackfruit (Ahmmed *et al.*, 2017), lemon pomaces (Azad *et al.*, 2014), cocoa hull (Mollea *et al.*, 2008), and soy hull (Kalapathy & Proctor, 2001). These sources can be used as an alternative to produce high-quality pectin for food production. In Thailand, many local fruits are available, for example breadfruit, papaya and santol. These fruits are commonly consumed with low price and they are very popular to be processed to produce many types of foods and desserts. In this regard, these indigenous fruits could be value-added products as well as a good source of pectin. Additionally, these alternatives may even be more sustainable and cost-effective than traditional pectin.

Therefore, the main objectives of this study were to extract and characterize the pectin from indigenous fruits in Thailand, including breadfruit, papaya and santol. Different parts of these fruits, including peel, pulp and abortive flower were used for pectin extraction and the pectin extracts were characterized for their chemical and physical properties compared to the commercial pectin. The knowledge gained from this study would explore novel and potential pectin sources for further use in the food industries.

2. Materials and methods

2.1 Raw material preparation

Fresh fruits, including breadfruit, papaya and santol were separated into different parts (peel, pulp and abortive flower): breadfruit's peel (Bp), breadfruit's pulp (Bpu), breadfruit's abortive flower (Baf), papaya's peel (Pp), papaya's pulp (Ppu), santol's peel (Sp), and santol's pulp (Spu). All samples were three-time washed, cut into small pieces, and then dried in the hot air oven at 60°C for 48 hours. Then, prepared samples were milled, sieved through sieving size of 80 meshes, and packed in airtight, moisture-proof bags at room temperature for further extraction process. Commercial

pectin (CP) (McGarrett, Thailand) was used as a reference in this study

2.2 Pectin extraction

All samples were extracted with 0.05 M hydrochloric acid in the ratio of 1:12 (sample: extraction solvent) in a shaker water bath at 90°C for 60 min. Samples were filtrated through a white cloth sheet and the extracts were collected. Each sample was extracted twice under the same condition. The extracts from the same samples were pooled. Pectin was precipitated by ethanol for 12 hours at room temperature. Pectin was obtained after ethanol removal at 65°C. Extracted pectin was ground into powder and measured for extraction yield (%).

2.3 Determination of ash content, moisture content and water activity

Ash content and moisture content were measured according to AOAC method (AOAC, 2000). Water activity was measured in triplicates per sample using the water activity meter (LabStart-AW, Novasina AG., Lachen, Switzerland). The color parameters of the pectin samples were determined using a Hunter Lab colorimeter by measuring L* (lightness), a* (redness) and b* (yellowness) values in the CIE system (Khamsucharit *et al.*, 2018).

2.4 Determination of galacturonic acid content

The galacturonic acid of pectin samples was measured according to the methods explained by Mesbahi *et al*. (2005) (Mesbahi *et al.*, 2005)

2.5 Determination of methoxyl content

Methoxyl content was analyzed according to the method described by Ranganna (1986) with small modifications (Ranganna, 1986). Briefly, 0.5 g of pectin sample was mixed with 25 mL of 0.25 N sodium hydroxide. The mixture was stirred and allowed to stand for 30 min at room temperature. Then, 25 mL of 0.25 N hydrochloric acid was added and titrated against 0.1 N sodium hydroxide. The percentage of methoxy content is calculated using the equation below:

$$\text{Methoxyl content (\%)} = \frac{\text{ml of NaOH x Normality of Naoh x 31 x 100}}{\text{weight of sample (mg) x 100}}$$

2.6 Determination of degree of esterification

The degree of esterification (DE) was analyzed, following the method described by Azad *et al.* (2014) and Castillo-Israel (2015) with small modifications (Azad *et al.*, 2014) (Castillo-Israel *et al.*, 2015). Briefly, 50 mg of pectin sample was added into 100 mL of deionized water. Then 2 mL of ethanol was added and stirred until completely dissolved. Five drops of phenolphthalein were added as an indicator before titrating against 0.1 M sodium hydroxide. The consumption was recorded as the initial titer (V_1). Ten milliliters of 0.5 M sodium hydroxide were then added to the sample and the sample was set at 30°C for 15 min. The sample was added with 10

mL of 0.5 M hydrochloric acid and then shaken until the pink color disappeared. Five drops of phenolphthalein were added as an indicator before titrating against 0.1 M sodium hydroxide. This volume titration was recorded as the saponification titer (V_2). The DE is calculated from the following equation:

$$DE\ (\%) = \frac{V_2 \times 100}{(V_1 + V_2)}$$

2.7 FT-IR spectroscopic method

FT-IR spectra of samples were obtained using a Fourier transform-infrared (FT-IR) spectrometer (Perkin-Elmer, Model Spectra Fourier and Spotlight 200i). Commercial pectin was used as a reference. (Singthong *et al.*, 2004).

2.8 Statistical analysis

All data were expressed as mean ± standard deviation. Data with normal distribution were analyzed using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) with a significance level of α=0.05.

3. Results

3.1 Pectin yield

Table 1 shows the percentage of yield, ash content, moisture content and water activity of pectin, extracted from different parts of breadfruit, papaya and santol. Yields of pectin extracted from different sources were significantly different ($p<0.05$) and varied from 8.52±0.05 to 49.96±0.89%. Breadfruit was more pectin-rich than papaya and salton, therefore it could become a potential source of pectin. The highest pectin yield was obtained from Bpu (49.96±0.89%), followed by Baf (39.57±0.78%) and Bp (35.44±0.47%), respectively whereas the lowest pectin yield was found in Ppu (8.52±0.05%).

The moisture content of all pectin samples ranged from 10.89±0.13 to 16.06±0.12%. Santol both in the pulp and peel contained more moisture content than those of papaya and breadfruit, as well as the commercial pectin. The highest moisture content was found from Spu (16.06±0.12%), followed by Sp (13.00±0.82%), whereas Bp gave the lowest moisture content (10.89±0.13%). In our study, a_w of all pectin samples was quite low, ranging from 0.42±0.01-0.49±0.01, which was comparable to the a_w of commercial pectin, which was 0.49±0.01. The ash content of all pectin samples varied widely in this study, ranging from 0.37±0.04 to 21.27±1.01%. Overall, santol and papaya had higher ash content than breadfruit. The highest ash content was found in Sp (21.27±1.01%), followed by Pp (20.89±1.04%). However, breadfruit peel, pulp and abortive flower contained a lower level of ash about 1.01±0.10, 0.37±0.04, and 0.53±0.04%, respectively. These values were significantly lower than that of commercial pectin (16.94±0.25%).

Table 1. Pectin yield, ash content, moisture content and water activity of commercial pectin and pectin extracted from breadfruit, papaya and santol

Samples	Pectin yield (%)	Moisture content (%)	Water activity	Ash content (%)
Bp	35.44±0.47^{c}	10.89±0.13^{d}	0.45±0.01^{bcd}	1.01±0.10^{d}
Bpu	49.96±0.89^{a}	11.09±0.22^{cd}	0.44±0.02^{bcd}	0.37±0.04^{d}
Baf	39.57±0.78^{b}	10.97±0.17^{cd}	0.42±0.01^{d}	0.53±0.04^{d}
Pp	10.95±0.12^{f}	11.80±0.47^{c}	0.46±0.03^{bc}	20.89±1.04^{a}
Ppu	8.52±0.05^{g}	11.55±0.74^{cd}	0.43±0.01^{cd}	3.56±0.37^{c}
Sp	14.33±0.18^{e}	13.00±0.82^{b}	0.47±0.02^{ab}	21.27±1.01^{a}
Spu	16.46±0.31^{d}	16.06±0.12^{a}	0.49±0.01^{a}	3.82±0.40^{c}
CP	-	11.15±0.08^{cd}	0.49±0.01^{a}	16.94±0.25^{b}

Bp=breadfruit's peel, Bpu=breadfruit's pulp, Baf=breadfruit's abortive flower, Pp=papaya's peel, Ppu=papaya's pulp, Sp=santol's peel, Spu=santol's pulp, CP=commercial pectin
Means with different letters within the same column are significantly different ($p<0.05$).

3.2 Color of pectin

Table 2 shows the color of pectin extracted from different parts of breadfruit, papaya and santol. By using a Hunter Lab, color parameters were measured and expressed as the lightness (L*), the redness (a*), and the yellowness (b*). The L* of pectin samples varied with significant difference ($p<0.05$), ranging from 20.55±0.12 to 85.74±0.02. The a* of pectin samples were significantly different ($p<0.05$) and their a* values ranged from 0.39±0.01 to 8.49±0.16. The b* of pectin samples also showed significantly different ($p<0.05$), ranging from 10.30±0.05 to 25.82±0.19. Results showed that the L* of pectin extracted from breadfruit was significantly greater than pectin extracted from papaya and santol.

Table 2. Color of commercial pectin and pectin extracted from breadfruit, papaya and santol

Samples	Color parameters			Pectin
	L*	a*	b*	
Bp	73.23±0.06^{c}	3.26±0.02^{f}	17.61±0.90^{c}	
Bpu	85.74±0.02^{a}	0.39±0.01^{h}	10.94±1.8^{3}f	

Table 2. Color of commercial pectin and pectin extracted from breadfruit, papaya and santol (cont.)

Samples	Color parameters			Pectin
	L*	a*	b*	
Baf	78.90±0.06[b]	1.35±0.01[g]	16.63±0.0[4]d	
Pp	39.84±0.13[f]	3.60±0.02[e]	10.30±0.0[5]f	
Ppu	55.16±0.54[e]	4.53±0.14[d]	11.56±0.02[e]	
Sp	32.25±0.54[g]	8.49±0.16[a]	21.58±0.27[b]	
Spu	20.55±0.12[h]	5.29±0.04[c]	25.82±0.19[a]	
CP	71.88±0.03[d]	6.32±0.03[b]	10.20±0.28[f]	

Bp=breadfruit's peel, Bpu=breadfruit's pulp, Baf=breadfruit's abortive flower, Pp=papaya's peel, Ppu=papaya's pulp, Sp=santol's peel, Spu=santol's pulp, CP=commercial pectin
Means with different letters within the same column are significantly different ($p<0.05$).

3.3 Chemical characterization of pectin

Table 3 shows chemical characteristics in terms of galacturonic acid content, methoxyl content, and degree of esterification of pectin extracted from different parts of breadfruit, papaya and santol. Overall, the results on the degree of esterification corresponded

to the results of methoxyl content. The degree of esterification was in the range of 23.34±0.22 to 96.70±0.17%, while the commercial pectin was 53.10±1.25%. The pectin extracted from Bp had the highest degree of esterification (96.70±0.17%), followed by those of Bpu (96.23±0.08%) and Baf (93.57±0.22%), respectively. On the other hand, pectin extracted from Spu and Sp were the second lowest degree of esterification with the values of 23.46±0.12 and 23.34±0.22%, respectively. The methoxyl contents from all pectin samples were in the range of 3.80±0.04 to 15.78±0.03%, while the commercial pectin contained 8.67±0.20%. Similarly, to the degree of esterification results, the highest methoxyl content was found in breadfruit. The third highest methoxyl content was Bp (15.78±0.03%), followed by Bpu (15.71±0.02%) and Baf (15.27±0.04%), respectively.

The galacturonic acid content of pectin extracted from different parts of breadfruit, papaya and santol ranging from 41.41±0.09-73.19±0.59% is presented in Table 3. The highest galacturonic acid content was found in Bp (73.19±0.59%), which was significantly higher than the commercial pectin (53.44±0.29%).

Table 3. Chemical characteristic of commercial pectin and pectin extracted from breadfruit, papaya and salton

Samples	Composition of the pectin extract		
	Galacturonic acid content (%)	Degree of esterification (%)	Methoxyl content (%)
Bp	73.19±0.5^{9}a	96.70±0.17^{a}	15.78±0.03^{a}
Bpu	54.47±0.36^{c}	96.23±0.08^{a}	15.71±0.02^{a}
Baf	56.86±0.55^{b}	93.57±0.22^{b}	15.27±0.04^{b}
Pp	42.59±0.48^{g}	33.67±0.59^{e}	5.50±0.10^{e}
Ppu	44.42±0.11^{f}	37.11±0.68^{d}	6.06±0.11^{d}
Sp	41.41±0.0^{9}h	23.34±0.22^{f}	3.80±0.04^{f}
Spu	46.62±0.45^{e}	23.46±0.12^{f}	3.82±0.02^{f}
CP	53.44±0.29^{d}	53.10±1.25^{c}	8.67±0.20^{e}

Bp=breadfruit's peel, Bpu=breadfruit's pulp, Baf=breadfruit's abortive flower, Pp=papaya's peel, Ppu=papaya's pulp, Sp=santol's peel, Spu=santol's pulp, CP=commercial pectin
Means with different letters within the same column are significantly different ($p<0.05$).

3.4 FT-IR spectroscopy

The chemical structures of pectin extracted from different parts of breadfruit, papaya and santol characterized by FT-IR and their spectra are presented in Figure 1-2. Figure 1 and 2 show the similarity of pectin extracted from pulp and peel of breadfruit, papaya, and santol compared to commercial pectin.

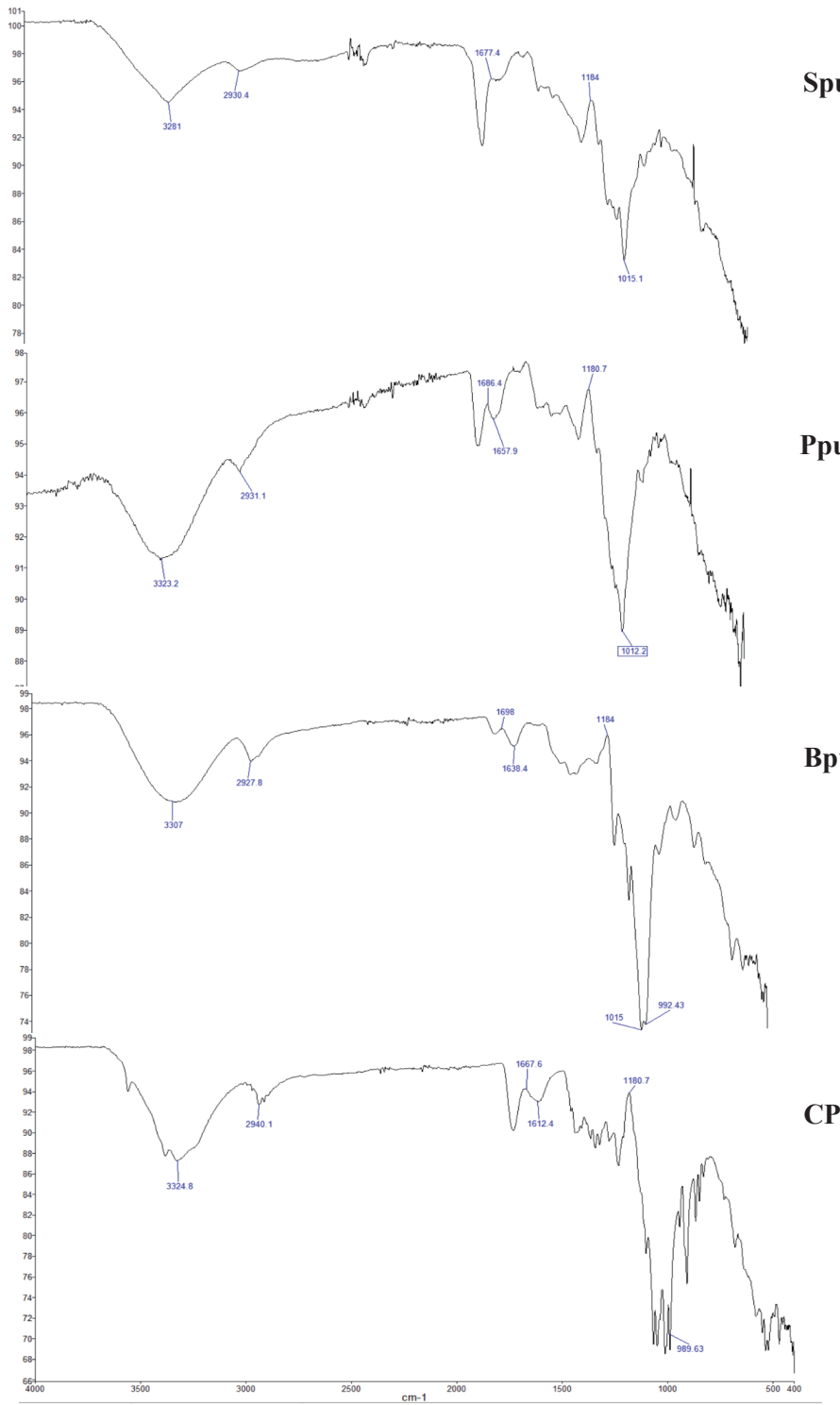

Figure 1. FT-IR spectra of extracted pectin from pulp of indigenous fruits compared to commercial pectin

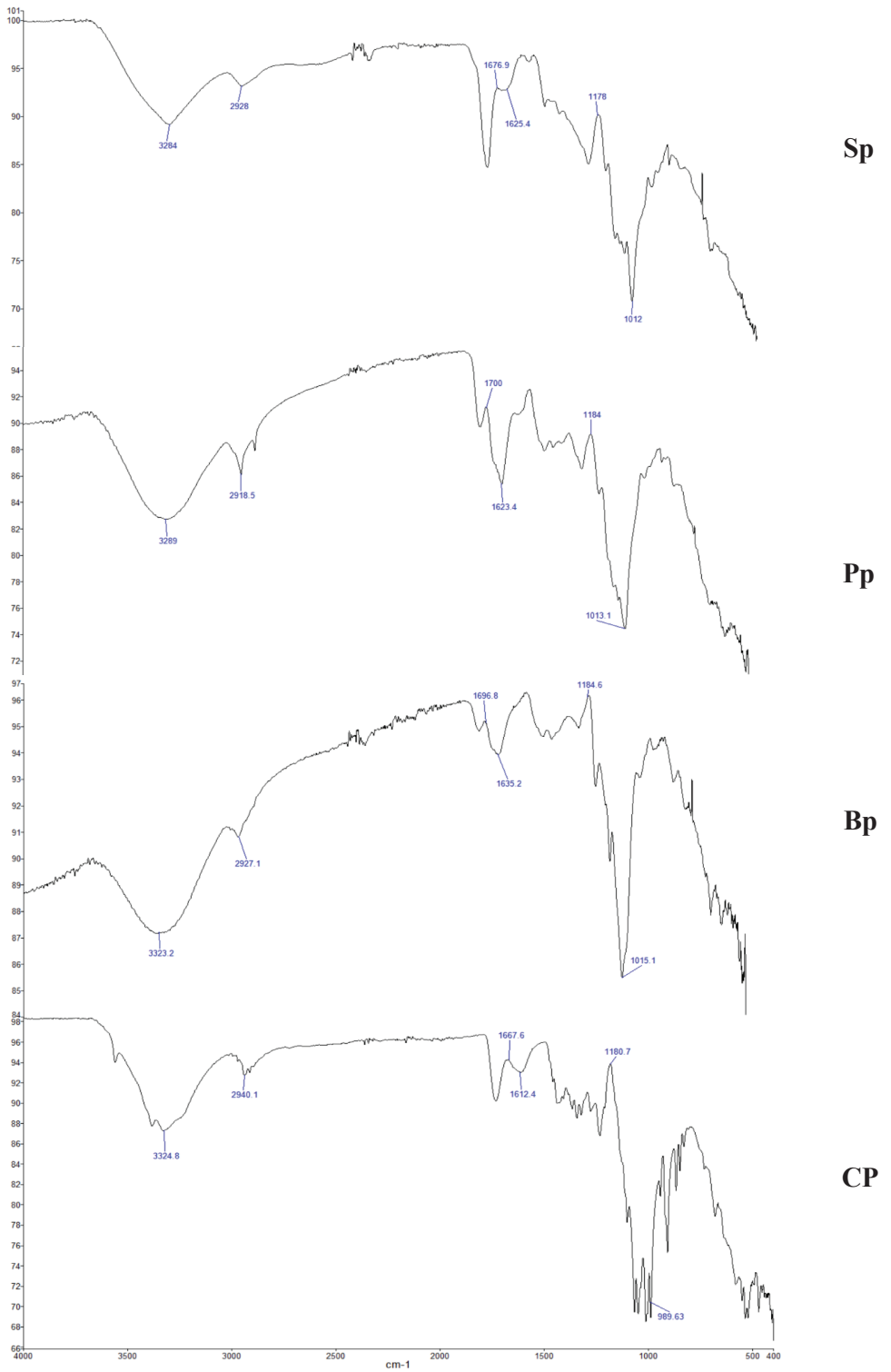

Figure 2. FT-IR spectra of extracted pectin from peel of indigenous fruits compared to commercial pectin

4. Discussion and conclusion

Results on pectin yield indicated that the source and part of the sample used for extraction play a vital role in pectin yield. Breadfruit had significantly higher pectin yield than papaya and santol. Among pulp, peel and abortive flower of breadfruit, the highest pectin yield was found in the pulp. Our results were in agreement with the study from Ji-u and Neamsorn (2022), which indicated that the yield of pectin obtained from the pulp of Namwa banana was significantly higher than pectin obtained from the peel and whole banana (Ji-u & Neamsorn, 2022). Interestingly, pectin yields from breadfruit were greater than the values obtained from many sources of pectin such as citrus peel (19.25±0.04%), apple pomace (10.91±0.02%), several banana peels such as Kluai Khai (16.24±0.13%), Kluai Leb Mu Nang (24.08±0.05%), Kluai Hom Tong (17.31±0.07%), Kluai Nam Wa (15.89±0.02%), and Kluai Hin (19.48±0.08%) (Khamsucharit *et al.*, 2018). This indicated the potential of breadfruit as an alternative source for commercial pectin production.

Consideration on the moisture content of pectin from different sources, it was found that the moisture contents from our extracted pectin were higher than those of banana peels from five different varieties, including Kluai Khai, Kluai Leb Mu Nang, Kluai Hom Thong, Kluai Nam Wa, and Kluai Hin (4.55±0.13-6.51±0.49%) (Khamsucharit *et al.*, 2018). However, it is noted that the moisture content could have been affected by the ripening stage. Azad *et al.* (2014) reported that premature dragon fruit showed the lower moisture content than the over ripen one (Azad *et al.*, 2014). Water activity (aw) is an important consideration in the food industry, as it affects the shelf-life and safety of food products. Water activity indicates the amount of water in the food which can be used by microorganisms. High water activity foods (aw>0.6) are more perishable and have a shorter shelf-life than low water activity foods (aw<0.6). In our study, aw of all pectin samples was quite low, ranging from 0.42-0.49. This result referred that the pectin obtained in this study was safe for storage. Because of their low aw, microorganisms such as bacteria and fungi might not be able to grow and decrease pectin quality.

Ash content of all pectin samples varied widely in this study, ranging from 0.37±0.04 to 21.27±1.01%. The highest ash content was found from Sp (21.27±1.01%), followed by Pp (20.89±1.04%) and commercial pectin (16.94±0.25%), respectively. However, it is important to keep in mind that the quality of pectin decreases as the ash content increases. Since ash content indicates the inorganic mineral residue that remains after a substance is completely burned or incinerated, it could be used to determine the purity of pectin (Ahmmed *et al.*, 2017) (Sikder *et al.*, 2019). Lower ash content in pectin means high purity and is good for gel formation. The maximum limit of ash content for good gel criteria is 10% (Ismail *et al.*, 2012). Therefore, Pp and Sp in this study probably not be good for gelatinization. In other words, all of the pectin samples other than Pp and Sp would possibly perform better gel formation than commercial pectin.

Results on the coloration of pectin showed a strong vivid of variety pectin colors from different sources. Overall, the color of pectin obtained from breadfruit was lighter than those of papaya and santol, as well as commercial pectin. The L* values

of pectin extracted from breadfruit were dramatically greater than pectin extracted from papaya and santol. Conversely, the a* values of pectin extracted from breadfruit were significantly smaller than pectin extracted from papaya and santol. Different color parameters of pectin from various sources might be caused by different color components contained in the raw materials (Khamsucharit *et al.*, 2018). However, the lighter pectin color obtained from breadfruit might be good as a food additive because it could not alter the color of food materials.

The galacturonic acid content, methoxyl content, and degree of esterification of pectin extracted from different parts of breadfruit, papaya and santol were examined. The degree of esterification and methoxyl content of pectin extracted from different sources were significantly different. The pectin extracted from Bp had the highest degree of esterification (96.70±0.17%), followed by those of Bpu (96.23±0.08%) and Baf (93.57±0.22%), respectively. On the other hand, pectin extracted from Spu and Sp were the second lowest degree of esterification with the values of 23.46±0.12 and 23.34±0.22%, respectively. The observations on the degree of esterification were similar to the methoxyl content. The higher degree of esterification means the higher methoxyl content. Pectin extracted from breadfruit (peel, pulp and abortive flower) had higher methoxyl content than those of papaya and salton. Based on the degree of esterification, pectin is divided into two groups; (1) high methoxyl pectin (pectin with the degree of esterification higher than 50%) and (2) low methoxyl pectin (pectin with the degree of esterification lower than 50%) (Khamsucharit *et al.*, 2018) (Mesbahi *et al.*, 2005). Therefore, the degree of esterification of different parts of breadfruit (peel, pulp and abortive flower) were classified as high methoxyl pectin, which was in the same category as the commercial pectin.

Galacturonic acid content influences pectin quality (Burana-osot *et al.*, 2010). Generally, the galacturonic acid content available in commercial pectin is greater than 65% in the ash-free dry weight, and molar masses vary from 100,000 to 200,000 Da (Joye & Luzio, 2000). In our study, different parts of breadfruit, papaya and santol revealed significant differences in galacturonic acid content. They ranged from 41.41-73.19%. The highest galacturonic acid content was found in Bp (73.19±0.59%), which was significantly higher than commercial pectin (53.44±0.29%). This result indicated that pectin extracted from Bp was in a good qualification.

The chemical structures of pectin extracted from different parts of breadfruit, papaya and santol were characterized by FT-IR and their spectra are presented in Figure 1-2. FT-IR is a technique that can be used to analyze and confirm pectin extracts from different sources. It can also be used to characterize the percentage of ester groups, which are functional groups that exhibit the quality level of the pectin (Yang *et al.*, 2018). Overall, the FT-IR spectra of all pectin samples and commercial pectin were similar, especially in the "fingerprint region". The region between 800 and 1300 cm-1 is considered the fingerprint region for carbohydrates of pectin. It can be used to identify major chemical groups that are specific to particular polysaccharides (Khamsucharit *et al.*, 2018) (Muhammad *et al.*, 2014) (Kamnev *et al.*, 1998). Figures 1 and 2 show the similarity between commercial pectin and pectin extracted

from the pulp and peel of breadfruit, papaya, and santol. However, according to previous studies, some differences found in the fingerprint region indicate variations in monosaccharide compositions of pectin from various sources (Kamnev *et al.*, 1998). Additionally, the study from Kamnev *et al.* (1998) and Filippov (1992) reported that the pyranose cycle vibration region are identical spectral parts, which consist of five bands at 1016-1019, 1052, 1076, 1104, and 1149 cm-1 characteristics. Therefore, this area might be different among pectin extracted from different plants (Kamnev *et al.*, 1998) (M.P, 1992).

The spectra at wavelengths 1730-1760 cm-1 and 1600-1630 cm-1 were spectra of the ester carbonyl group (C=O) and carboxyl groups (COO-), respectively. These spectra were broad and dominant, so it can also indicate an increase in the percentage of the degree of esterification in the pectin (Kyomugasho *et al.*, 2015). However, the spectra may be shifted in the presence of high polysaccharide groups. Therefore, most of the time, the apparent spectra of 1650 and 1750 cm-1 or nearby were used to analyze the percentage of ester groups (Bichara *et al.*, 2016). In addition, the spectral band 2900 cm-1, which demonstrates the presence of a methoxy group (-OCH3), could indicate a greater gelling ability of pectin with higher spectral resolution (do Nascimento Oliveira *et al.*, 2018) (Santos *et al.*, 2013). The FT-IR spectra and degree of esterification were in good agreement, which strongly indicated that the pectin extracted from Bp was of good quality.

According to our results, the pectin extracted from Bp was the most potential pectin for further use. In general, the peel of breadfruit is costless and normally discarded, while the pulp is popularly consumed. Therefore, it would be good for the renewable peel of breadfruit as an alternative source of pectin because of its high yield of pectin. The quality of pectin extracted from Bp was as good as commercial pectin. It could perform better gelatinization as its high degree of esterification and methoxyl content.

Additionally, a high glucuronic acid content supported the quality of pectin from Bp.

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