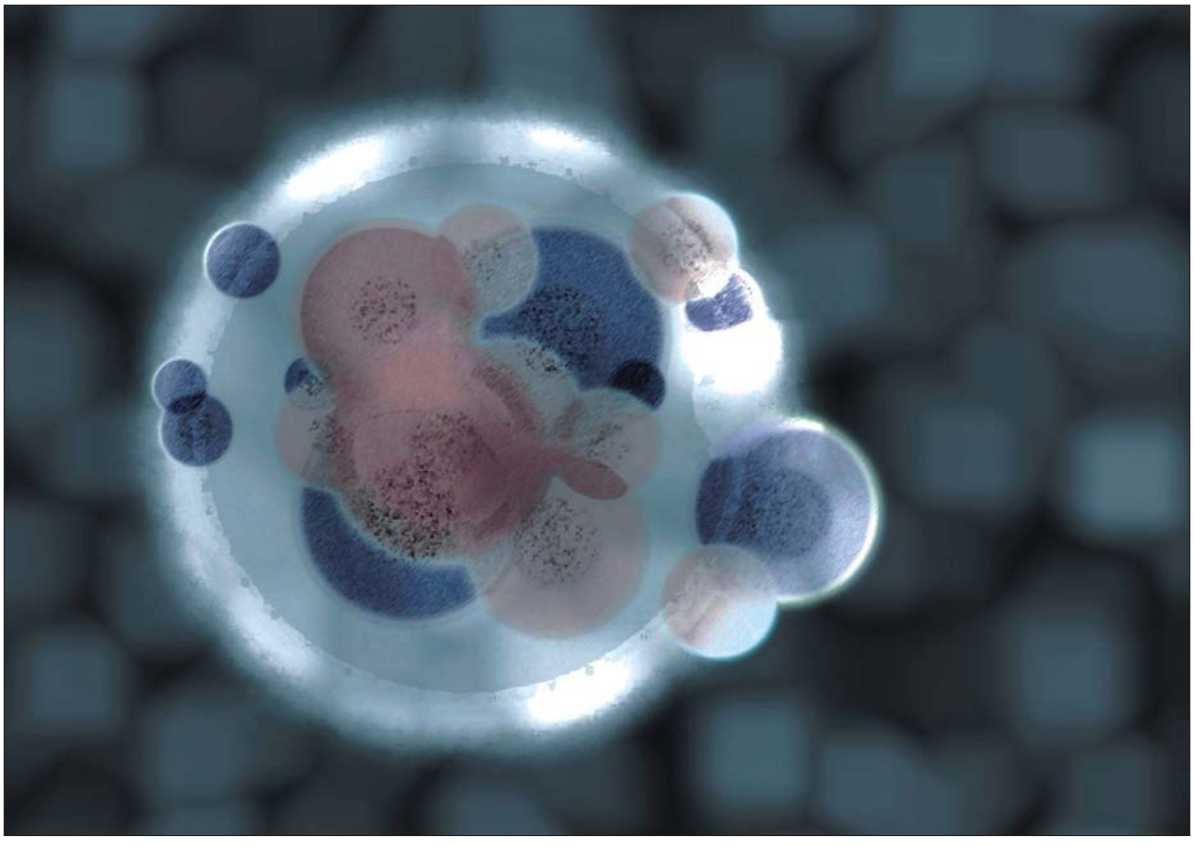

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Pectin Extraction and Characterization from Red Dragon Fruit (*Hylocereus polyrhizus*): A Preliminary Study

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Abstract: The present study was focused on the potential of red dragon fruit (*Hylocereus polyrhizus*) peel to be a source of pectin. The peel of red dragon fruit was collected from the local beverages factory and oven dried before pectin extraction. Dried fruit peels were first blended with distilled water and further acidified with different volume of citric acid to pH 3.0, 3.5 and 4.0, respectively. The acidified samples were then treated at 75°C in different time interval (30, 60 and 120 min) to extract pectin from the fruit peel. Highest yield with a total of 14.86% was obtained in 60 min extraction, pH 3.5. Characterization of the extracted pectin in terms of glucose content and degree of esterification was conducted. Highest glucose content was observed in sample extracted for 60 min, pH 4.0, whilst sample with the highest degree of esterification (DE) was obtained from treatment at pH 4.0, 120 min.

Key words: Fruit pectin, carbohydrate polymer, hydrocolloid, degree of esterification, purple pitaya

INTRODUCTION

Pectin is a carbohydrate polymer consisting α-D-galacturonic acid linked through the 1 and 4 positions. In some cases, the carboxyl groups are esterified with methanol (Walter, 1991). This heterogeneous grouping of acidic structural polysaccharides can be found in primary cell wall and intercellular space (middle lamella) of fruits and vegetables. According to the literature, fresh weight of plant material accomplishes 0.5-4.0% of pectic substances (Faravash and Ashtiani, 2008; Kashyap *et al*., 2001; Sakai *et al*., 1993). Sources of pectin include banana, beets, cabbage, carrots etc. However, the main raw materials used to produce commercial pectin are apple pomace and citrus peels (Wang *et al*., 2007). Recently, sugar beet and sunflower have been utilised as sources of pectin extraction.

The major application of pectin is in food and beverages industrial due to its gelation properties which served as the basis of jam and other fruit preserves (IFT, 1991; Walter, 1991). Gelation of pectin is due to partial dehydration of the pectin molecule to a degree in which the molecule stayed in a form of an intermediate between solution and precipitation. The gel formation property is very much influence by the degree of esterification (DE) and the molecular weight (Thakur *et al*., 1997). Therefore, pectin is generally categorised into two groups based on the DE, High Methoxy (HM) pectins and Low Methoxy (LM) pectins. Pectin with degree of methylation (DM) higher than 50% is grouped under high methoxyl pectin (HM). The HM pectins can be deesterified to LM pectins with the aid of acid, alkali, ammonia, or pectin methyl esterase (Ralet *et al*., 2001). Both category of pectin has different requirement in forming gel. The HM pectin requires heating in sugar solutions at concentration higher than 55% and pH lower than pH 3.5 for gel formation. On the other hand, formation of gel with a low methoxyl pectin (LM; DM< 50%) requires the presence of calcium ions. The gelling properties of LM enable its application in some low sugar food applications, such as low-calorie jams and jellies, confectionery jelly products. Despite the gelling properties of LM, its application in food can be further extended in bakery jams and jellies for glazing, retorting, microwaving, baking and sterilizing or pasteurizing, due to the heat reversibility of the gels. Besides, pectin is also applied in the pharmaceutical, dental and cosmetic industries for its gellifying properties (Endress, 1991).

It has been reported that the worldwide annual consumption of pectin is estimated approximately 45 million kilograms (Willats *et al*., 2006). Due to some major demand of pectin in food and pharmaceutical industries, numerous investigations have been carried out

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to determine the composition of pectin in different plants (Arthey and Ashurst, 1996; Jayani *et al*., 2005; Kalapathy and Proctor, 2001).

Researches concerning the extraction methods and characterization of fruit pectin have been reported elsewhere (Fissure *et al*., 2009; Kurita *et al*., 2008; Mollea *et al*., 2008; Yapo *et al*., 2007) however, using red dragon fruit as a source of pectin has not been reported so far. Information concerning the pectic substances of dragon food is limited. There are two main varieties of dragon fruit cultivated in Malaysia, purple skin white flesh dragon fruit and purple skin with purple flesh dragon fruit, which the latter is commonly used for production of juice. In red dragon fruit juice production, only the flesh is processed, the left over peels will be discarded. The disposal of such waste products has become the burden to the industry as well to the environment. Hence, pectin extraction from red dragon fruit peels might provide an alternative way to reduce the expenses for waste disposal and at the same time generate side incomes.

Pectin extraction method applied to date usually involved treating plant samples with hot dilute mineral acid to reduce pH to approximately pH 2.0. Pectin extraction is a multiple-stage physical-chemical process in which the hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, pH and time (Kertesz, 1951).

In the present study, red dragon fruit peel was selected as the source of pectin. The fruit peel will be subjected to various pH and heat treatment duration to obtain optimum extraction conditions and the effect of extraction parameters to the yield of pectin will be determined.

MATERIALS AND METHODS

Pectin extraction and characterisation was carried out in Universiti Tunku Abdul Rahman in between 2008 to 2009.

Pectin extraction: A total of 20 g dragon fruit peel was dried in an oven at 55°C until constant weight. Dry weight was recorded for pectin yield determination. The dried dragon fruit peel was then blended with 250 mL distilled water and acidified with different volume of 0.1 N citric acid (35, 65 and 105 mL) and pH of the mixtures were recorded. The pectin extraction procedure was continued with treating the acidified samples at 75°C in different time interval (30, 60 and 120 min). Heat treated samples were then filtered and 95% ethanol was added in to allow pectin precipitation. The procedure was carried out in dark at 25°C. Precipitated pectin was recovered by centrifugation at 5000 x g for 10 min, subsequently washed twice with 70% ethanol. Acetone was then added in a drop wise manner to remove unwanted colour. Acetone was added until the top liquid phase was completely clear. The resulted pectic substance was dried at 65°C. The percentage yield of the dried dragon fruit pectin was determined.

Determination of glucose content in dragon fruit pectin: Glucose oxidase (1 mL) was added into 10 μL pectic sample. The reaction mixture was allowed to react in dark at 25°C for 20 min. The released of glucose was observed at 505 nm.

Determination of degree of esterification: The degree of esterification (DE) of dragon fruit pectin was determined using potentiometric titration method according to Bochek *et al.* (2001) with slight modification. Dried pectin (50 mg) was wetted and stirred for complete dissolved. The resulted solution was titrated with 0.1 N NaOH and a few drops of phenolphthalein. The titration volume was recorded as the initial titre (It). Subsequently, another 30 mL of 0.1 N NaOH solution was added and the mixture was stirred at room temperature for 30 min to de-esterified the pectin. Next, 0.1 N H_2SO_4 (30 mL) was added to neutralised the NaOH. The mixture was further titrated with 0.1 N NaOH in the present of phenolphthalein. The total titration volume (Ft) of NaOH was recorded. The DE was calculated using the equation below:

$$\%DE = \left[\frac{Ft}{(Ft + It)}\right] \times 100\% \quad (1)$$

Statistical analysis: All measurements were done in triplicate. Analyses of Variance (ANOVA) were conducted by using SPSS version 16.0 for Windows (SPSS, Chicago, IL, USA). Duncan test was performed to test the significant differences between the mean values for treatments ($p<0.05$).

RESULTS AND DISCUSSION

Pectin yield: Figure 1 shows the influence of pH on the yield of pectic substances. Generally the yield of pectin increased accordingly from pH 3.0 to 3.5 and decreased at pH 4.0 regardless of the extraction time. The lowest yield (4.25%) was obtained from sample adjusted to pH 3.0 and heat treated for 120 min. On the other hand, optimum yield (14.86%) was obtained from sample adjusted to pH 3.5 heat treated for 30 min. Generally, the time duration for sample heat treatment has influence towards pectin yield.

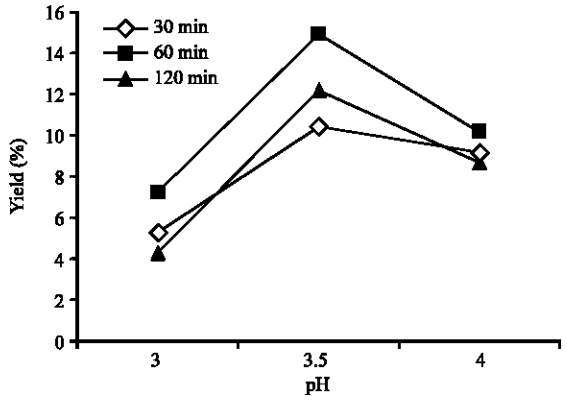

Fig. 1: Yield of red dragon fruit pectin from varies pH and heat treatment conditions

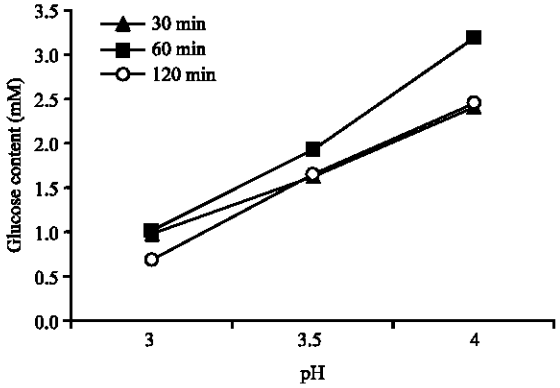

Fig. 2: Glucose concentration of red dragon fruit pectin treated with varied pH and extraction time

Heat treatment within 60 min showed significant high yield disregards of pH. As heat treatment prolong, a decreased in approximately 2.5% pectin yield was observed at pH 3.0 and 3.5. However, extraction time did not play a major influence on pectin yield. Based on the yield obtained in this study, pH 3.5-4.0 exhibited a promising recovery in terms of yield. Indicated that extreme pH (pH 3.0) might not be suitable for maximum extraction for pectin. Results from the statistical analysis indicated that pH and the heat treatment time period influence the pectin yield obtained. The analysis results show that pH 3.5 with the extraction time of 60 min is the optimum condition for high pectin yield in this study.

Glucose content: In terms of glucose content, sample with 60 min heat treatment demonstrated higher glucose content ranging from 3.2 to 1.0 mM as compared to treatment at 30 and 120 min (Fig. 2). Based on the results on the time period for heat treatment and pH values of the samples, it was found that sample at pH 4.0, treated with

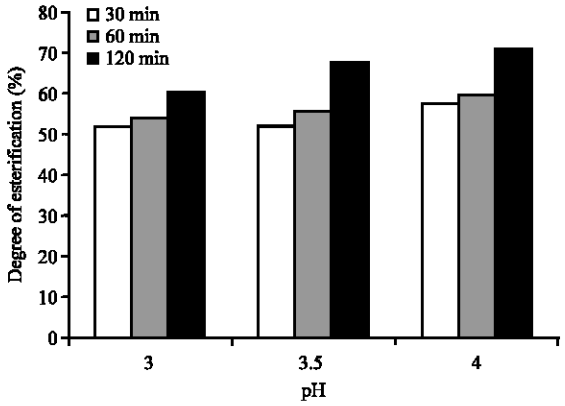

Fig. 3: Effect of pH and extraction time on the degree of esterification (DE) of red dragon fruit pectin. E

heat for 60 min showed the optimum glucose content of 3.2 mM. In contrary, sample in pH 3.0 with prolong heat treatment up to 120 min exhibited the lowest glucose content. The results indicated that heat treatment time and pH did contribute to the glucose content in the sample pectin. Statistical analysis on the relationship to extraction time and pH towards glucose content exhibited that pH and extraction time effect the glucose content in the extracted pectin. According to the analysis, high glucose content could be obtained at pH 4.0 with the extraction time of 60 min.

Degree of esterification: Degree of esterification was closely related to the duration of heat treatment. As the heat treatment prolong, degree of esterification was found to increase at the same time. The increased in pH also demonstrated higher degree of esterification in the samples. Highest degree of esterification was observed in sample treated in heat for 120 min at pH 4.0, which was more than 70.0% (Fig. 3). In contrary, only 51.0% was obtained in sample treated in heat for 30 min at pH 3.0. Based on the statistical analysis, pH and extraction time was the factors affected the DE of the extracted pectin. Extraction time of 120 min at pH 4.0 demonstrated the highest DE among all the parameters tested.

Generally, precipitation of pectin is pH dependent. Therefore, optimum yield might not be obtained if the precipitation is not carried out at the optimum pH. Results obtained from the present study indicated that pectin yield the highest from pH 3.5 (Fig. 1) which is agreeable to some studies. Kertesz (1951) reported that high concentration of hydrogen ions present in the solvent (at low pH) stimulates the hydrolysis of protopectin At lower pHs, the highly hydrated carboxylate groups are repressed in the larger hydrogen ion concentrations and therefore, converted into slightly hydrated carboxylic acid

groups (BeMiller, 1986). The lost of charge is able to reduce the repulsion of the polysaccharide molecules which promote the gelation properties of pectin giving more precipitated pectin at lower pH. Thus, the decreased in pH is able to promote the liberation of pectin molecules from the peel during acid-washing stage because of the interaction of pectins to the hemicellulose fractions are cleaved (Rombouts and Thibault, 1996). The reduction of yield at higher pH (pH 4.0) might be due to some pectin is still attached to the cell wall components, although pectin molecules can be partially solubilised from plant tissues without degradation by weakly acidic aqueous solvents (Voragen *et al*., 2003). In order to improve the yield, this type of pectin constituent (protopectin) is suggested to be hydrolysed by acid. Based on some studies, the use of hydrochloric acid (Kalapathy and Proctor, 2001; Hwang *et al*., 1998; Dinu, 2001) and nitric acid (Pagan *et al*., 2001) for pectin extraction is recommended. However, extraction with citric acid is reported to produce the highest average yield (13.75 %) in apple pomace pectin. In the present study, citric acid is used for pectin extraction, the yield obtained in this study is comparable to soy hull pectin where the yields decreased with increasing acid strength (Kalapathy and Proctor, 2001).

Effect of extraction time on the yield of pectin: Despite pH, the results obtained in this study indicated that extraction time as well is one of the factor affected the yield. Pectin yield increased initially but declined after 60 min of extraction (Fig. 1). The decreased in the pectin yield by the increased in extraction period may be due to the thermal degradation of the extracted pectin. The degradation is mainly caused by the depolymerisation mechanism of galacturonan chain of pectin, which is known as beta-elimination (Albersheim *et al*., 1960). Thus, the pectin cannot be recovered by precipitation with alcohol (Kertesz, 1951). The results obtained from this study are in agreement with Robert *et al*. (2006) and Garna *et al*. (2007) working on chicory roots and apple pomace, respectively. Pectin yield increased from 10.37 to 14.86% from 30 min treatment to 60 min treatment at pH 3.5, but decreased to 12.11% when treatment increased to 120 min (Fig. 1). Similar trend was observed in another study on the pectin obtained from sunflower head demonstrated an increased in pectin yield to 10.97%, whilst declined to 10.73% during extended extraction time (Sahari *et al*., 2003).

Estimation of glucose content in dragon fruits pectin: Glucose content was predominantly influenced by different volume of citric acid. The pectin extracted at lower amount of citric acid (higher pH) contained more glucose than those at higher amount of citric acid (Fig. 2), suggesting that glucose content of pectin increased with increasing pH.

Glucose is one of the content of neutral saccharides (rhamnose, galactose, arabinose, xylose, mannose and glucose) in pectin. In the present study, the neutral sugars content obtained is lower than that reported for pectins sourced from prickly pear fruit peel and potato pulp (Habibi *et al*., 2004). On the other hand, glucose content of red dragon fruit pectin is higher than those reported from fresh sugar beet (295-528 mg g^{-1}) (Levigne *et al*., 2002). In general, the neutral sugar side chains are linked to rhamnogalacturonan segments within the pectin molecule (Seymour and Knox, 2002). Low glucose content may suggest the existence of long chains polygalacturonate region (homogalacturonan), which is essential for the gelling process. The presence of glucose and xylose could be explained, in part, by the presence of xyloglucans, which have been shown to be associated with pectic material (Selvendran and Du Pont, 1980) or byxylogalacturonan (Kikuchi *et al*., 1996).

Degree of esterification of pectin: In this study, pH 4.0 was the optimum pH for extraction of pectin with high percentage of DE (58.8%-71.0%) (Fig. 3). Similar trends could be observed for majority of low pH pectin extraction procedure (Faravash and Ashtiani, 2008). Hence, based on this study it is suggested that pH 4.0 is preferable for obtaining pectin of commercial quality as compared to the DE value (55-75%) of those commercially available pectin from citrus or apple pomace (Severian, 1998). The DE of pectin extracted at pH 4.0 was also close to those pectins extracted from passion fruit peel using hydrochloric acid as extractor (69.7-73.2%) (Corona *et al*., 1996; D'Addosio *et al*., 2005; Matsumoto and Otagaki, 1990) and apple pomace (68.8%) with citric acid as extractor (Canteri-Schemin *et al*., 2005). Results also indicated that DE was significantly influence by the pH and extraction time (Fig. 3). In this study, highest DE was obtained by sample treated for 120 min at pH 4.0. According to Faravesh and Ashtiani (2008) and Pagan *et al*. (2001), prolong extraction time will lead to higher pectin quality under a constant pH and temperature. Therefore, it could be assumed that extraction time of 120 min favoured the extraction of high-quality pectin and would be the most suitable extraction time for obtaining pectin with high quality.

Besides, other factor such as the addition of alcohol during extraction is believed to affect the DE. Alcohol is believed to change the thermodynamic condition of monophase system into two-phase gel-liquid system such

as the interaction between water molecules, carboxylic groups of pectin and alcohol functional groups (Faravash and Ashtiani, 2007).

CONCLUSION

Pectin has been successfully extraction from the red dragon fruit peel with different extraction parameters. The results from the present study indicated that pH and extraction time were the major factors affecting the pectin yield, glucose content and the degree of esterification. pH 3.5 and 60 min heat treatment demonstrated a promising yield for pectin extraction.

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