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Original Research Article

Comparative Physicochemical Properties of Isolated Pectin from Various Tropical Fruit Peel Wastes

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ABSTRACT

Pectin is widely used as the main polymer in cosmetics, pharmaceuticals, and biomedical applications. The pectin utilization in diverse fields can contribute to fruit waste processing since waste such as fruit peel serves as a pectin source. This study is aimed to analyze the extraction temperature effect on the physicochemical properties of extracted pectin from various tropical fruit peel waste. In this study, we determined the extracted pectin's physicochemical properties from peel wastes of tropical fruits like papaya, lime, lemon, and dragon fruit using 3% citric acid at 65 and 90°C. A higher pectin yield resulted from a higher extraction temperature of 90ºC (8.78 to 27.03 %) than that obtained at 65ºC (5.78 to 17.71 %). The spectra of Fourier transform infrared (FT-IR) mainly showed the degradation of pectin, specifically in the cases of papaya, lime, and lemon, indicated by the higher OH peak intensity. All samples extracted were high methoxyl pectin (HM), with degrees of esterification (DE) ranging from 56.98 to 89.63%. Among the fruit peels studied, only the lemon peel pectin extracted at 90°C, with anhydrouronic acid (AUA) content of 77.35%, meets the criteria for food additives and pharmaceutical purposes.

Keywords: Pectin, degree of esterification, FT-IR, AUA, fruit peels

Introduction

Pectin is a carbohydrate polymer, a complex heteropolysaccharide that plays several important functions in the cell wall and exists in higher plants in the cell wall and middle lamella.[1] Galacturonic acid units comprise about 70% of the pectin composition.[2] Rhamnose, galactose, arabinose, glucose, xylose, mannose, and fucose are other monosaccharides in pectin.[3] The backbone of pectin comprises D-galacturonic acid units, connected via α-1,4-glycosidic linkages or modifying units of residues α-1,4-linked d-galacturonic acid and α-1,2-linked l-rhamnose.[4,5] Pectin classification is commonly defined by the degree of esterification (DE) and is expressed as the percentage of methyl-esterified carboxyl groups to the total number of galacturonic acid units ratio. High methoxyl (HM) pectin is defined as pectin with DE higher than 50%; on the other hand, pectin with DE less than 50% is known as low methoxyl (LM) pectin.[2] The high variability of pectin polysaccharides determines the pectin properties in any pectin application.[1.]

Pectin is utterly used in the food industry, especially in producing jams, marmalades, jellies, and fruit juices as gelling agents, thickeners, stabilizers, and coatings owing to its gelling properties.[1,6] It is widely considered the main polymer used in cosmetics, pharmaceuticals, biomedical, and tissue engineering applications because of its gelling and thickening capabilities.[3]

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In tissue engineering applications, pectin acts as a scaffold in which cells are reseeded to form functional tissues. The gelation mechanisms and properties, different for HM and LM pectins, are affected by intrinsic and extrinsic factors such as DE, charge distribution in the polymer, molecular weight, temperature, pH, ionic strength, and the presence of co-solutes. HM pectins require specific gelation conditions, like an acidic pH solution (pH 2.5 to 3.5) and a minimum quantity of soluble carbohydrate, specifically sucrose (55 to 75%), or other similar co-solutes, such as polyethylene glycol and sorbitol. In contrast, LM pectins have easily gelled in the presence of divalent cations, commonly Ca^{2+}.[7] Depending on the DE value, pectins may have various applications. Therefore, determining the characteristics of pectin is crucial for its food, biomedical, and tissue engineering applications.

The production of pectin started in the 1920s to process waste from juice industries.[2] A significant amount of the inedible part of the fruit peel, generated by fruit processing steps, is a good source of pectin.[8] It exists in the cell wall as a protopectin that is insoluble in water. Acid hydrolysis at high temperatures breaks the bonds between the side-chain sugars and cell walls. Subsequently, pectin is released and water-soluble.[3] The application of organic acids, for instance, citric acid, has been reported as an option extraction method for obtaining pectin from clementine peel,[9] apple peel,[10] cocoa husks,[11] sweet lemon peel,[12] sugar beet pulp,[13] passion fruit by-products,[14] and grape pomace.[15] Citric acid is effective for pectin extraction resulting in improved physicochemical properties,[6,10] including preferable pectin color.[16]

In this study, this work extracted pectin from various tropical fruit peels, such as papaya (*Carica papaya* Linn.), lime [*Citrus aurantifolia* (Christm.) Swingle], dragon fruit [*Hylocereus polyrhizus (Weber*)] and lemon (*Citrus limon* Linn.), at different temperatures: 65°C and 90°C using citric acid (pH 3). Previous studies have reported that pectin yield increased with increasing temperature. However, it decreased when pectin started to hydrolyse.[17,18] Yanilka and colleagues found that the extraction temperature affected the DE values but did not affect the yield and viscosity of the pectin extracted from mango peel.[19] However,

another study revealed that the greatest influential factor for pectin yield was the pH, while time affected the DE values.[14] Thus, the temperature effect on the pectin, physicochemical characteristics, including DE and viscosity properties, has not yet been well-studied.

Hence, this study investigated the temperature extractions' effect on the physicochemical properties of pectin extracted from papaya, lemon, lime, and dragon fruit peels. Our study presented that a higher pectin yield has resulted at the temperature of 90 °C (8.78 to 27.03%) than that obtained at 65 °C (5.78 to 17.71%); the Fourier transform infrared (FT-IR) spectra mainly showed the degradation of pectin, specifically for papaya and lime, indicated by the higher OH peak intensity.

Materials and Methods

Materials

Papayas were obtained from Borobudur (Central Java, Indonesia), lemons were collected from Sukoharjo, Central Java. Lime was obtained from a local farm in Sleman (Yogyakarta, Indonesia), and dragon fruits were harvested from Banyuwangi (East Java, Indonesia). All the fruits were harvested and collected in May 2022. The fruits sample were identified in the Herbal Medicine Garden Laboratory, Faculty of Pharmacy, Sanata Dharma University. The collected fruit was cleaned and washed, then saved in a jar with alcohol as a wet herbarium in the Pharmacognosy Phytochemistry Laboratory, Faculty of Pharmacy, Sanata Dharma University. Standard pectin was purchased from Sigma-Aldrich (P9135). Technical-grade citric acid and 96% ethanol were used to extract pectin from the tropical fruit peel.

Pectin extraction

The tropical fruits as our samples were harvested and washed; then, the peels were cut into small pieces (< 1 cm) and dried at 65°C in the oven. After completely dried, the peels were powdered by using a blender and then stored in a sealed polyethylene bag till further use. Pectin extraction was done by using the conventional method using an acidic solution and heat.[6,12,19] The powders of the fruit peels (10 g) were mixed with a 1% citric acid solution (1:30). In the water bath, the solutions were heated to the desired temperature (65°C or 90°C) and constantly mixed for 90 mins. The pectin extract solutions were filtered using a 100-mesh nylon filter and then with a cotton cloth to obtain as much filtrate as possible. The as-obtained filtrates were cooled to 25°C, and a solution of 96% ethanol was poured, equivalent to the filtrate quantity, under continuous stirring to create contact between the pectin extract solutions and ethanol. The solutions were left overnight, and the precipitated pectin samples were filtered using a 400-mesh nylon filter. The pectin precipitates were washed with 96% ethanol until the ethanol solution was clear. The pectin samples were left at room temperature until most of the ethanol evaporated, and then they were crushed then dried at the range of 45 to 50 °C in the oven. The pectin yields were calculated using the following formula:

$$yield\ (\%) = \frac{weight\ of\ dried\ pectin\ (g)}{weight\ of\ peel\ powder\ (g)}\ x\ 100$$

Pectin characterization

Equivalent weight

The pectin equivalent weight was determined using the procedure and formula described by Ranganna (1995).[20] 1 mL ethanol was used to moisten pectin powder (0.5 g), and the procedure continued by dissolving the moistened pectin powder in 20 mL carbon dioxide-free water under continuous stirring until complete dissolution of pectin occurred. NaCl (0.2 g) and phenolphthalein were dropped, and the resulting solution was then titrated with 0.1 N NaOH until a persistent light pink color was obtained. The following equation was then used to calculate the equivalent weight:

$$Equivalent\ weight\ (g/mol) = \frac{weight\ of\ sample\ (g)}{volume\ of\ titre\ (mL)\ x\ titre\ normality}\ x\ 1000$$

Methoxyl content

1 mL of 0.1 N NaOH used as the saponification titre during the determination of DE is equivalent to 3.10 mg of -OCH_3. The following formula was used to calculate the percentage of methoxyl groups in pectin samples:[20]

$$Methoxyl\ content\ (\%) = 3.10\ x\ \frac{V_2}{w}\ x\ 100$$

V_2 = saponification titre (mL)

w = weight of the pectin sample (mg)

Anhydrouronic acid (AUA) content

The pectins' AUA content was determined by calculating the titrant volumes from the calculation of equivalent weight and methoxyl content. The formula for calculating the AUA content is as follows:[21]

$$AUA\ (\%) = \frac{176\ x\ 0.1a\ x\ 100}{w\ x\ 1000} + \frac{176\ x\ 0.1b\ x\ 100}{w\ x\ 1000}$$

where one molecular unit of AUA = 176 g, a = volume of titre obtained from the equivalent weight, b = volume of titre obtained from the methoxyl content, and w = samples' weight (g).

Degree of esterification (DE)

With a little modification, the titrimetric method from USP43-NF38 (2020) was used to determine the DE values of the extracted pectin samples. The pectin peel powder (0.1 g) was transferred to a 100 mL flask, then 2 mL ethanol was used to moisten the pectin peel powder, followed by dissolving them in 20 mL carbon dioxide-free water under continuous stirring at 40 °C using a magnetic stirrer until complete dissolution of pectin occurred. NaCl (0.2 g) was added to sharpen titration results. The phenolphthalein (some drops) was added, then 0.1 N NaOH was used to titrate the solution until the color of the solution changed to a faint pink. The color persisted even after vigorous shaking. The volume of NaOH was recorded as the initial titre volume V_1 (mL). Thereafter, 5 mL of 0.25 N NaOH was transferred to the flask, and the mixture was stirred at room temperature for 30 mins continuously. NaOH (5 mL) was added to the flask, followed by titration with 0.1 N NaOH until a persistent faint pink color was obtained. The volume of NaOH was recorded as the saponification titre V_2 (mL). The DE value was determined using the following formula:

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

Functional group analysis and DE calculation using FT-IR spectroscopy

For functional group analysis, attenuated total reflectance (ATR) FT-IR spectroscopy was performed using a Shimadzu IR Spirit FT-IR spectrometer; 64 scans (wavenumber range of 600-4000 cm^{-1}) were accumulated. The pectin functional groups' signal range was established using the similar setting that was used to record the spectra of pectin in previous studies. The FT-IR data was also used to determine the DE value from the areas of the ester carbonyl (1745–1760 cm^{-1}) and free carboxylate groups (1620–1640 cm^{-1}). The DE values were calculated and analyzed using Origin 9.0. The equation used for calculating the DE values is as follows:[22,23]

$$DE = \frac{area\ of\ esterified\ carboxyl}{area\ of\ esterified\ carbonyl\ groups + area\ of\ nonesterified\ carboxyl\ groups}$$

Viscosity of pectin solution

A Rion VT-04F viscometer with rotor number 3 was used to measure the viscosities of the pectin solutions. The experiment was adapted from a previous study.[9] The fruit peel pectin was converted to 1% (w/v) aqueous solution by adding 2 mM $CaCl_2$ and then dissolved in deionized water under constant stirring using a magnetic stirrer until complete dissolution of pectin occurred. A standard pectin solution was prepared using the same method as the control. The pectin solutions were kept overnight in a cool room at 4°C prior to the measurement. The viscometer was used to measure the pectin solutions' viscosity, and the viscosity data was reported in dPa·s.

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Results and Discussion

In this study, we extracted pectin from tropical fruits of Indonesia, such as papaya (Carica papaya), lime (Citrus aurantifolia), lemon (Citrus limo), and dragon fruit (Hylocereus polyrhizus) peels, which are good sources of pectin.[12,24,35] We adopted a green extraction method using citric acid (at 65 and 90°C) rather than traditional ones involving inorganic acids, such as HCl and HNO_3. These acids release corrosive effluents into the environment and may leave toxic traces in the final product.[9] The heated acid dissolves pectin and other cell wall pectin components (protopectin), thus increasing pectin yield.[26] Figure 1 shows the schematic of pectin extraction from the various tropical fruit peel wastes and its subsequent purification. The fruits were peeled and dried at 65°C in the oven. The peels of papaya and dragon fruit required a longer drying time than those of the other samples. The dried peels were powdered using a blender prior to extraction. Compared to peel powders of the citrus fruits, which were yellowish white, those of papaya and dragon fruit were orange and pinkish, respectively. The pectin extracts were then precipitated and refined by washing them with ethanol. After drying at the range of 45 to 50°C, the physicochemical properties of the refined pectin, for instance, DE, functional groups, and viscosities, were determined (Figure 1).

The ethanol-precipitated pectin samples of papaya and dragon fruit were reddish-brown, while those of lime and lemon had a yellow-white colour (Figure 2a). This is possibly due to the removal of impurities such as betacyanin, red pigment, anthocyanin, and the total phenolic compounds from the peel extract of pectin.[27,28] The wet pectin from papaya had an orange-cream colour, whereas those from the others had a yellowish-white colour (Figure 2b). The drying temperature for all the pectin samples was in the range of 45 to 50 °C, which is considered to be optimum owing to the highest extraction yield obtained.[29,30] After drying, the as-obtained pectin samples were brown (Figure 2c); however, according to the International Pectin Producers Association (2009), pectin is usually light in colour. The brownish colour observed in a previous study was reported to be possibly due to the presence of pigments and polyphenol compounds.[31] Thus, oven-drying has indicated some destructive effects on the extracted pectins' structure.[14]

The yield of extracted pectin with citric acid increased with increasing temperature, and the maximum yield was obtained at 90°C, which was higher than that required to induce pectin hydrolysis. Because the short pectin polymer could not be precipitated in ethanol, it resulted in a lower pectin yield, according to a previous study.[17] This study revealed that the yields of extracted pectin from papaya, lime, lemon, and dragon fruit peels using citric acid at 65°C were 7.34 ± 0.09, 9.62 ± 0.05, 5.78 ± 0.24, and 17.71 ± 0.67%, respectively, which significantly increased to 8.78 ± 0.61 ($p < 0.05$), 27.03 ± 0.63 ($p < 0.001$), 12.10 ± 0.36 ($p < 0.001$), and 23.24 ± 0.76% ($p < 0.001$), respectively, as the extraction temperature was increased to 90 °C (Figure 2d). A higher extraction yield of pectin from fruit peel waste resulted from a higher temperature of extraction.[31,11]

Furthermore, the physicochemical properties of the extracted pectin from the different fruit peels were evaluated. Table 1 shows the equivalent weights and methoxyl and AUA contents of standard pectin and pectin extracted from the different fruit peels.

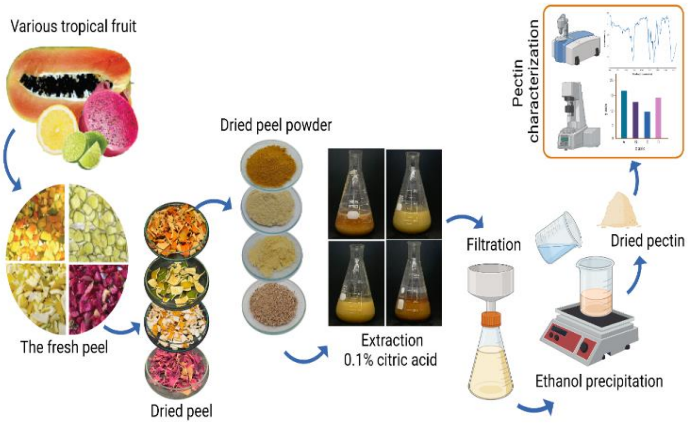

Figure 1: Schematic of pectin extraction, purification, and characterization from various tropical fruit peel wastes.

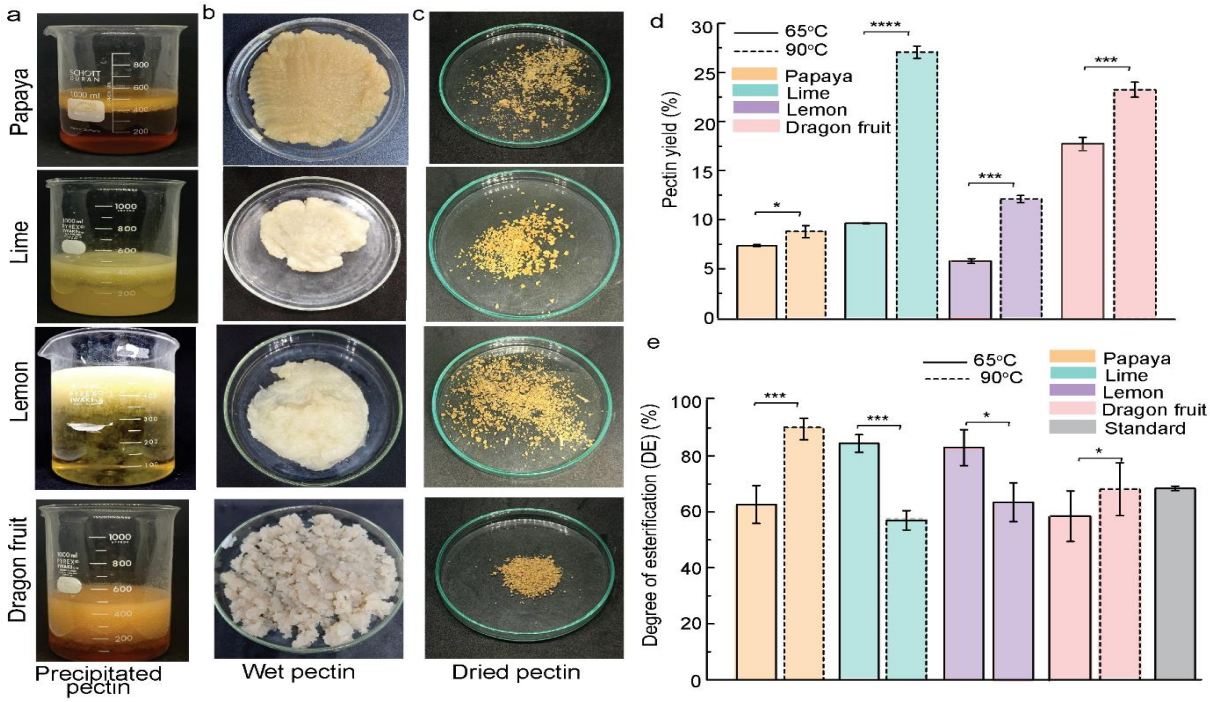

Figure 2: Physicochemical properties of refined pectin. a. Ethanol-precipitated pectin; b. Wet pectin; and c. Dried pectin from various fruit peels. d. Yield of isolated pectin obtained from various fruit peels (n = 6), e. DE of the isolated pectin at different extraction temperatures (n = 4). Statistical significance was determined using one-way ANOVA followed by Tukey's test; *$p < 0.01$, and ***$p < 0.001$. Error bars indicate mean ± standard deviation (S.D.)

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The equivalent weights of extracted pectin from papaya, lime, lemon, and dragon fruit peels at 65°C were 1131.75, 1426.71, 1272.12, and 1251.80, respectively, while they were lower for pectin extracted at 90°C, that is, 971.80, 1085.54, 797.5621, and 892.68, respectively. This study also revealed that the pectins' methoxyl content isolated at 90°C was higher than pectin isolated at 65 °C for all the samples except those extracted from dragon fruit peel. The proportion of methoxyl group (%) of pectin extracted at 65 °C from papaya, lime, lemon, and dragon fruit peels was 4.15, 4.31, 4.37, and 5.64, respectively, while that of pectin extracted at 90 °C was 19.30, 8.89, 10.21, and 4.03, respectively. The AUA content of the extracted pectin samples was significantly diverse, from 29.81 to 77.35%. Pectin samples extracted from papaya, lime, lemon, and dragon fruit peels at 65°C showed AUA contents of 29.81%, 32.74%, 56.35%, and 46.23%, respectively, while those of the pectin samples extracted at 90°C were 41.93%, 60.54%, 77.35%, and 43.78%, respectively. The AUA content suggested the purity level of isolated pectin with a minimum recommended value of 65% for pectin applications in the pharmaceutical or food industry. Thus, according to this study, only the lemon peel pectin extracted at 90°C meets the requirements for food additives and pharmaceutical purposes.

DE is an important property that influences pectin application as a scaffold in tissue engineering. To form the porous structure of pectin, it must be cross-linked with a suitable Ca^{2+} electrolyte or sugar in low pH solutions.[3,4] In this study, the DE value was determined in two different ways: titrimetric and FT-IR spectral. As determined by the titrimetric method, the pectins' DE values extracted at 65°C from papaya, lime, lemon, and dragon fruit peels were 62.62 ± 6.71, 84.46 ± 3.16, 82.99 ± 6.38, and 58 ± 9.05, respectively (Figure 2e). At a higher temperature of 90 °C, the DE values significantly declined to 56.98 ± 3.43 ($p < 0.001$) and 63.0 ± 6.90 ($p < 0.05$) for lime and lemon peel pectin samples, respectively. However, they increased to 89.63 ± 3.81 ($p < 0.001$), and 68.11 ± 9.47 ($p < 0.05$) for papaya and dragon fruit peel pectin samples. The DE value of the standard pectin sample was 68.40 ± 0.82 (Figure 2e).

Further, the FT-IR data was used to determine the DE values. In previous studies, these were determined from the areas of the esterified carbonyl (1745–1760 cm^{-1}) and free carboxylate groups (1620–1640 cm^{-1}).[22] The steps of the DE calculation based on the FT-IR data are presented in Supplementary Information. Prior to the measurement of the area under the curve, background subtraction was applied to normalize the FT-IR peaks (Supplementary 1a). The peaks of the esterified carbonyl and free carboxylate groups were observed at 1741 and 1610 cm^{-1}, respectively (Supplementary 1b). The area under the curve (AUC) was then selected and calculated (Supplementary 1c and 1d). At 65 °C, the DE values were 88.38, 83.58, 84.73, and 48.09 for papaya, lime, lemon, and dragon fruit peel pectin samples, respectively, while they decreased to 57.54, 77.32, and 83.36 for pectin extracted from papaya, lime, lemon peels at 90°C. However, the pectin extracted from dragon fruit peel DE value increased to 79.26, whereas that of the standard pectin was 59.21. Overall, all the pectin samples from the various fruit peels were HM pectin. The decrease in the DE values of lime and lemon pectin samples at the higher temperature was possibly due to the hydrolysis of the ester carboxyl. Thus, the possible causes of DE variation are differences in growing environments, plant varieties, and pectin extraction conditions.[32]

A strong band in the range of 3200–3600 cm^{-1}, belong to the hydroxyl group (-OH) of the pyranose ring, indicates the specific characteristics of pectin. The bands at approximately 1730–1744 cm^{-1} (Figure 3a) and 1733 cm^{-1} (Figure 3b) were attributed to C=O ester stretching vibration, while the band at approximately 1600 cm^{-1} was ascribed to the stretching vibration of the methyl ester of the carboxyl group (COO-R) However, COO asymmetric stretching vibrations for pectin samples extracted at 65 and 90°C were observed at 1400–1440 cm^{-1} and 1414–1460 cm^{-1}, respectively. The carbohydrate fingerprints were investigated in the range of 950–1200 cm^{-1}.[22] as confirmed by the yellow highlighted area in Figures 3a and 3b. The major broad peak at 3252–3400 cm^{-1} was indicated to the stretching vibration of the hydroxyl group with intermolecular H-bond of galacturonic acid polymer of all pectin samples extracted at 65 and 90°C (Figure 3a and 3b). A stronger OH band was observed for the papaya, lime, and lemon pectin samples extracted at 90°C than for those extracted at 65°C. The methoxy group of papaya, lime, and lemon pectin samples possibly start to hydrolyze at a higher temperature.

The extraction and application of pectin are influenced by viscosity properties. The higher the molecular weight of pectin means the higher its grade and viscosity.[33] Molecular weight, degree of methylation, concentration, and temperature are factors that influence viscosity.[33,34] A 1% solution of Ca^{2+} was used to measure the viscosity. Papaya pectin was darker than any of the other extracted pectin samples (Figure 4a).

Table 1: Characteristics of pectin isolated from the various fruit peel wastes

Characteristics	Papaya		Lime		Lemon		Dragon Fruit		Standard
	65°C	90°C	65°C	90°C	65°C	90°C	65°C	90°C	
Weight equivalent (g/mL)	1131.75	971.80	1426.71	1085.54	1272.12	797.5621	1251.80	892.68	892.77
AUA	29.81	41.39	32.74	60.54	56.35	77.35	46.23	43.78	47.17

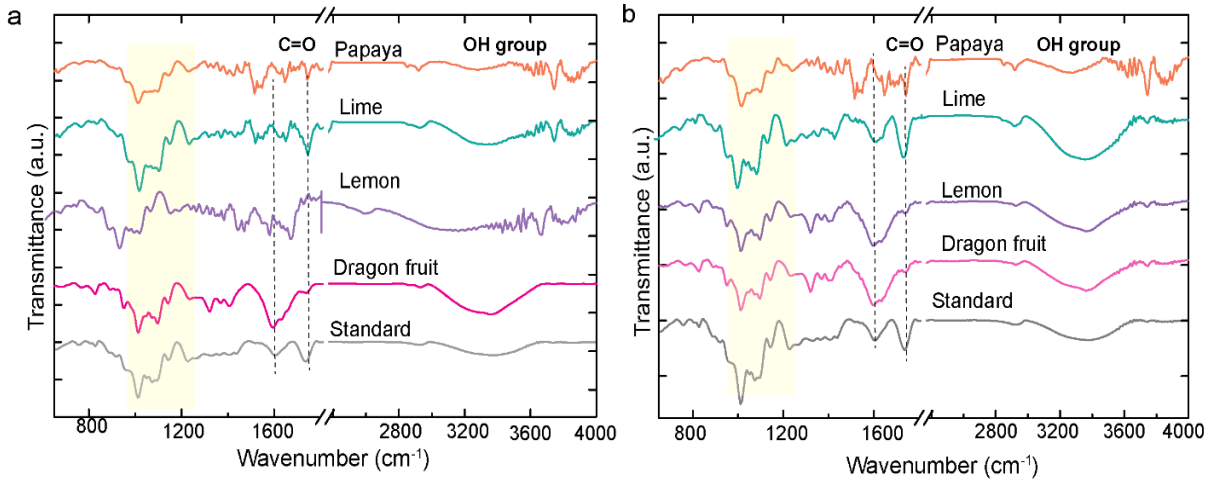

Figure 3: FT-IR spectra of pectin samples extracted at: a. 65°C, and b. 90°C.

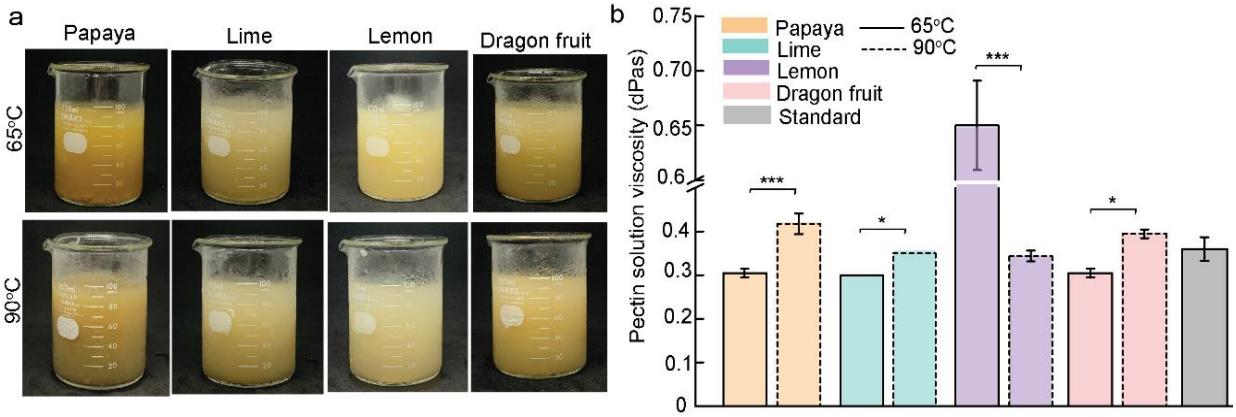

Figure 4. Viscosity behaviour of the pectin solutions. a. Appearance of pectin solutions in water containing Ca^{2+} b. Viscosity characteristics of pectin extracted at different temperatures (n = 3). Statistical significance was determined using a one-way ANOVA followed by Tukey's test; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Error bars indicate means ± standard deviation (S.D.)

The viscosities of the pectin solutions were 0.31 ± 0.01, 0.30 ± 0.00, 0.65 ± 0.04, and 0.31 ± 0.01 for pectin extracted from papaya, lime, lemon, and dragon fruit peels at 65°C, while those of the pectin solutions extracted at 90 °C were 0.42 ± 0.02, 0.35 ± 0.00, 0.34 ± 0.01, and 0.39 ± 0.01, respectively (Figure 4b). Most of the pectin solutions exhibited a significant increase in viscosity when extracted at a higher temperature, except that of dragon fruit peel. All the pectin samples were highly methylated pectin however, the viscosities were affected by the divalent ions.

Comprehensively, the pectin samples extracted at a higher temperature from all the tropical fruit peels used in this study exhibited comparable properties with those extracted at a lower temperature. All the extracted pectin samples were categorized as HM pectin, similar to the pectin standard obtained from Citrus peel. The AUA content of lemon peel pectin extracted at 90°C was 77.35% and met the criteria for food additives and pharmaceutical purposes.

Conclusion

All the tropical fruit peels used in this study exhibited higher pectin extraction yields at 90°C than at 60°C. The pectin extracted from papaya, lime, lemon, and dragon fruit peels were categorized as HM pectins, similar to the standard pectin obtained from Citrus peel. The AUA content of lemon peel pectin extracted at 90°C (77.35%) meets the criteria for food additives and pharmaceutical purposes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

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