

Extraction and characterization of pectin from jackfruit (*Artocarpus heterophyllus Lam*) waste

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Abstract: This study focuses on the potential of jackfruit (*Artocarpus heterophyllus Lam*) waste to be a source of pectin. Dried alcohol-insoluble residues of jackfruit waste were treated separately with 0.25% ammonium oxalate/oxalic acid at pH 4.6 and temperature 85°C; 0.1 N H₂SO₄ at pH 2.5 and temperature 80°C; and 0.3% sodium hexametaphosphate/ HCl at pH 2.2 and temperature 80°C for 1h with continuous stirring. Pectins obtained by these treatments were compared in terms of yield, physicochemical properties, rheological properties and chemical structure. Extraction processes have significant effect ($p < 0.05$) on pectin yield, colour, material properties but insignificant effect on chemical composition. Ammonium oxalate and sulfuric acid extracted pectin showed similar rheological and structural properties to commercial pectin. It is concluded that jackfruit waste could be considered as rich source of good quality pectin.

Keywords: Jackfruit waste, pectin, physicochemical properties, material properties; viscosity, FTIR

Date of Submission: 11-11-2017

Date of acceptance: 07-12-2017

I. Introduction

Pectin is a complex heteropolysaccharide found in primary cell wall and middle lamellae of higher plant [1]. It is mainly composed of D-galacturonic acid covalently bonded by α (1-4) glycosidic linkage to form the linear backbone [2]. Around 300 to 1000 unit of galacturonic acid may present in a chain of pectin [3] which correspond to average molecular weight 50000-150000 Dalton [4]. Sometimes pectin backbone interrupted by (1-2) linked L-rhamnose, at some distinguished area and side chain of various neutral sugars is observed [5] as side chain.

Normally this galacturonic acid contain carboxyl group part of which esterified by methanol [6]. Around 80% carboxyl groups of galacturonic acids are esterified by methanol in nature but in can be decrease during pectin extraction. The gelling behavior of pectin greatly varies with this ratio of esterified to non-esterified galacturonic acid. Based on this ratio pectins are classified as high methoxy pectin (HMP; DM> 50%) and low methoxy pectin (LMP; DM<50%) [7]. High methoxy pectin can form gel at low pH (<3.5) in presence of high sucrose concentration (>55%) and low methoxy pectin can form gel comparatively at higher pH (3-4.5) in presence of calcium ions by the egg box mechanism [8].

Pectins have many applications in food, pharmacy and cosmetics as gelling, stabilizing, emulsifying and binding agents [9]. Consumption of this polysaccharide reduces cholesterol, blood sugar, ulcer, cancer and stimulates immune response [10]. Commercially apple pomace and citrus waste (peel and pulp) are used as source of pectin [11]. However, other food industrial waste and indigenous species can be potentially explored for producing this hydrocolloid.

Jackfruit (*Artocarpus heterophyllus Lam*) is the national fruit of Bangladesh and produce abundantly in all over the country. Nutritionally rich jackfruit is very popular to the people. Ripe fruit contain edible portion (sweet bulb, seed) and non-edible portion (outer prickly rind, inner perigones and central core). About 60% of the whole fruit is unutilized waste [12] is throw to environment or use as cattle feed which contain higher amount of pectin than the edible portion [13], So Jackfruit can be a potential source of pectin.

The aim of the study was to evaluate the impact of different extraction methods on the yield and characterization of jackfruit waste pectin and their suitability as a source of industrial pectin.

II. Materials and Methods

2.1 Raw materials

Jackfruit was collected from Germplasm Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh. Bulb and seed were segregated from the inedible portion and finally stored in the deep freezer at -20°C.

2.2 Alcohol Insoluble Residue (AIR)

Jackfruit waste was washed 3-4 times with water to remove all adhering substances, cut into small pieces, dried in cabinet drier at 60°C. The dried waste was grinded to powder using a mechanical grinder (Buhlar Gold, USA). The AIRs were prepared following the methods as reported by Kuobala et al. [9]. The ground powder was then suspended in 85% (v/v) ethanol at 70°C for 20 min in a shaking water-bath. The resulting alcohol-insoluble-residue was collected and air-dried at 50°C.

2.3 Pectin extraction

Pectin was extracted from AIR by using three different extraction conditions and compared to determine the best condition for good quality pectin recovery. AIR of 5g was heated with 200 ml of following each extraction solution: ammonium oxalate (0.25%), pH 4.6 ±0.01 adjusted by 0.01 N oxalic acid (14) at 85°C for 1 hour; acidic solution pH 2.5 ±0.01 adjusted by 0.1 N sulfuric acid at 80°C for 1 hour [15]; 0.6 g of freshly ground sodium hexametaphosphate solution (200 mL) pH adjusted 2.2±0.01 by 3N HCl heat at 80°C [16]. The extracts were separated from the AIR residue by filtering through four fold cheese cloth and cooled immediately by chilled water, dispersed in an equal volume of 95% ethanol, stirred 5 min for proper mixing and allowed to stand for 1 h. In case of sodium hexametaphosphate, extract was dispersed in an equal volume of 95% ethanol containing 0.5 M HCl. The precipitate was collected, washed 3-4 times by 70% acidic ethanol (0.5% HCl), 70% ethanol and finally 95% ethanol. The product was dried at 40°C in an air oven to constant weight. The dried pectin was ground to powder and stored at normal temperature for further experiment.

2.4 Analysis for moisture and ash

Pectin (1g) was weighed and dried in an air oven at 105°C for 24 hours to a constant weight to determine moisture content. For ash content 1 g pectin was weighed in crucible and then ignited in a muffle furnace at 600°C for four hours. The residue was cooled in a desiccator and weighed [17].

2.5 Color measurement

The color of extracted pectin was measured by using a color reader (CR-10; Konica Minolta Sensing America's Ltd., Ramsey, NJ, USA). The lens of the color reader was placed on the sample powder and recorded the value of L*, a*, and b* displayed on the liquid crystal display (LCD). Triplicate samples were analyzed. The color of different extracted pectin powder was compared with commercial pectin.

2.6 Material properties

True density of pectin powder was determined by gas pycnometer (Accupyc II 1340; Micromeritics, Norcross, GA, USA). The volume of the powder was measured based on a gas displacement method. Triplicate samples were analyzed. A 10 ml measuring cylinder was used to measure the bulk density. The weight and volume of the powder was taken inside the measuring cylinder and the bulk density of the powder was determined by the equation (1). After recording the initial volume, the cylinder was tapped mechanically up to reach the constant volume. The tap density is calculated by the equation (2). Hausner ratio and Carr's index (compressibility index) are used to quantify the flowability of obtained powder [18,19]. They are both computed on the basis of bulk density of the powder by using the equation (3 & 4)

$$\text{Bulk density } (\rho_B) = m/V_b \quad (1)$$

$$\text{Tap density } (\rho_T) = m/V_t \quad (2)$$

$$\text{Hausner ratio (HR)} = (\text{tapped density}) / (\text{bulk density}) \quad (3)$$

$$\text{Carr index (CI)\%} = (\text{tapped density} - \text{bulk density}) \times 100 / (\text{tapped density}) \quad (4)$$

Where m is the weight of powder, V_b and V_t are the volume of powder before and after tapping respectively.

2.7 Particle size

The mean particle size for extracted pectin and commercial pectin were measured using a Malvern Mastersizer 2000 particle size analyzer (Malvern Instrument Ltd, Worcestershire, U.K.).

2.8 Chemical composition of pectin

Equivalent weight, methoxyl and anhydrouronic acid (AUA) contents were determined by the method described by Owens et al (20). The values of equivalent weights will be used for calculating the AUA content and the degree of esterification (DE). A 0.5 g pectin was weighed in a 250 mL conical flask and moistened it with 0.5 mL of ethanol. One gram of sodium chloride, 100 ml carbon dioxide free water and 6 drops of phenol red indicator were added. The mixture was stirred rapidly to dissolve, titration was made 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 s. The neutralized solution was used for the methoxyl determination. The equation (5) was used to calculate the equivalent weight. 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. 25 mL of 0.25 N HCl was then added and titrated to the same end point (pink) as before. The equation (6) was used to calculate the methoxyl content. When the equivalent weight and methoxyl content of pectin was known, its anhydrouronic acid (AUA) was calculated by equation (7 and 8)). After getting %AUA and %MeO the degree of esterification of pectin was determined by equation (9).

$$\text{Equivalent weight} = (\text{Weight of sample}) \times 100 / (\text{ml of alkali} \times \text{normality of alkali}) \quad (5)$$

$$\% \text{ MeO} = (\text{meq of sodium hydroxyde} \times 31) \times 100 / \text{wt of sample (mg)} \times 100 \quad (6)$$

where 31 is the molecular weight of the methoxyl group

$$\% \text{ AUA} = 176 \times 100 / Z \quad (7)$$

Where 176 is the molecular weight of AUA and

$$Z = \text{wt of sample (mg)} / (\text{meq of alkali free acid} + \text{meq of alkali for methoxyl}) \quad (8)$$

$$\% \text{ DE} = (176 \times \text{MeO}\%) \times 100 / (31 \times \text{AUA}\%) \quad (9)$$

2.9 Rheology of pectin solution

The rheology of 3% pectin solution in deionized water (wt/wt) was determined with a control stress Rheometer (AR G2, UK) with the cone plate measuring system (cone dia. 60 mm truncation 30 micron) [21]. All rheological experiments were carried out at a constant temperature (20°C). The shear rate was increased from 0 to 1000 1/s by the software control, and strain measurement was recorded. Each sample was tested three times. Rheology curves were prepared to show shear rate vs viscosity.

2.10 Surface Structure analysis

Fourier transform infrared spectra (FTIR) of jackfruit waste pectin samples were collected using the perkin Elmer, GX spectrum model with wavelengths ranging from 400-4000 1/cm. FTIR spectra were analyzed for surface chemical functional groups and compared with the commercial pectin.

2.11 Statistical analysis

Results of three replicates were used. The values were expressed as the mean \pm standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) (version 21) 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% ($P < 0.05$).

III. Results and Discussion

3.1 Pectin yield, moisture and ash content

The yield, moisture content and ash content of pectin extracted from jackfruit waste are presented in Fig. 1. The yield of pectin from jackfruit waste varies significantly ($P < 0.05$) under different extraction condition. Among the extraction methods, sodium hexametaphosphate/HCl treatment yielded the highest pectin, followed by ammonium oxalate and acid treatment gave the lowest yield. However, irrespective of extraction methods used, the yield of pectin in this study is lower than the previous study [16, 22]. The reason could be the different extraction condition and variety or maturity of jackfruit. Previous study also revealed the effects of extraction conditions (extraction temperature, pH, time, solubilizing agents) on pectin yield from same sources [16, 22]. Pectin extraction is a multiple stage physical-chemical processes in which the hydrolysis and extraction of pectin macromolecules from plant tissue occur sequentially. Different factors, mainly extraction temperature, pH, time and solubilizing agents affect the extraction of pectin [3]. Higher yield obtained by sodium hexametaphosphate and ammonium oxalate solvent may be due to pectins are physically bound in situ via

metallic cation, sequestering agents such as sodium hexametaphosphate and ammonium oxalate which readily bind those cations and help to release the pectins from cell walls [3].

The moisture and ash content of extracted pectin was observed 7.72-14.73% and 3.71-8.15% respectively. Sodium hexametaphosphate extracted pectin showed highest moisture content among all extracted pectin as well as commercial pectin (Fig.1). Ammonium oxalate and sulfuric acid extracted pectin contained lower moisture content than commercial pectin. Pectin should contain minimum moisture content. High moisture content may cause quality deterioration due to production of pectinase enzyme [23]. Ash content indicates the inorganic impurities present in pectin. Highest ash content (8.15%) was observed in sodium hexametaphosphate extracted pectin and lowest (3.71%) in acid extracted pectin. Ash content of commercial pectin was significantly lower ($P < 0.05$) than that of extracted pectin from jackfruit waste. Though ash content in pectin could vary from 0.76 to 10.69% [24], lower ash content is preferable for gel formation. Higher ash content in extracted pectin could be reduced by washing with acidify alcohol. Previous study also reported that the high ash content in ammonium oxalate [25] and sodium hexametaphosphate extracted pectin [16]. This could be due to the ability of chelating agents to solubilize indigenous minerals in the jackfruit waste.

3.2 Colour of pectin

The CIE LAB color was studied, the following color coordinate was determined: lightness (L^*), redness (a^* , red-green) and yellowness (b^* , yellow-blue) [26]. L^* , a^* and b^* values of crude pectin are presented in Table 1. Color values (L^* , a^* , and b^*) of all extracted pectin were significantly different ($P < 0.05$) from commercial pectin. Sulfuric acid and ammonia oxalate extracted pectin colour were found quite similar (a^* value) whereas sodium hexametaphosphate extracted pectin color was darker. Highly colored pectin may have the presence of polyphenols or other water soluble pigments trapped inside the pectin during precipitation. Moreover, drying process also affects the color of final product [27]. In this study extracted pectin was dried in air oven at lower temperature (40 °C) for long time which may cause the darker color of final pectin sample.

3.3 Material properties of pectin

Values for material properties of different extracted pectin and commercial pectin are presented in Table 2. Flowability characteristics can be assisted by Hausner ratio and Carr index. Lower Hausner ratio (1.00-1.11) and Carr index (0-10) indicate excellent flowability, on the contrary higher Husner ratio (1.46-1.59) and Carr index (32-37) indicate very poor flowability [28]. Pectin extracted from jackfruit waste exhibited Husner ratio 1.17-1.18 and Carr index 14.51-15.71 which indicate good flowability whereas commercial pectin showed fair flowability. Particle size of extracted pectin was much more higher (324-363 μm) than commercial pectin (160 μm) which effect on the powder flowability. Generally particle size influences the powder compaction, flowability, segregation and other factors, powders with smaller particles exhibit poor flow properties [29]. Previous study also revealed that the flowability of pectin powder vary from passable to fair and reduced with the decreasing particle size [30].

3.4 Chemical composition of pectin

Three extraction conditions were compared to determine the characteristics of the jackfruit waste pectin. The results are shown in Table 3. Methoxyl content of extracted pectin was between 5.37-6.72% and did not show significant different ($p < 0.05$) with commercial pectin. All extracted pectin showed higher %AUA compared to commercial one. These values are higher than the AUA obtained from similar source by Mohammed and Hasan [16]. Higher AUA value indicates that jackfruit waste could be an excellent source of commercial pectin. The content of AUA indicates the purity of pectin and is suggested to be not less than 65% ash and moisture free basis [31]. AUA content in this study were less than 60% as the weight was included moisture and ash. Degree of esterification of pectin in this study was 45 to 65%. DE of ammonium oxalate and acid extracted pectin was higher than 50% which indicate as high methoxyl pectin. Amonium extracted pectin showed higher equivalent weight, methoxyl content and DE than acid extracted pectin. Similar result reported by Ramli and Asmawati [25] for cocoa husk. Though sodium hexametaphosphate extracted pectin contain higher AUA and it showed lower degree of esterification. This result may be due to hexametaphosphate and high acidity hydrolyzed some methoxyl content present in main galacturonic acid chain.

3.5 Rheology of pectin

The viscosity properties of different pectin extracted by different extraction condition from jackfruit waste and commercial pectin are presented in Fig. 2. In this figure viscosity of pectin were plotted as a function of shear rate. All pectin sample demonstrated shear thinning behavior (the viscosity decreased with the increase of shear rate), which is typical behavior of pectin solution. This is due to the polymer chain of pectin being stretched out and elongated in the direction of flow, therefore less resistance to flow [32]. Ammonium oxalate and Na-hexametaphosphate extracted pectin demonstrated highest and lowest viscosity among all pectin

sample as the changes in viscosity were larger and smaller respectively. Sulfuric acid extracted pectin showed viscosity similar to commercial pectin. Previous study reported that the extraction process significantly affect the viscosity of jackfruit waste pectin [33]. The study found that the degree of esterification has great impact on viscosity. Higher degree of esterification shows higher viscosity [21]. Since sodium hexametaphosphate extracted pectin shows lower degree of esterification and higher ash content and consequently it showed very low viscosity.

3.6 Surface structure

The FTIR spectra of jackfruit waste extracted pectin and the commercial pectin sample are presented in Fig. 3. The FTIR spectra shows the functional groups and provides structural information of different extracted pectin and the pure pectin in the wavelengths between 400 and 4000 1/cm. The major functional groups in pectin are usually in the region between 1000 and 2000 1/cm of the FTIR spectra [34] which provide structural information that could be used to compare different type of pectin. The free and esterified carboxyl groups were observed by the bands at 1630-1650 and 1740-1760 1/cm respectively [35]. Higher intensity and area of esterified carboxyl than free carboxyl group indicate higher degree of esterification. Like commercial pectin ammonium oxalate and sulfuric acid extracted pectin showed higher degree of esterification but sodium hexametaphosphate extracted pectin showed lower degree of esterification. This finding agrees the results from the Table 3 showing the low degree of esterification of sodium hexametaphosphate extracted pectin by titration method.

IV. Figures and Tables

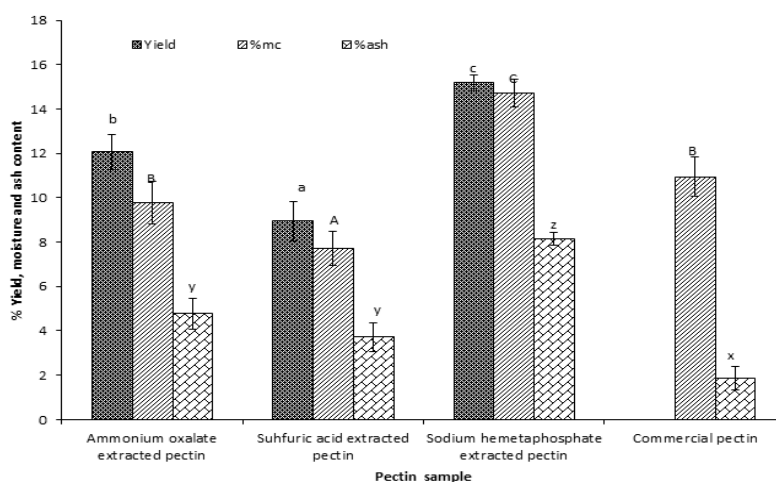


Fig. 1 Yield, moisture content, ash content of pectin extracted from jackfruit waste

Yield of commercial pectin was not done. Mean value from three replicates were used to draw bar chart with error bar. Bars with different superscript (a,b,c...for yield, A,B,C.. for moisture content, x,y,z...for ash content) are significantly different ($p < 0.05$).

Table 1: Color of jackfruit waste pectin and commercial pectin

Pectin sample	L	a	b
Ammonium oxalate extracted pectin	49.77±0.12 ^b	11.60±0.44 ^a	29.10±0.30 ^b
Sulphuric acid extracted pectin	47.60±0.35 ^c	11.43±0.25 ^a	26.83±0.21 ^c
Sodium Hexametaphosphate extracted pectin	32.93±0.15 ^d	5.03±0.15 ^c	15.5±0.21 ^d
Commercial pectin	73.06±0.23 ^a	6.80±0.17 ^b	30.17±0.06 ^a

Mean value from triplicate measurement. Values with different superscript in the same row are significantly different ($p < 0.05$).

Table 2: Material properties of pectin powder

Properties	Ammonium oxalate extracted pectin	Sulfuric acid extracted pectin	Sodium hexametaphosphate extracted pectin	Commercial pectin
Bulk density (Kg/m ³)	640.93±1.05 ^b	598.01±0.48 ^d	630.11±0.28 ^c	687.56±1.36 ^a
Tapped density (Kg/m ³)	760.23±1.09 ^b	705.85±0.68 ^d	737.12±0.86 ^c	830.27±0.59 ^a
True density (Kg/m ³)	1500.26±0.70 ^c	1414±0.36 ^d	1543.03±0.15 ^b	1574.56±0.35 ^a
Hausner's ratio (Hausner 1967)	1.18	1.18	1.17	1.20
Carr Index (%) (carr 1965)	15.71	15.27	14.51	17.18
Flowability	Good	Good	Good	Fair
Particle size (µm)	324.71	363.62	350.36	160.54

Mean value from triplicate measurement. Values with different superscript in the same row are significantly different (p < 0.05).

Table 3: Chemical composition of jackfruit waste pectin extracted by different condition and commercial pectin

	Ammonium oxalate extracted pectin	Sulfuric acid extracted pectin	Sodium hexametaphosphate extracted pectin	Commercial pectin
Equivalent weight	919.3 ^b	835.66 ^b	530.23 ^a	787.42 ^b
Methoxyl content, %	6.72 ^a	6.14 ^a	5.37 ^a	5.43 ^a
AnhydroUronic acid content (AUA), %	57.34 ^a	56.11 ^a	65.42 ^a	54 ^a
Degree of esterification (DE), %	62.77 ^b	58.53 ^b	44.85 ^a	57.38 ^b

Values are means of triplicate measurements. Values with the different letter in the same row are significantly different (P < 0.05)

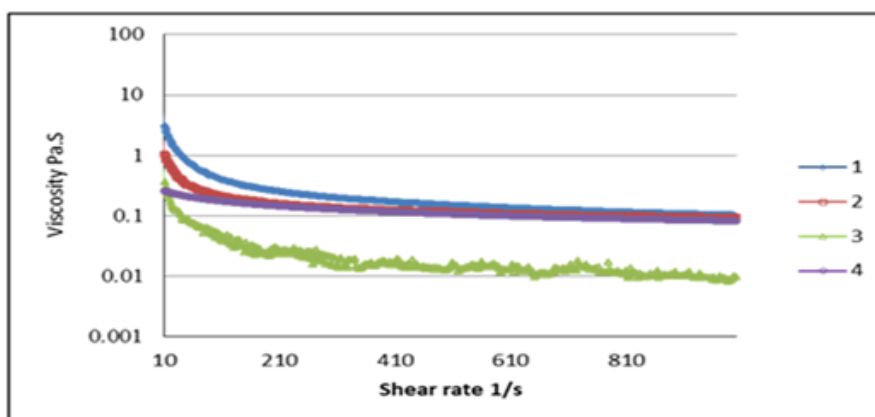


Fig. 2 Changes in viscosity (Pa.S) as a function of increasing shear rate (s⁻¹) of pectin solutions: Ammonium oxalate (1), sulfuric acid (2), sodium hexametaphosphate (3) extracted pectin and commercial pectin from Lubachemiepvt.Ltd. (4).

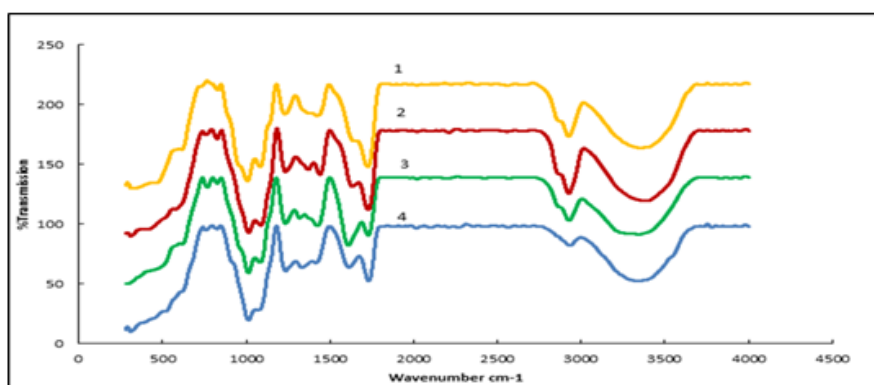


Figure 3 FTIR spectra of jackfruit waste pectin extracted by ammonium oxalate (1), sulfuric acid (2) sodium hexameta phosphate (3) and commercial pectin from Lubachemiepvt.Ltd. (4).

V. Conclusion

Jackfruit waste was investigated as a source of pectin. Ammonium oxalate as extractant is highly suitable for the recovery of pectin from jackfruit based on yield, functional and chemical properties. Extracted pectin contains high level of AUA, DE and viscosity. However, it showed darker color compared to commercial pectin. Future investigation need to check the gelation properties of these pectin.

Acknowledgements

The authors would like to acknowledge Ministry of Education, Bangladesh (project code: 2000/99/MoE) and Organization for Women in Science for the Developing World (OWSD) (Grant no. 3240266462) for their financial support..

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Rokeya Begum "Extraction and characterization of pectin from jackfruit (*Artocarpus heterophyllus* Lam) waste." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* , vol. 12, no. 6, 2017, pp. 42-49.