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Extraction and Characterization of Pectin from Dragon Fruit (*Hylocerens polyrhizus*) Peel Using Different Concentration of Ammonium Oxalate

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Abstract: The study was focused on the red dragon fruit (*Hylocerens polyrhizus*) peel which can be used as a source of pectin .The physicochemical properties were studied moisture, protein, fat, ash, total soluble solid TSS, pH and titratable acidity TA .Using three different concentration of ammonium oxalate 0.5 ,1 ,2 gm. At pH 4.9, temperature 90 °C, time 90 min to extract pectin. Highest yield with about 0.5 gm. Concentration ammonium oxalate was 26.64% following 23.75%, 15.88% yields at concentration 1 and 2 gm. ammonium oxalate .There were significant differences in yields at ($p< 0.05$). Characterization of the extracted pectin in terms of equivalent weight, methoxyl content, degree of esterification, an hydrouronic acid AUA, Fourier-transform infrared spectroscopy FTIR, viscosity, setting times were studied. Based on the value of methoxyl content 2.54% and degree esterification pectin can be categorized as low methoxyl.

Keywords: Ammonium oxalate, Dragon fruit peel, Extraction, Pectin.

Introduction

Dragon fruit is a member of the family cactaceae family, and it was called as pitaya or pitahaya (Haber, 1983; Mizrahi *et al*., 1997). Based on peel and pulp color, dragon fruit can be divided into three types, i.e., *Hylocereus undatus* (red peel ,white pulp), *Hylocereus costaricensts* (red peel and red pulp) and *Hylocereus megalanthus* (yellow peel and white pulp) (Nerd *et al*.,2002; Hoa *et al*., 2006). It is commercially available world-wide for improving many healthy problems and is well known for the rich nutrient contents such as protein, carbohydrate, fat, crud fiber, vitamin C, vitamin B_1, vitamin B_2, and vitamin B_3, phenolic, carotene, thiamin, niacin, pyridoxine, flavonoid, kobalamin, betacyanins, iron, phosphorus and phytoalbumin (Le Bellec *et al*., 2006). It is also rich with plytoalbumins, which are exhibiting higher capacity for their antioxidant properties (Mahattanatawee *et al*., 2006). Fruits are usually processed into juice, beverage, squash and syrups. During processing, peel is contributing almost 5-2% of the total fruit .These by-products are also rich with beneficial compoundes such as polymethoxylated flavonoid and hydroxycinnamtes, which are found in peel (Hamapitour *et al.,* 2004).

Pectin is polysaccharide contening 1,4 linked □-D-galacturonic acid residues (Levigne *et al.,*2002). It is found in the cell walls and middle lamellae. There is about

300-1000 chain of galacturonic acid units consist of these polysaccharides (Yeoh *et al*., 2008). Pectin is widely used in the food industry as emulsifier, stabilizer, thickener, fermented dairy products, used jellies as a gelling agent, also used fruit drink (Tsoga *et al*., 2004). Pectin reduces the risk of heart disease by controlling blood pressure and cholesterol levels (Liu *et al*., 2006). Pectin can be classified into two types, high methoxyl pectin (D.E.> 50%) and low methoxyl pectin (D.E. < 50%) (Zhang and Taihua, 2011). Pectin quality and purity depending on degree of estrification, ash content an hydrogalacturonic acid, and molecular weight (Chakraborty and Ra, 2011). The study was aimed to evaluated the different extraction concentration from ammonium oxalate on yield of dragon fruit pectin and characterize pectin.

Picture (1): Dragon fruit.

Materials and Methods

Sample Collection and Preparation

The fresh dragon fruits were collected from Field Horticulture of Agriculture Directorate Basrah in 20-8-2016.The samples were prepared according to method described by Ismail *et al*. (2012). The fruits were cleaned and washed using the tap water, the fruit pulp was removal from peel then cut into small parts, thereafter it was dried in oven at 50°C.The dried peel was grinded in an electronic grinder. The powder was sieved and stored until uses.

Physiochemical Properties

The Physiochemical Properties: moisture, ash, protein, fat, pH, titratable acidity, total soluble solid were determined according to method (A.O.A.C., 1990). Viscosity and Setting time were determined according to method described by Yoo *et al*. (2006) and McCready (1970) respectively.

Extraction of Pectin

5gm of peel powder was mixed with120 ml of each concentration of ammonium oxalate (0.5, 1, 2 %) at pH 4.9 (adjusted with oxalic acid), the mixture was heated and stirred at 90 °C for 90 min .The extractions were filtered using a nylon cloth, and the pectin was coagulated with absolute ethanol (99%) in the ratio of 1:1 (w/v) and remained for 60 min at refrigerator, the pectin was filtered using filter paper what man No.4 and then dried in oven at 50 °C, the dried pectin was stored in polyethylene bag.

Picture (2). Dragon fruit peel pectin.

Pectin Yield

The yield of pectin was calculated using the formula**:**

$$\text{pectin\%} = \frac{\text{amount of extracted pectin in (g)}}{\text{amount dried peel used (5g)}} \times 100$$

Determination of Equivalent Weight

Equivalent weigh was determination according to Rangann (2008). 0.5gm sample was moistening it with 5 mL of ethanol .1gm of sodium chloride, 100 ml of distilled water and 6 drops of phenol red were added, mixture was stirred for dissolving the substance, titrated with 0.1 N NaOH until the color changed to pink. This neutralized solution was used for methoxyl content determination. The equation was following to calculate the equivalent weight :

$$\text{Equivalent weight} = \frac{\text{weight of sample} \times 1000}{\text{ml of alkali} \times \text{normality of alkali}}$$

Determination of Degree Esterification (D.E.)

The D.E. was done using method described by F.C.C. (2004). A 200 mg sample which was mixed with 2 ml ethanol and dissolved in 20 ml of distilled water for 2 h at 40 °C. The solution was calibrated with 0.1 N NaOH. The calibration volume was considered as the initial titre. Add 10 ml of 0.1 N NaOH to the solution and mixed well and let settle for 15min, then mixed with 0.1 N HCl (10 ml) .The solution was stirred until the pink color disappears. Excess HCl was calibration with 0.1 N NaOH even the faint pink color appeared. The calibration volume was recorded as the final titre. The D.E. was calculated as follow:

$$DE\ \%=\frac{\text{the final titer}}{\text{the in tital titer} + \text{the final titer}} \times 100$$

Determination of Total Anhydrouronic Acid Content (AUA)

The following formula was calculated the content of pectin from AUA (Mohamed and Hasan, 1995).

$$AUA\%=\frac{176 \times 0.1\ z \times 100}{W \times 1000}+\frac{176 \times 0.1\ y \times 100}{W \times 1000}$$

The molecular weight of AUA =176gm

Z=ml (titre) of NaOH from estimation equivalent weight.

Y=ml (titre) of NaOH from estimation methoxyl content

W=weight of sample

Determinationm of Methoxyl Content (Meo).

Determination of Meo was assigned according to Ranganna (2008). 25 ml of NaOH (0.25 N) was add to the solution used in the estimate equivalent weight, which was stirred and let settle for 30 min at room temperature. Then 25 ml of 0.25 N HCl was added calibration with 0.1N NaOH. Methoxyl content was calculated by following formula:

$$\text{methoxyl content}\ \%=\frac{\text{ml of alkalix} \times \text{normality of alkali} \times 3.1}{\text{weight of sampl}}$$

Structural Analysis

The FTIR spectra were used to obtain information on chemical structure with wave lengths 4000-400 using the method described by Singthong *et al*. (2004).

Statistical Analysis

Results were statistically analyzed using F test and the design complete random (C.R.D.) and using the program Excel 2013 at significant level (0.05).

Results and Discussion

Physiochemical properties of dragon fruit and peel

The table 1 showed the physiochemical properties of dragon fruit peel. The percentage of fat, protein, moisture, ash content in mature peel using dried sample were (0.5%), (5.25%), (7.47%), and (23.71%), respectively. These values were in agreement with the study done by Rahati *et al*.(2015) who, found the percentage of moisture, fat, protein were (5.2%, 0.8%, 6.6%), respectively.

Table (1): Physiochemical contents of dragon fruit peel

Moisture%	Protein%	Fat %	Ash%	pH	TSS(°Brix)	Titratable acidity%
7.47	5.25	0.5	23.71	4.26	4.5	0.38

The result showed the pH, total soluble solid (TSS) and titratable acidity content in peel were (4.26), (4.5), (0.38%), respectively .The results were in concord with Jamilah *et al*. (2011).

Pectin yield

The yield of pectin content of the dragon fruit peel was shown in fig. (1). The yield of pectin was depended on the concentration of ammonium oxalate. The highest yield of pectin was (26.64%) which was obtained from 0.5% ammonium oxalate. The extraction with 1% ammonium oxalate gave (23.75%) of pectin. While, extraction with 2% ammonium oxalate gave (15.88%) of pectin. Statistical results showed significant differences between treatments at (p>0.05). Ammonium oxalate works as a calcium binding that helps the pectin to release from the cell wall (Yeoh *et al*., 2008).

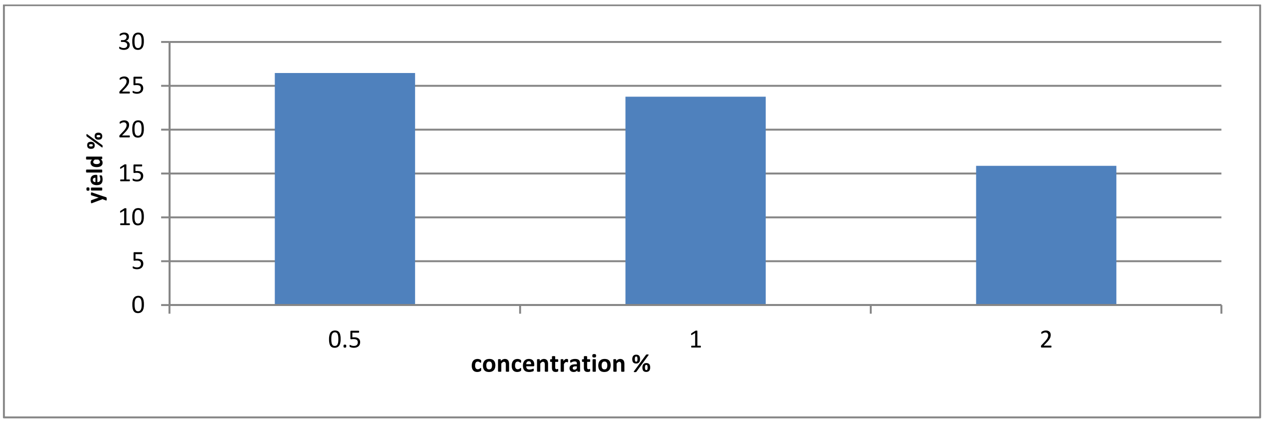

Fig. (1): Yield of dragon fruit peel pectin produced using different concentration ammonium oxalate.

Characterization of pectin

Degree of Esterification (D.E.)

Characterization of dragon fruit peel pectin was shown in Table (2). The degree esterification of dragon fruit peel pectin was (44.82%). Pectin extracted in this study can be classified as low methoxyl pectin due to it has a D.E. % that is lower than 50% and a methoxyl content 2.5%. The increase in maturity is accompanied by a decrease in degree of esterification. The low D.E. due to the conversion of pectin into proto pectin during the maturation which increases the sugars and the fruit softer (Bartley and Knee, 1981; Redgwell *et al*., 1997). The D.E. depends on, tissue, stages of maturity and species (Sundar Raj *et al*., 2012).

Methoxyl Content

The results showed that the dragon fruit pectin had lower methoxyl content (2.54%). These value was approximately similar to (Ismail *et al*., 2012) who found that dragon fruit pectin provide with 2.98% .The methoxyl content decreased with increase of maturity, due to ripening the sugar content of the fruits are increased and the methoxyl content decreased (Sirisakulwat *et al*., 2008). Based on the methoxyl content value, dragon fruit peel pectin was considered to have low methoxyl content.

Equivalent weight

The results are appearing in Table 2. The equivalent weight obtain was (3125). The extract pectin showed lower equivalent weight may be back to degradation of pectin, depends on increase or decrease of the

Total Anhydrouronic acid (AUA) Content

The AUA considered as a measure for purity pectin and its value should not be less than < 65% (Food Chemical Codex, 1996). In this study the highest AUA content was (84.68%). The AUA less than < 65%, which evidence that pectin is not pure (Ismail *et al*., 2012).

Viscosity

Table 2. Shows the viscosity of dragon fruit peel pectin. The viscosity of dragon fruit peel pectin was (3.2). There are some factors responsible of pectin viscosity such as degree estrization, temperature, pH and molecular weight, the viscosity increase in present ion calcium until pectin estrization low 75% (Sakai *et al*., 1993).

Setting time

The sitting time of dragon fruit peel pectin was (12.5min) (Table 2). The sitting time increased with decrease of degree estrization, methoxyl content, equivalent weight and number side chain content pectin (Cardoso *et al*., 2003). Methoxyl content is an important factor in controlling the setting time of pectin and the ability of the pectin to form gel (Shaha *et al*., 2013).

Picture (3): Setting time Dragon fruit peel pectin.

Ammonium oxalate (0.5%)	
Equivalent weight	3125
Methoxyl content %	2.54
D.E. %	44.82
AUA %	84.68
Viscosity	3.2
Setting time/min	12.5

equivalent weight upon quantity of free acid (Ramli and Asmawati, 2011).

Table (2): Characterization of dragon fruit peel pectin.

FTIR (Spectra of Dragon fruit peel pectin)

The Fourier Transform Infrared (FTIR) Spectra show the main functional groups of dragon fruit peel pectin and commercial apple pectin (Figure 2). The absorption band in the region at 800 and 1300 cm^{-1} were similar to fingerprint region. The absorption band in the region at 1000 and 2000 cm^{-1} were similar to major functional groups found in pectin (Kalapathy and Proctor, 2001). The broad absorption band in the region at 2500 and 3600 cm^{-1} was attributed OH stretching vibration the absorption was due to hydrogen bonding of the galacturonic acid, these OH stretching vibrations occurred with in abroad rang of frequencies, followed by the absorption bound at roughly 2900 cm^{-1} , which was due to CH stretching of CH_2-CH_2 or CH_3 (Singthong *et al*., 2004). The absorption bands in the region 1630-1660Cm^{-1} was ascribed to methyl esterified carbonyls (C=O) and carboxylate anions (COO^-) stretching vibration. Strong absorption band in (COO^-) couple with a weak absorption band in C=O were ascribed to low methoxyl and D.E. pectin (Chatijigakis *et al*., 1998; Gannasin *et al*., 2012). This indicates that, the sample pectin extract had low D.E., besides the carboxyl late group showed an extra weaker symmetric stretching band of COO^- near 1230-1420 Cm^{-1} in addition to asymmetric stretching COO^{-1} band (Singthong *et al*., 2004). The dragon fruit peel pectin structure was similar to those of the commercial apple pectin sample.

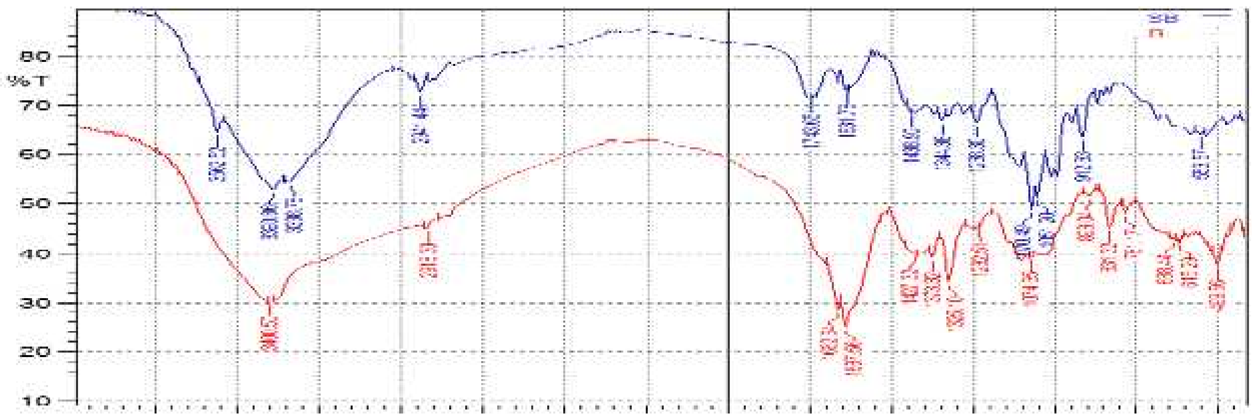

Fig. (2): FTIR Spectra of (B) apple pectin, (SB) dragon fruit pectin produced by ammonium oxalate (0.5%).

Conclusions

Pectin was extracted from dragon fruit using different concentration of ammonium oxalate, the concentration 0.5% showed high yield with the higher content anhydrouronic acid (AUA) and low methoxyl type. Dragon fruit peel, which is considered to be rich in pectin, was proven to exhibit a high quality of properties and could be used in food industries.

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