Hot Acid Extraction, Characterisation and Scavenging Activity of Pectin from *Hylocereus polyrhizus*

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Abstract: Gelatin is extensively added to the food products for quality improvements of food entities. The role of gelatin as food thickener, texturizer, stabilizer, ingredient and as an animal based source has restricted its liberal use. However, the usage of this animal-based food quality improver has become less popular due to religious constraints and health restrictions. In fact, it is now direly needed to replace animal-based gelatin by plant-based. Pectin, the basic building material of cell walls in the terrestrial plant has great potential to be gelatin replacer as it can work as a gelling agent, thickener and also a stabilizer. Dragon fruit contains pectin which has high-value functional food as well as health-enhancing properties to substitute gelatin's function in foods production. The current study aims to extract pectin from dragon fruit peels by using hot acid extraction. The optimum conditions for extraction were found to be at 75ºC and pH 3.5 based on the highest percentage of pectin yield (33%). The FTIR result proved that dragon fruit peel contained pectin, which can be used as gelatin replacer are free from any religious and health-wise prohibitions. Pectin extracted was characterized in terms of moisture (14.03 ± 1.925) and ash content (8.73 ± 1.218). The extracted pectin of dragon fruit peel acts as the best gelatin replacer compared to commercial pectin and gelatins from the market. The prepared fruit peels also exhibit high DPPH scavenging activity (57.94%) with methanol extract (2mg/ml).

Keywords: Pectin, gelatin, *Hylocereus polyrhizus*, antioxidant, hot acid extraction.

INTRODUCTION

Gelatin is a product of collagen hydrolysis extracted from parts of the animal such as skin, bones and connective tissues. This insoluble protein consists of 50.5% carbon, 25.2% oxygen, 17% nitrogen and remaining 6.8% hydrogen. The outstanding property of thermos-reversibility in the food gel, which changes in the oral cavity is very effectively utilized. The cost effectiveness and easy access to this plant origin product for liberal (no religious limitations) utility is a commendable effort. In other words, gel in the gelatin able to melt at a lower temperature compared to other polysaccharides [1]. Opposite to the collagen features, gelatin has a distinctive nature as it is soluble in hot water. Commercial sources of porcine and bovine are skin, bone and hides of pigs and cattle, respectively. According to the statistic, highest production of gelatin was derived from pig due to a cheaper production cost and easily accessibility source as compared to cattle [2]. This polysaccharide has been widely employed in various industries such as food, pharmaceutical, cosmetic and also photography. In food productions, gelatin is regarded as one of the important ingredients used for several functions, be it as an emulsifier, water binder, gelling agent, thickener, elasticity and chew-ability improver, texture improver and many more. The good thing about gelatin is that it does not affect the flavour and colour of the food. Baked products, confectionaries, ice-creams and desserts are some examples of gelatin-food products. Nevertheless, the consumption of porcine and bovine gelatin is forbidden to certain consumers especially Muslims, Jews and Hindus for religious issues. In addition, the outbreak of mad cow disease which attacked European countries in the 1980s was drawing attention towards finding a gelatin alternative. Hence, the search for gelatin substitute is gaining interest among the researchers. Marine sources including fish and poultry (chicken and duck skin, feet and bones) are found to have chances to replace mammalian gelatin [3]. Besides, several finding also stated that gelatin can be derived from insects [1,4,5]. Besides animal, replacement is also studied onto plant materials. Carrageenan, agar- agar, pectin and konnyaku are also observed to have gelatin's characteristics. However, among the list, the common practice is to replace gelatin from plant-based pectin as it is easily extracted from fruits. The best source of plant based pectin turned out to be dragon

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fruit and other origins are marine based. *Hylocereus polyrhizus* or famously known as Red dragon fruit appears to be a good example that can be benefited from in terms of pectin content [6]. This tropical fruit is belongs to the cactus family, Cactaceae. Dragon fruits are also cultivated around Southeast Asia as a food source and widely consumed in the form of juice. However, dragon fruit peels will be discarded as a result of the peeling process in the juice industry and create a waste for the environment. Surprisingly, that peel which constitutes to 22% of the whole fruit contains the highest level of pectin which can be used as gelatin replacer. A pectin is a group of complex hydrophilic colloidal hetero-polysaccharide with a molecular weight ranging from 50, 000 to 150,000 Daltons. The component of this complex polysaccharide is located at the primary cell wall in plants. It is a polymer of 1-4 linked α-D-galacturonic acid with a variable number of methyl ester groups. D-galacturonic is an oxidized form of D-galactose and presented as polygalacturonic acid in pectin [7].

Various studies have confirmed that pectin has the ability to form gel and thus possesses significant nutritional and technological properties [8]. Gelling properties of pectin are affected by the Degree of Esterification (DE) which also determines the group of pectins. High methoxyl pectin (HM pectin) is pectin with DE > 50 and low methyl pectin (LM pectin) have DE <50 [9]. Pectin is widely used in food, pharmaceutical and also cosmetic industries. In food products, pectin is commonly used as a gelling agent in jellies, jams, marmalade, ice- creams, marshmallow, dairy products, and processed meats. Most of the commercially supplied pectins are produced by apple pomace and citrus peel [10]. Therefore, the need of utilizing a waste material as a source of pectin is indeed important. This study aimed to assess the physicochemical properties of pectin extracted from dragon fruit and compare it with the commercial gelatins from bovine and porcine.

MATERIALS AND METHODS

Sample Preparation

Approximately 500-550 g of *Hylocereus polyrhizus* (dragon fruit) peel samples were freeze-dried for 72 hours, after which the samples were ground into fine powder and kept at -20°C until further analysis.

Extraction of Pectin

8.0 g of powdered sample was rehydrated with distilled water with pH adjusted from 5.6, 5.0, 3.5, 2.0 and finally 1.4 with 1M of hydrochloric acid. The extraction was carried out in a time-dependent manner at 11, 30, 75, 120 and 139 minutes and heated at 70ºC, optimum temperature for extraction using the stirring hot plate (500 rpm) [10,11]. The mixture was then centrifuged for 5 min at 10 000 rpm. Following that, double volume of 95% ethanol was added for overnight precipitation. The next day, pectin was separated from the alcohol solution using a double layer of cheesecloth and the samples were washed three times with 70% alcohol and once with undiluted alcohol to remove any impurities such as monosaccharide and disaccharide [12]. Then, pectin yields were dried using freeze dryer to remove all moistures. Samples were cooled, weighed, ground and stored in small plastic sample bags prior to analysis. The pectin yield was calculated as the ratio of the weight of dried pectin to the dried powder taken for extraction for each parameters.

Determination of DE and Confirmation of Pectin Identity

The degree of esterification (DE) was determined using the Fourier Transform Infrared (FTIR) spectroscopy method adapted from [13]. DE is defined as the percentage of number of esterified carboxylic groups over number of total carboxylic groups. The samples were analysed using FTIR to confirm the identity of pectin extracted from dragon fruit and to estimate the DE. Commercial pectin with known DE of 63% from citrus pectin was used as standard. The spectra was recorded in the absorbance range from 4000 to 400 cm^{-1} (mild- infrared region). DE calculations were made as follows:

DE = (Area of esterified carboxyl groups / area of esterified carboxyl groups + area of non-esterified carboxyl groups) x 100%

Determination of Ash and Moisture Contents

The ash content was determined by weighing 2 g of samples in a tared crucible. The crucible was then heated in a muffle furnace at 600ºC. The residue was cooled in desiccator and weighed to constant weight. Moisture content determination was done by drying the samples at 105ºC for 4 hours until it reaches a constant weight. Then, the samples were removed into desiccators to cool down and weighed again with the moisture dish. The following equations were used to calculate ash and moisture contents:

Ash content (%):

[(moisture dish + wet sample) - (moisture dish + dry sample)] / (wet sample) x 100 %

Moisture content (%):

[(moisture dish + wet sample) - (moisture dish + dry sample)] / (wet sample) x 100 %

Determination of Antioxidant Activity using DPPH Scavenging Assay

DPPH Solution Preparation

To prepare 0.2 mM of DPPH solution, 0.0788 g was dissolved in 1 ml of solvent. The solution of DPPH was prepared according to the method used in [14]. This test required around 25 ml of the DPPH solution, therefore 2.00 g of DPPH was weighed and mixed with 25 ml of MeOH to produce 0.2 mM DPPH solution.

Ascorbic Acid Standard Preparation

100 µl of MeOH was pipetted using multichannel pipette (6 tips) into holes B1-B6, C1-C6, until H1-H6 of 96- well micro plate. Then, 200 µl of ascorbic acid with 1 mg/ml concentration dissolved in MeOH were pipetted into holes A1-A6. The samples were then diluted using serial dilution technique by pipetting 100 µl of ascorbic acid samples from holes A1-A6 and transferring into holes B1-B6. The step was repeated from holes B1-B6 to the next holes until holes H1-H6. 100 µl of the solution from the last holes were discarded. After that, 100 µl of prepared DPPH solution were transferred into holes A4-A6 until F4-F6, and 100 µl MeOH into holes A1-A3 until H1-H3. The microplate was covered and placed in a dark cabinet at room temperature for 40 minutes before the absorbance analysis was done using UV-Vis spectrophotometer at 517 nm.

RESULTS AND DISCUSSION

Pectin Yield

Table **1** established that the yield of the pectin extracted from the dragon fruit peel ranged from 13 to 33% in relation to different exposure of time and pH value. Extraction time at 75 minutes and pH value at 3.5 using hydrochloric acid was observed to produce substantially highest percentage of pectin yield that is 33%. This is in agreement with previous study that stated extraction time and pH conditions are two key factors affecting pectin yield [15-17].

Hydrochloric acid was used since it yields the highest percentage of pectin, defeating nitric acid and citric acid from guava peel, citrus fruits, banana and coco pods [18,19]. Hydrolysis of pectin from proto pectin was enhanced by higher concentrations of hydrogen ions as it enhances the capability of pectin to precipitate. This could be happening when greater ionic strength acids have better attraction to cations such as Ca^{2+} which stabilizes the pectin molecule. Table **1** shows that highest percentage of pectin yield in average was obtained by sample E (33%) under conditions of pH 3.5 and 75 minutes of extraction since previous studies reported that pH medium between 2.8 to 3.5 is important for jellification [20,21]. Lowest percentage of pectin yield was obtained by sample C (13%) at 120 minutes' extraction time and at the pH value of 2.0, which is contradicts with the results observed in previous study that the best pectin yield was at 1 hour of extraction and lower yield at longer extraction time of more than 60 minutes [6,22,23]. Similar results were also observed in the extraction of pectin from orange peel using microwave field [24]. Nevertheless, the extracted pectin is ready to be commercialized as long as the yields are more than 10% [17].

Determination of DE and Confirmation of Pectin Identity

The determination of pectin identity in extracted samples was made by comparing the FTIR spectra with standard commercial pectin. The finding shows

Table 1: Percentage of Pectin Yield in Response to Different Exposure of Time and pH Value

Sample	Parameter		Average yield (%)
	pH	Time (min)	
A	1.4	75	25
B	2.0	30	19
C	2.0	120	13
D	3.5	11	30
E	3.5	75	33
F	3.5	139	19
G	5.0	30	22
H	5.0	120	17

that absorption pattern of samples shared similarities with the standard, and thus confirmed the samples identity as pectin. Figure **1** established that stronger areas of absorption between 3600 and 3000 cm^{-1} indicated O-H stretching due to linking of intra- and inter- molecular bonds. Several features of a compound, including free hydroxyl groups stretching bonds that occurred in vapour phase and bonded O-H bands of carboxylic acid were also shown in these absorption areas. Further analysis showed that absorption band at 2900 cm^{-1} was due to C-H stretching of CH_2 groups. Area between 1740 and 1630-1600 cm^{-1} showed weaker absorption due to ester carbonyl groups (C=O) and carboxyl ions (COO-) stretching [25]. To identify the major chemical group in polysaccharides, absorption area between 1300 and 800 cm^{-1} was determined as it is the finger print region for carbohydrates [13,26-29].

The calculation of pectin DE was analysed by using the peak area in relation to the free carboxyl groups (1630- 1600 cm^{-1}) and esterified groups (1740 cm^{-1}).

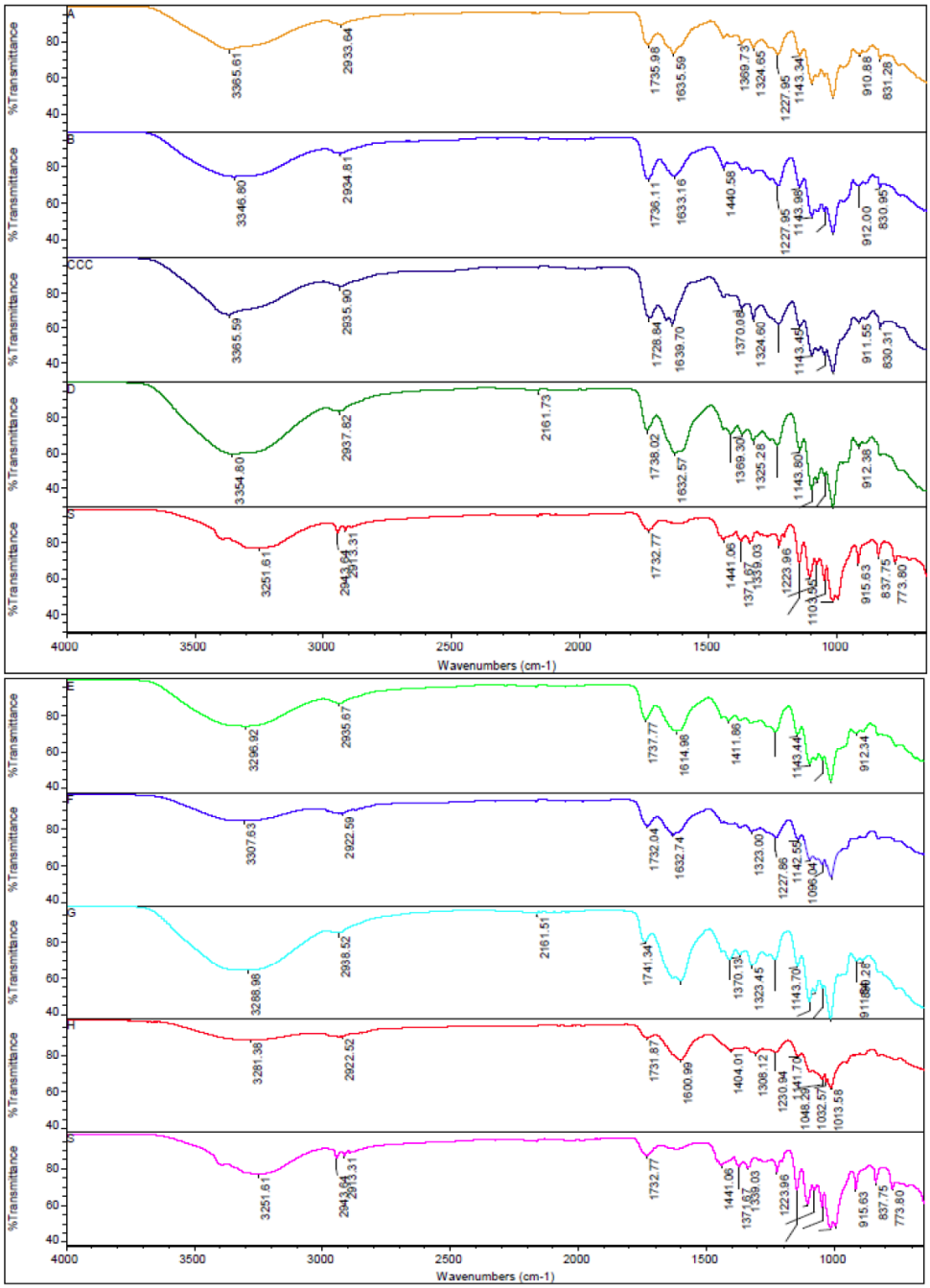

Figure 1: The FT-IR spectra comparison of dragon fruit peel pectin samples (A, B, C, D, E, F, G, H) and commercial pectin standard (S).

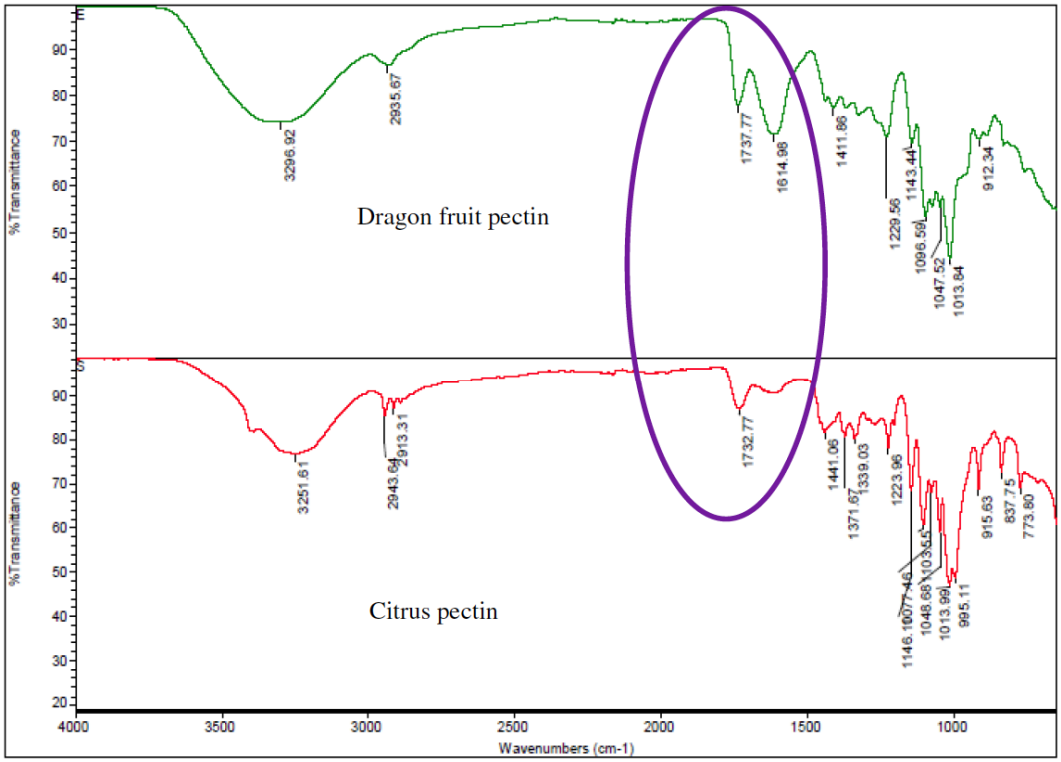

Figure 2: The FT-IR spectra comparison of dragon fruit peel pectin sample (DE 52%) and standard commercial citrus pectin (DE 63%).

Figure **2** revealed that the FT-IR spectrum of standard commercial citrus pectin with DE 63% is high methyoxyl (HM) pectin, as the absorbance is higher at 1750 cm^{-1} than at 1650 cm^{-1}. Dragon fruit peel pectin was also having the same absorbance pattern as the standard, and thus it also falls under HM pectin group. The estimated DE of dragon fruit peel pectin was 52%, which proves that it is HM pectin as the DE is more than 50%. Low methoxyl (LM) pectin would exhibit lower absorbance at 1750 cm^{-1} as compared to 1650 cm^{-1}. Previous study also stated that dragon fruit peel pectin is categorized as HM pectin [30].

Determination of Ash and Moisture Contents

Ash content in dragon fruit peel pectin was found to be 8.73 ± 1.21. Previous studies reported that different ash content of dragon fruit peels ranged from 6.88%, 7.10%, 10.02%, 11.55% and 11.95 resulted from different methodologies used [27,28]. Ash content would determine the purity of pectin and percentage below 10% indicated good criteria for gel formation [18,22,27,31]. Thus, pectin extracted from dragon fruit peel was comparable to standard commercial pectin (9.08 ± 0.28), as well as gelatins (bovine (7.75 ± 1.00) and porcine (5.91 ± 1.61). The moisture content was varied among four different samples ranging from 7.23 ± 0.41 (standard commercial pectin) to 14.03 ± 1.92 (dragon fruit peel pectin). The value of moisture content in dragon fruit peel pectin was slightly higher compared to finding from previous study which observed that the amount was between 11.13 to 13.13%. Pectin quality is moisture content dependent where increasing percentage of moisture content will promote microorganism growth caused by pectinase enzyme production [22,28].

Table 2: Percentage of Ash and Moisture Contents of Pectin and Gelatins from Different Sources

Samples	Ash	Moisture
Dragon fruit peel pectin	8.73 ± 1.21	14.03 ± 1.92
Commercial citrus pectin	9.08 ± 0.28	7.23 ± 0.41
Bovine gelatin	7.75 ± 1.00	9.22 ± 0.45
Porcine gelatin	5.91 ± 1.61	10.07 ± 0.12

Scavenging Activity of Dragon Fruit Peel Pectin

Table **3** represent 50% inhibition value of dragon fruit peel pectin and ascorbic acid as a standard. DPPH radical activity become diminish in the presence of antioxidant and is reflected in decreasing absorbance reading at 517 nm. This DPPH assay was used to determine the ability of dragon fruit peel to donate

Table 3: DPPH Antioxidant Test on Different Concentration of Pectin

Betalain concentration (mg/ml)	Ascorbic acid (standard)	Inhibition (%)
1.0	84.30	57.94
0.5	84.11	53.64
0.25	83.74	52.80
0.125	82.80	51.59
0.063	83.18	51.68
0.032	82.62	50.93

hydrogen to DPPH radical. Reduction of DPPH capability was determined by purple discoloration of DPPH methanol solution at 517 nm. At this time, the initial, stable free radical of DPPH concentration is reduced to 2, 2-diphenyl-1- picrylhydrazine (yellow colour) when it interacted with antioxidant [32,33]. 50% inhibition activity of dragon fruit peel had positive correlation with DPPH concentration as the value decreased from 57.94% to 50.93%. Betacyanin was found to be the highest free radical hunter present in the sample. Besides, some phenolic compounds were also correlated with high antioxidant activity of dragon fruit peel [33]. For standard ascorbic acid, 50% inhibition activity also showed a decreasing trend from 84.30% to 82.62% by decreasing concentration of DPPH. These values proved that ascorbic acid has more free radical scavenging potential than the sample as explained by previous study [28].

CONCLUSION

Pectin, the basic building material of cell walls in the terrestrial plant has great potential to be replace gelatin as it can work as a gelling agent, thickener and also a stabilizer. Dragon fruit contains pectin which has high-value functional food as well as health-enhancing properties to substitute gelatin's function in foods production. Extraction time at 75 minutes and pH value 3.5 using hydrochloric acid was observed to produce substantially highest percentage of pectin yield at 33%. The FTIR result proved that dragon fruit peel contained pectin which can be used as gelatin replacer are free from any religious and health-wise prohibitions. Ash and moisture contents in dragon fruit peel pectin were found to be 8.73 ± 1.21 and 14.03 ± 1.925 respectively. Ash content would determine the purity of pectin and percentage below 10% indicated good criteria for gel formation. The extracted pectin of dragon fruit peel acts as the best gelatin replacer compared to commercial pectin and gelatins from the market. The prepared fruit peels also exhibit high DPPH scavenging activity (57.94%) with methanol extract (2mg/ml).

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