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Extraction and Characterization of Pectin from Dragon Fruit (*Hylocereus polyrhizus*) using Various Extraction Conditions

(Pengekstrakan dan Pencirian Pektin daripada Buah Naga (*Hylocereus polyrhizus*) Menggunakan Pelbagai Keadaan Pengekstrakan)

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ABSTRACT

*The extraction of pectin from dragon fruit (*Hylocereus polyrhizus*) peels under three different extraction conditions was identified as an alternative source of commercial pectin. In this work, dried alcohol-insoluble residues (AIR) of dragon fruit peels were treated separately with 0.25% ammonium oxalate/oxalic acid at a pH of 4.6 at 85°C; 0.03 M HCl at a pH of 1.5 at 85°C; and de-ionized water at 75°C. The pectin obtained from these methods was compared in terms of yield, physicochemical properties and chemical structure. Fourier Transform Infrared Spectroscopy (FTIR) was used in the identification of dragon fruit pectins. The results showed that the pectin yield (14.96-20.14% based on dry weight), moisture content (11.13-11.33%), ash content (6.88-11.55%), equivalent weight (475.64-713.99), methoxyl content (2.98-4.34%), anhydrouronic acid (45.25-52.45%) and the degree of esterification (31.05-46.96%) varied significantly ($p < 0.05$) with the various extraction conditions used. Pectin extracted with ammonium oxalate gave the highest yield of pectin, with high purity and low ash content. Based on the value of methoxyl content and the degree of esterification, dragon fruit pectin can be categorized as low-methoxyl pectin.*

Keywords: Alcohol insoluble residue; dragon fruit; extraction conditions; pectin

ABSTRAK

*Pengekstrakan pektin daripada kulit buah naga (*Hylocereus polyrhizus*) dengan menggunakan tiga keadaan pengekstrakan yang berbeza telah dikenalpasti sebagai sumber alternatif kepada pektin komersial. Dalam kajian ini, pepejal tak larut alkohol (AIR) kulit buah naga telah diperlakukan secara berasingan dengan 0.25% ammonium oksalat/ asid oksalik pada pH 4.6 pada suhu 85°C; 0.03 M HCl pada pH1.5 pada suhu 85°C; dan air ternyahion pada suhu 75°C. Pektin yang diperolehi daripada kaedah ini dibandingkan dari segi hasil, ciri fiziko-kimia dan struktur kimia. . Spektroskopi infra- merah (FTIR) digunakan dalam penentuan pektin buah naga. Keputusan menunjukkan hasil pektin (14.96- 20.14% berdasarkan berat kering), kandungan air (11.13- 11.33%), kandungan abu (6.88- 11.55%), beratara (475.64- 713.99), kandungan metoksil (2.98- 4.34%), asid anhidro uronik (42.25- 52.45%) dan darjah pengesteran (31.05- 46.96%) berbeza secara signifikan ($p< 0.05$) dengan pelbagai keadaan pengekstrakan yang digunakan. Pektin yang diekstrak dengan ammonium oksalat memberikan hasil pektin tertinggi, dengan ketulenan tinggi dan rendah kandungan abu. Berdasarkan kandungan metoksil dan darjah pengesteran, pektin buah naga boleh dikategorikan sebagai pektin metoksil rendah*

Kata kunci: Buah naga; keadaan pengekstrakan; pepejal tak larut alkohol; pektin

INTRODUCTION

Dragon fruit (*Hylocereus polyrhizus)* or red pitaya is one of the tropical fruits that belongs to the cactus family, Cactaceae. The fruits contain high amounts of vitamin C and water-soluble fiber (Ruzainah et al. 2009). In Malaysia, dragon fruits are known as "buah naga" or "buah mata naga", which were first introduced in 1999 in Setiawan, Johor and Kuala Pilah regions. The national acreage of pitaya production in 2006 was 927.4 ha with a total production of 2,534.2 tons (Cheah & Zulkarnain 2008). The 'scales' or bracts on the surface of dragon fruit give it a 'dragon-like' appearance (Hoa et al. 2006). The peel of the fruit is usually red, and the pulp is purple-red with numerous small, soft seeds (Nerd et al. 1999). The peels are mostly waste materials resulting from the dragon fruit juice processing industry and are normally discarded. These discarded peels may cause an environmental problem, particularly water pollution. Thus, in addition to being fed to animals, the peels can be used in the production of pectin, which would then increase the potential return for the dragon fruit juice processing industry.

Pectin is the methylated ester of polygalacturonic acid that contains 1,4-linked α-D-galacturonic acid residues (Levigne et al. 2002). It is commonly found in the cell walls and middle lamellae of higher plants. These polysaccharides consist of 300-1,000 chains of galacturonic acid units (Yeoh et al. 2008). Pectin is widely used in the food industry as a thickener, emulsifier,

texturizer and stabilizer. Pectin is usually added in jams and jellies as a gelling agent. It has also been used as a fat substitute in spreads, ice-cream and salad dressings. In terms of nutrition, pectin has been shown to lower blood cholesterol levels and low-density lipoprotein cholesterol fractions, which is beneficial for human health (Liu et al. 2006). According to the FAO (1969), pectin is considered to be a safe additive that can be taken daily without limits.

Pectin can be obtained from many sources with a variation in the percentage yield. Commercial pectins are primarily extracted from citrus peels and apple pomace using an acid extraction method with yields of about 25 and 12% pectin, respectively. Sugar beet and sunflower head residues consist of 10 to 20% pectin (Miyamoto & Chang 1992). Other sources for pectin include cocoa husk, with about 9% of the dry weight being pectin (Mollea et al. 2008), and soy hull, with pectin contents at about 26-28% (Kalapathy & Proctor 2001). To date, little or no research has been done on the extraction of pectin from dragon fruit. The increasing world market demand for pectin has been in excess of 30,000 tons annually (Yeoh et al. 2008). For these reasons, we are interested in producing pectin from the dragon fruit peels as an alternative source for apple and citrus pectin.

The extraction of pectin basically involved the aqueous extraction of pectin from the raw material (plant), the isolation of the extracted pectin and purification (Joye & Luzio 2000), followed by drying process. The pectin extraction process should use a suitable method to obtain the maximum yield and quality of pectin. The yield of pectin usually depends on the extraction conditions, such as temperature, extraction time, pH, type of extraction solvents (Yeoh et al. 2008), and drying method (Monsoor 2005). Before extraction begins, an alcohol-insoluble residue is prepared to remove low-molecular weight compounds, including any traces of free galacturonic acid (Happi et al. 2008). Pectin can be divided into two types based on the degree of esterification (DE) of the pectin: high methoxyl pectin (DE > 50%) and low methoxyl pectin (DE < 50%) (Mesbahi et al. 2005).

The objectives of this study were (i) to evaluate the impact of different extraction conditions on the yield of dragon fruit pectin, (ii) to characterize the dragon fruit pectin, and (iii) to define the structure of pectin using infrared spectroscopy (FTIR).

Materials and Methods

Sampling and Sample Treatment

The dragon fruit peels were obtained from MARDI, Malaysia. The peels of the fruits were removed and chopped into 1 cm^2 pieces using a stainless steel knife; these pieces were then dried in an air convection oven at 50°C. Next, the dry peels were ground in a laboratory dry blender. Finally, alcohol-insoluble residue (AIR) was prepared by treating the dried peels four times with isopropanol (85 vol.%) at 70°C for 20 min following the procedure modified from Koubala et al. (2008a).

Extraction Procedure

Three different extraction conditions were applied and compared to determine the best condition for recovering pectin from dragon fruit: the use of ammonium oxalate (0.25%), pH 4.6 ± 0.01 (adjusted with oxalic acid) at 85°C for 1 h; hydrochloric acid (0.03 M; pH 1.49 ± 0.02) at 85°C for 1 h; and de-ionized water at 75°C for 1 h. About 50 g of AIR were stirred with 1 L of each of the above extracting solutions. The extract was separated from the AIR residue by filtering through a nylon cloth, and the pectin was coagulated with isopropanol. The coagulated pectin was then washed several times with isopropanol and acetone before drying in an oven at 50°C for a few hours to a constant weight and a finely ground consistency. The yield obtained was reported as the % yield (g dried pectin per g dried peels). Each of the extraction conditions was carried out in triplicates. This pectin was used for further analysis.

Analysis and Characterization of Pectin

Determination of Ash and Moisture Contents

The ash content was determined by weighing 1 g of pectin in a tared crucible and then heated in a muffle furnace at 600°C for four hours. The residue was cooled in a desiccator and weighed to constant weight. In determining the moisture content, 1 g of pectin was weighed and dried at 100°C for four hours to a constant weight (AOAC 1980).

Determination of Equivalent Weight

The determination of methoxyl and AUA contents and the equivalent weight were conducted following the method described by Owens et al. (1952). The values of equivalent weights were used for calculating the anhydrouronic acid (AUA) content and the degree of esterification. Equivalent weights were determined by weighing 0.5 g pectin in a 250 mL conical flask and moistening it with 5 mL of ethanol. One gram of sodium chloride was added to sharpen the end point. Free carbon dioxide distilled water (100 mL) and six drops of phenol red indicator were added. The mixture was then stirred rapidly to ensure that all the pectic substance has dissolved and no lumps are retained on the sides of the flask. Titration was done slowly (to avoid possible deesterification) with 0.1 N standardized NaOH until the color of the indicator changed to pink (pH 7.5) and persisted for at least 30 seconds. The neutralized solution was used for the methoxyl determination. The following equation was used to calculate the equivalent weight:

$$\text{Equivalent weight} = \frac{\text{weight of sample (g)} \times 1000}{\text{ml of alkali} \times \text{Normality of alkali}}$$

Methoxyl Content Analysis

The determination of the methoxyl (MeO) content was performed by adding 25 mL of 0.25 N NaOH to the titrated

solution, which was shaken thoroughly, and allowed to stand for 30 min at room temperature in a stoppered flask. Twenty-five milliliters of 0.25 N HCl was then added and titrated to the same end point (pink) as before. The following equation was used to calculate the methoxyl content:

$$\text{MeO}\% = \frac{\text{meq of sodium hydroxide} \times 31 \times 100}{\text{Weight of sample (mg)}}$$

where 31 is the molecular weight of the methoxyl group.

Anhydrouronic Acid (AUA) Analysis

By using the values of the equivalent weight and the methoxyl content, the anhydrouronic acid content was calculated from the expression given below:

$$\%\text{AUA} = \frac{176 \times 100}{2}$$

where 176 is the molecular weight of AUA and

$$z = \frac{\text{wt of sample (mg)}}{\text{meq of alkali for free acid + meq of alkali for methoxyl}}$$

Degree of Esterification

The degree of esterification (DE) of pectin was calculated as follows:

$$\%\ \text{DE} = \frac{176 \times \text{MeO}\% \times 100}{31 \times \text{AUA}\%}$$

where MeO is the % methoxyl content.

Structural Analysis

The FTIR spectra were used to obtain information on chemical structure. Fourier transform infrared data were obtained using the Perkin Elmer, GX spectrum model with wavelengths ranging from 4,000-400 cm^{-1}.

Statistical Analysis

The analysis of pectin samples was done in triplicate. The values were expressed as the mean ± standard deviation. Statistical analysis was performed using the Statistical Analysis System "SAS" (version 6.12) for Windows. The Duncan test was performed to evaluate the significant differences between mean values. The confidence limits used in this study were based on 95% ($P < 0.05$).

Results and Discussion

Pectin Yield

The yield of the extracted dragon fruit pectin varied from 14.96 to 20.14% of the dried peels, depending on the extraction conditions used. The pectin yield, moisture, and ash content of the dragon fruit pectin that was produced under various extraction conditions are presented in Table 1. The highest yield of pectin was 20.14 ± 0.43%, which was obtained from ammonium oxalate/oxalic acid extraction. Ammonium oxalate is a calcium chelating agent that aids in pectin release from the cell wall (Yeoh et al. 2008). The extraction with de-ionized water gave 15.37 ± 0.44% of pectin, whereas, extraction with 0.03 M HCl gave 14.96 ± 0.36% of pectin. There was no significant difference between the yields for acid- and water-extracted pectin. In comparison, the yields from ambarella peels (10 to 13%) and mango peels (4.6 to 18.5%) obtained using the same conditions were lower using water extraction (Koubala et al. 2008a; Koubala et al. 2008b). The pectin obtained in this study was more than 10%, which makes it feasible for commercial use.

Characterization of Dragon Fruit Pectin

The moisture contents of all the samples (Table 1) were quite high (11.13-11.33%) and did not show any significant differences ($p < 0.05$). Pectin should have as low a moisture content as possible for safe storage and to inhibit the growth of microorganisms that can affect the pectin quality due to the production of pectinase enzymes (Muhamadzadeh et al. 2010). Table 1 shows that ammonium oxalate-extracted pectin contained a lower ash content than the acid- and water-extracted pectin did. Low ash content is good for gel formation. The maximum limit of ash content for good quality gel criteria is 10%.

Three extraction conditions were compared to determine the characteristics of the dragon fruit pectin. The results are shown in Table 2. The equivalent weights obtained were used in the calculations of the % AUA and % DE. Under all of the extraction conditions, dragon fruit pectin showed lower methoxyl content and degree of esterification as compared to commercial apple pectin. The dragon fruit pectin produced in this study can be categorized as low methoxyl pectin (LMP) because it has a % DE that is lower than 50% and a methoxyl content between 0.5 - 7.0%. The types of pectin determine the mechanism for gel formation. LMP can form gels with the addition of a low amount of sugar or without sugar in the presence of divalent cations. LMP are highly calcium sensitive and useful in low-sugar applications such as in diet jams and jellies. The content of AUA indicates the purity of the extracted pectin and is suggested to be not less than 65% (Food Chemicals Codex 1996). However, the AUA content obtained under all of the extraction conditions was < 65%. Result indicates that the extract may not be sufficiently pure due to the possible presence of proteins, starch and sugars in the precipitated pectins.

FTIR Spectra of Dragon Fruit Pectin

The Fourier Transform Infrared (FTIR) spectra show the functional groups and provides structural information

TABLE 1. Extraction yields (% dry peels), moisture and ash content for dragon fruit (*Hylocereus polyrhizus)*

	Ammonium oxalate/oxalic acid 0.25 %	0.03 M HCl	Deionized water
Yield of pectin, %	20.14 ± 0.43[a]	14.96 ± 0.36[b]	15.37 ± 0.44[b]
Moisture, %	11.19 ± 0.25[a]	11.13 ± 0.7[a]	11.33 ± 0.69[a]
Ash content, %	6.88 ± 0.42[b]	11.95 ± 1.55[a]	11.55 ± 0.13[a]

[a-b] Mean value from triplicate measurement ± standard deviation. Values with different superscripts in the same row are significantly different ($p < 0.05$).

TABLE 2. Chemical composition of dragon fruit pectin and commercial apple pectin

	Ammonium oxalate/ oxalic acid 0.25 %	0.03 M HCl	De-ionized water	Commercial apple pectin
Equivalent weight	475.64 ± 0.95[d]	713.99 ± 0.47[b]	636.65 ± 0.56[c]	892.56 ± 0.51[a]
Methoxyl content, %	2.98 ± 0.07[d]	3.43 ± 0.31[c]	4.34 ± 0.19[b]	7.34 ± 0.03[a]
AUA, %	54.44 ± 1.08[b]	45.25 ± 0.88[d]	52.45 ± 1.41[c]	61.72 ± 0.53[a]
DE, %	31.05 ± 0.72[c]	45.69 ± 1.36[b]	46.96 ± 0.8[b]	67.71 ± 0.4[a]
Color of pectin	Brown	Light brown	Light pink	Yellow white

[a-d] Mean value from triplicate measurement ± standard deviation. Values with different superscripts in the same row are significantly different ($p < 0.05$).

about the extracted dragon fruit pectin obtained from the different extraction methods and the commercial apple pectin in the wavelengths between 400 and 4,000 cm^{-1} (Figure 1). The major functional groups in pectin are usually in the region between 1,000 and 2,000 cm^{-1} of the FTIR spectra (Kalapathy & Proctor 2001). The carbonyl bands at 1,630-1,650 and 1,740-1,760 cm^{-1} indicate the free and esterified carboxyl groups, respectively (Gnanasambandam & Proctor 1999). The increase in DE values will also increase the intensities and band area of the esterified carboxyl groups. This could be used to compare the different types of pectins. The absorption bands between 1,100 and 1,200 cm^{-1} were from ether (R-O-R) and cyclic C-C bonds in the ring structure of pectin molecules. No major structural difference in the FTIR spectra of the pectin samples produced by various extraction conditions were observed, and the dragon fruit pectin structures were similar to those of the commercial apple pectin sample. The broad band, from 2,400 to 3,600 cm^{-1}, was due to absorbed moisture in the pectin samples.

CONCLUSION

In the present study, different extraction conditions were used to obtain pectin from dragon fruit peels. Dragon fruit, which is considered to be rich in pectins, has a yield of

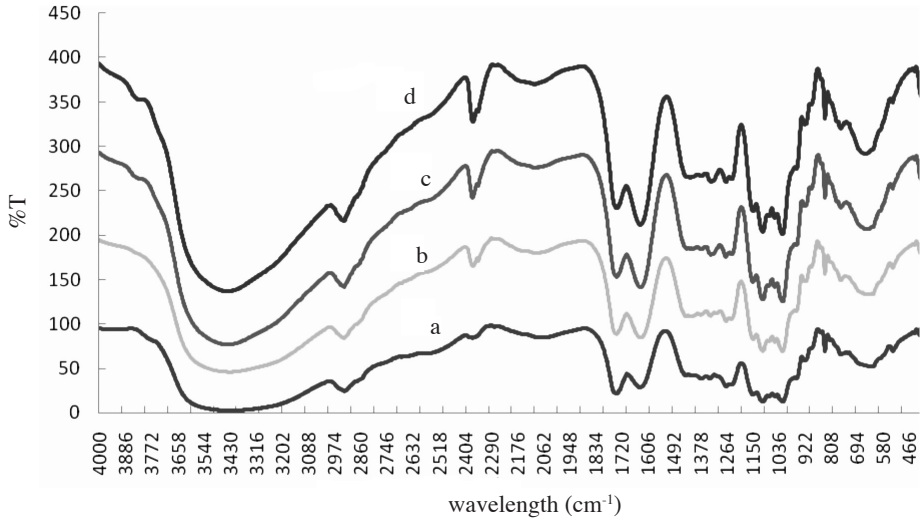

FIGURE 1. FTIR spectra of (a) apple pectin from Sigma Inc., (b) dragon fruit pectin produced by ammonium oxalate extraction, (c) dragon fruit pectin produced by 0.03-M HCl extraction, (d) dragon fruit pectin produced by de-ionized water extraction

about 20% and is of the low methoxyl type. The extraction conditions had a major impact on the extraction yields and the pectin's physicochemical properties but did not make a significant difference in the pectin's structure. Further investigations need to be directed at the gelling properties of these pectins by means of rheological studies.

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