BCSIR

Pectin from ripe peels of mango cultivars

K. Nahar*[1], M. Z. Haque[2], K. Nada[1], M. N. Uddin[1], M. Abdullah Al-Mansur[1], N. Khatun[1] and S. A. Jabin[2]

[1]*Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka. BCSIR, Dhaka-1205, Bangladesh*
[2]*Institute of Food Science & Technology (IFST), BCSIR, Dhaka-1205, Bangladesh*

Abstract

The present study focused on the influence of different extraction conditions of pectin isolated from ripe mango peel of four Bangladeshi mango varieties (*Amrapali, Fazlee, Langra & Kharsapat*). The correlation between biochemical properties and protein content were also observed. Five different extraction conditions were applied where the optimum conditions for highest yield and purity were 'H_2SO_4-$(NH_4)_2SO_4$ buffer /pH 1.5 /80 °C /1h /*Amrapali*' and 'Tem. 99.8 °C/ HCl-NaOH buffer/ pH1.5/ 1h/ *Amrapali*' respectively. The best source among four varieties was *Langra* as it contained highest purity (AUA, 72.27±0.03%) under a specific condition. The best extractant was HCl-NaOH buffer as the pectin extracting with it contained minimum impurity (3.10±0.06). This study also showed that pectin from *Amrapali* contained both Low and High Methoxyl pectin with the same extraction condition other than extractant. The proximate, elementary, microbiological, spectroscopical and structural investigations were also conducted in this study.

Keywords: Pectin; Different mango cultivars; Buffer solution; Extraction condition; Biochemical properties; Protein content

Introduction

Pectin is methylated ester and are highly heterogeneous with respect to their galacturonic acid and neutral sugar contents, their methylation and acetylation degrees, and their molar mass (Ralet and Thibault, 2002). They are widely used as gelling and stabilizing agents in food, pharmaceutical and cosmetic industries. Based on the degree of esterification (DE) it can be divided into two types: high methoxyl pectin (HM pectin) which have DE > 50 % and low methoxyl pectin (LM pectin) which have DE < 50 % (Mesbahi *et al.*, 2005). The major raw materials used for the production of commercially acceptable pectins are citrus peel and apple pomace (May, 1990). Other sources include sugar beet residues (Yapo and Koffi, 2013), cacao husks (*Theobroma cacao* L.) (Nazaruddin, 2011), red dragon fruit (*Hylocereus polyrhizus*) (Woo *et al.*, 2010), Kaffir Lime (*citrus hustrix*) (Shaha *et al.*, 2013), chicory roots (*Chicorium intybus* L.) (Robert *et al.*, 2006), mango peels (Koubala *et al.*, 2008). However, most of these could not satisfy the industrial requirements which include high yields, best quality gels and functionality (Daniel *et al.*, 2014). Mango peels represent about 16 -19 % of the total weight of the fruit (Kansci, *et al.* 2003). These peels are thrown as garbage or used for animal feeding. They have been reported to be a potential source of pectins (Koubala *et al.*,

2008). There are nearly 100 cultivars of mango available in Bangladesh. The study have been done with four variety, named; *Amrapali, Fazlee, Langra* and *Kharsapat. Amrapali* is a hybrid variety of *Dasheri* and *Neelum. Langra* is medium size pyramidal shape light green color fruit. *Kharsapat* is conical shape green with red patches color and *Fazlee* is Pyramidal green with red patches color. The individual weight of *Langra, Kharsapat* and *Fazlee* are about 238-244g, 229-260g and 503-648g respectively (Kobra *et al.*, 2012). In Bangladesh, 32011 hectares of land are used for the cultivation of mango with an annual production of 1047849 metric tons estimated by Bangladesh Bureau of Statistics, 2011(Barua *et al.*, 2013). In mango processing industries, the peels are discarded as wastes.

The aim of this study was to search optimized extraction condition where the quality pectin could be isolated from different mango cultivars in Bangladesh and also to develop a suitable technology for the waste management through utilizing the byproduct from mango processing industries.

Materials and methods

Mangoes (varieties: *Amrapali, Fazlee, Langra and Kharsapat*) were collected from Shaheb Bazar, Rajshahi, Bangladesh.

*Corresponding author e-mail: kamrunbcsir@bcsir.gov.bd, knahar139@yahoo.com

Pectin extraction method

Peels were removed from mangoes and chopped (1 sq.cm) and dried in oven at 90 ℃ for 6 h and then powdered and sieved (mesh no. 80). The moisture content was 8-10%. Several extraction conditions were considered; 1) variable sources (*Amrapali, Fazlee, Langra and Kharsapat*)/ H_2SO_4-$(NH_4)_2SO_4$ buffer/ pH 1.5/ 80 ℃/ 1h , 2) variable extractants (H_2SO_4-$(NH_4)_2SO_4$ buffer, HCl-NaOH buffer and Citric acid buffer) /pH1.5 /80°C /1h /*Amrapali* , 3) variable pH(1.5, 2.0, 2.5)/ HCl-NaOH buffer/ 80 °C1h/ Amrapali, 4) variable temperature (80 °C, 90 °C & 99.8 °C)/ HCl-NaOH buffer/ pH1. / 1h/ *Amrapali*, 5) variable extraction time (1h, 1.3 h, 2h, 2.3h)/ HCl-NaOH buffer/ pH1.5/ 80 °C/ 1h/ *Amrapali*. In all, 25 g of powder was mixed with 500 ml of extractant and processed following different extraction conditions. The extracts were filtered through a silk cloth and pectins were precipitated with double volume of methanol and dried at 60 °C in an oven for 4 hours and then powdered and stored at 25 ℃. The yields were 10.92-30.34 % on dry basis.

Spectroscopical and structural investivation (FTIR, SEM) of Pectin

Fourier Transform infrared analysis was carried out by ATR (Attenuated total reflection) method. The sample was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer (BRUKER, ATR crystal was germanium). The SEM micrograph of was taken by SEM (Hitachi-2600SN, Japan) by non-destructive method and the images were taken at voltage of 15.0 KV at 68.0 mm x 250 mm.

Biochemical properties

Equivalent weight (Eq. wt.) was determined by Ranganna's method. About 0.5 g of sample was taken in a 250 ml conical flask and 5 ml of ethanol, 1 g of sodium chloride and 100 ml of distilled water were added. Penol red or Hinton's indicator (6 drops) was added for sharpen the end point. The solution was titrated with 0.1 N NaOH. The end point was indicated by turning the yellowish color to purple color at pH 7.5. This neutralized solution was stored for determination of Methoxyl (MeO) content. The neutral solution was collected for determination of Eq.wt. and 25 ml of NaOH (0.25N) was added. The mixer was stirred thoroughly and kept at room temperature for 30 min. After then, 25 ml of 0.25N HCl was added and titrated against 0.1 N NaOH to the same end point as before like in Eq.wt. titration. Estimation of Anhydrouronic acid (AUA) content was essential to determine the purity and DE and to evaluate the physical properties (Mohamed and Hasan, 1995). The percentage of

DE was determined on the basis of percentage of MeO content and percentage of AUA content (Azad *et al*., 2014).

Proximate analysis

Moisture and total ash content were determined using Fischer and Ranganna's method respectively. Fiber, residual fat and crude protein were determined by Fritted Glass Crucible (AOAC 978.10), Hexane Extraction (AOAC 2003.06) and Automated Kjeldahl method (AOAC 976.05) respectively.

Measurement of elementary analysis

The minerals were determined using Atomic Absorption Spectrophotometric method (AOAC, 968.08). The minerals Ca, Mg, Fe and Zn. were measured with GBC 908 Atomic Absorption spectrophotometer. P was determined by the ammonium molybdate method (Shimadzu ultraviolet -visible spectrophotometer 1601 PC). K and Na were analyzed using a Corning 410 Flame Photometer.

Microbiological analysis

Enumeration of Total Aerobic Plate Count (Maturin and Peeler, 1998), Total Fungal Count (Tournas *et al*. 1998), *Escherichia coli* (Hitchins *et al*. 1998), *Salmonella*, (Andrews and Hammack, 1998) and *Staphylococcus aureus* (Bennett and Lancette, 1998) were carried out following Bacteriological Analytical Manual (1998). Total Plate Count was enumerated by Pour Plate Method on melted nutrient agar/ plate count agar. Enumeration of fungas was carried out by spread plate technique on potato dextrose agar (PDA) media. Enumeration of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were carried out by selective method where Baired-Parker ager medium and cetrimide agar plates were used for *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. *Escherichia coli* count was carried out by three-tube most probable number (MPN) method. Lactose broth and eosin methylene blue (EMB) agar were used for *E. coli*. *Salmonella* count was carried out using Enrichment method where Lactose broth, selenite broth and bismuth sulphite agar were used. Total Plate Count of *Escherichia coli*, *Staphylococcus aureus* and Fungas were enumerated on plate count agar. Incubation temperature and time were 37℃ and 24h. Suspected pathogens were biochemically confirmed.

Statistical analysis

Descriptive statistics of parameters of yield%, Eq.wt., MeO%, AUA%, DE% and Protein% were computed at first. In order to test the equality of different extraction conditions,

one way analysis of variance (ANOVA) test was used. Since parameters varies significantly ($p<0.05$) in different extraction conditions, Duncan Multiple Rank Test (DMRT) of Post Hoc was performed. SPSS of its version 17.0 was used for data analysis.

Results and discussion

Structural and spectroscopical analysis

Fig.1 shows the fingerprint of HM pectin, with a high degree of esterification. The approach was effective to confirm the pectin functional identity with esterified carboxylic groups (1744.90 cm-1) and free carboxyl groups (1642   cm-1) which were consistent with the literature of Fertonani (2006).
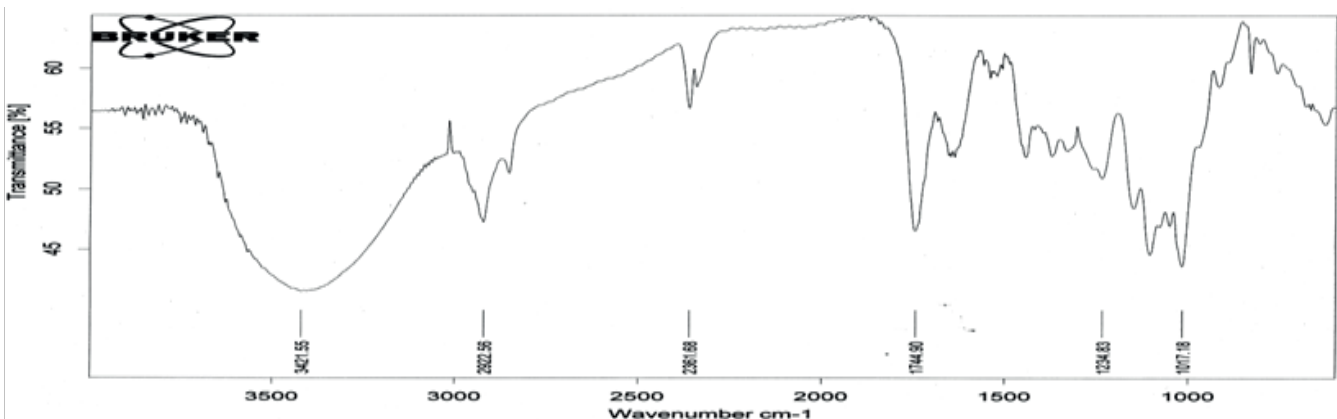
Effect of extraction condition on pectin yield

The yields varied from 10.76-30.43 % (dry basis) depending on the extraction condition used. The maximum yield was 30.43 % at H_2SO_4-$(NH_4)_2SO_4$ buffer /pH 1.5 /80 $^\circ$C /1hour /*Amrapali*. With the extraction condition: 'variable sources/ (H_2SO_4-$(NH_4)_2SO_4$ buffer/ pH 1.5/ 80 $^\circ$C/ 1h)', the yields were 30.43, 25.53, 20.62 and 17.31 % (g/g) from *Amrapali, Fazlee, Langra* and *Kharsapat* respectively (Table I1). Yields from *Fazlee* and *Langra* were similar to the work with *Améliorée and Mango* mango peels where yields were 20.4 and 26.3 % (g/g) respectively with HCl (pH 1.5) as extractant (Koubala *et al*. 2008). Using HCl-NaOH buffer instead of H_2SO_4-$(NH_4)_2SO_4$ buffer, the yield from Amrapali was decreased (Table I[2]). This result was consistent to (Rehman *et al*., 2004) where the production decreased when HCl used instead of H_2SO_4. Citric acid and HCl-NaOH buffer

Fig. 1. FTIR analysis of pectin (Langra) (condition: H_2SO_4-$(NH_4)_2SO_4$ buffer /pH 1.5/80 $^\circ$C/1h)

SEM images were obtained to characterize the microstructure of pectins from Amrapali, Kharsapat & Langra mango cultivars. The images showed the morphology of irregular particle size with rough surface textures. The images proved that pectins are amorphous.

extracted almost the same amount (18.85 and 18.49 % respectively) (Table I[2]) which was relevant to work of Shaha (2013). It was observed that the yield was decreased with increasing pH of extractant (*Amrapali* pectin decreased from 18.49-10.99 % with increasing pH of HCl-NaOH buffer from 1.5-2.5) (Table I[3]). Low pH is able to promote the liberation
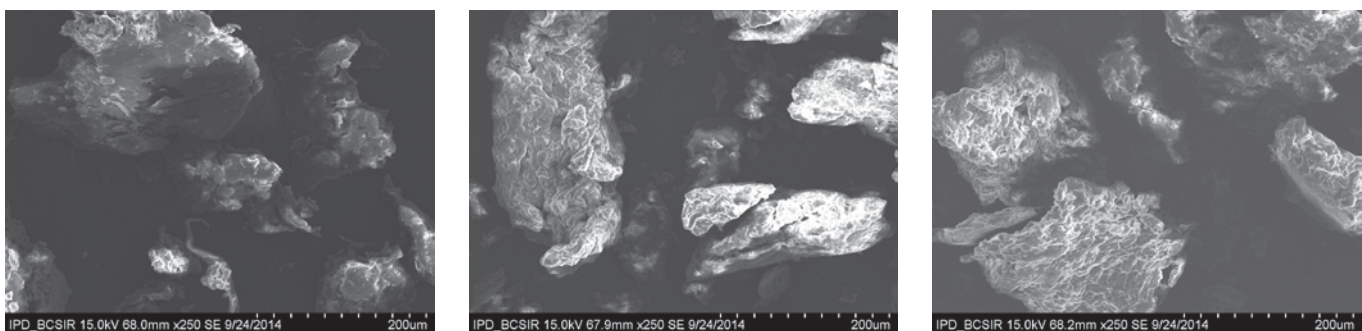

Fig. 2. SEM images of Pectins from *Amrapali, Kharsapat* and *Langra* mango cultivar respectively

of pectin molecules from the peel during acid-washing stage because of the interaction of pectins to the hemicelluloses fractions are cleaved (Rombouts and Thibault, 1986).The reduction of yield at higher pH might be due to some pectin is still attached to the cell wall components (Voragen, 2003). Keeping all other conditions constant other than temperature, yield was decreased from 18.49-10.76 % with increasing the heat from 80-99.8 °C for *Amrapali* (Table-I[4]). The cause of yield reduction at higher temperature could be attributed to break down of pectin molecules (Woo *et al*., 2010) and also by the depolymerisation mechanism of galacturonan chain of pectin, which is known as beta-elimination (Albersheim *et al*., 1960). Yields were varied with increasing extraction time. At 'HCl-NaOH buffer/ pH1.5/ 80°C/ *Amrapali*', the yield decreased slightly with enhancing extraction time from 1-1.3 h but again increased after 1.3-2.3 h. The maximum yield was 18.49 % for 1 h and the minimum yield was 14.55 % for 1.3 h (Table I[5]). Almost similar trend was observed in another study on pectin from sunflower head which demonstrated an increased yield to 10.97 %, whilst declined to 10.73 % during extended time (Sahari *et al*., 2003). Pectin yield increased from 10.37-14.86% from 30-60 min treatment at pH 3.5, but decreased to 12.11% when treatment increased to 120 min (Woo *et al*., 2010).

Effect of extraction condition on Eq.wt.

Eq.wt. indicates whether the pectin have got partial degradation or not. In this study, it was varied from 506.58-2142 depending on the extraction condition used. The highest Eq.wt obtained 2140.70 from *Amrapali* (condition: 'HCl-NaOH buffer/ pH 2.5/ 80 °C/ 1h'). On the other hand, the lowest was 507.28 under the condition: 'HCl-NaOH buffer/ pH 1.5/ 99.8 °C/ 1h', this is because the partial degradation occurred at 99.8° C. Eq.wt. of lemon pomace pectin range from 368-1632 (Azad *et al*., 2014), which was relevant to the result of this study. At condition: 'variable sources/ H2SO4-(NH$_4$)2SO$_4$ buffer/ pH1.5/ 80 °C/ 1h', Eq.wt. from *Amrapali, Fazlee, Langra* & *Kharsapat* were 755.61, 795.61, 815.18 & 686.05 respectively (Table -I[1]). In this condition, *Langra* contained the highest Eq.wt which was consistent to the study by (Rehman *et al*., 2004) and observed the Eq.wt 820 with the condition: 'H$_2$SO$_4$/ 1.5/ 80 °C/ 1h'. By changing the extractant, e.g. using HCl-NaOH buffer instead of H$_2$SO$_4$-(NH$_4$)2SO$_4$ buffer, Eq.wt. of *Amrapali* pectin did not notably decrease (from 755.61-737.51), (Table I[2]). The variation of Eq.wt. might be also dependent upon the amount of free acid (Nazaruddin, 2011). Eq.wt. was increased significantly with increasing pH and obtained 737.51, 1871.07 & 2142.70 for pH 1.5, 2.0 & 2.5 respectively (Table I[3]) with the condition: 'HCl-NaOH

buffer /80°C /1h/ *Amrapali*'. The similar increasing tendency (up to pH 2.5) was observed in another study (Rehman *et al*., 2004). Eq.wt. was decreased with increasing temperature and time (Table I[4] and Table I[5]) where it was observed that the highest and lowest Eq.wt. were 737.51 (at 80°C) and 506.58 (at 99.8°C) for 1h. The lower value could be for higher partial degradation of pectin (Azad *et al*., 2014).

Effect of extraction conditions on MeO

MeO content is an important factor in controlling the setting time of pectins and the ability of pectin to form gels (Constenla and Lozano, 2003). Pectin's spreading quality and sugar binding capacity were increased with increasing MeO content (Madhav and Pushpalatha, 2002). MeO decreased with increase of sugar content with pectin (Sirisakulwat *et al*., 2008). In this study, the MeO content significantly varied with the variation of extraction conditions and sources. Among four varieties, MeO was highest for *Langra* (8.94%) and the lowest for *Amrapali* (2.85 %) with 'H$_2$SO$_4$-(NH$_4$)2SO$_4$ buffer/ pH1.5/ 80°C/ 1h', where as *Fazlee* and *Kharsapat* contained 7.05 and 4.22 % respectively (Table I[1]). The values of Fazlee and Langra were approximately similar to pectins from the peel of mango (7.33%), banana (7.03%) and pumelo (8.57%) (Madhav and Pushpalatha, 2002) and the values of *Amrapali* & *Kharsapat* were almost similar to dragon fruit pectin (2.98-4.34%) (Ismail *et al*., 2012). The MeO content of orange peel pectin using citric acid and nitric acid was 5.89 and 5.58 % respectively (Devi *et al*., 2014). Only altering the extractant e.g. using HCl-NaOH buffer instead of H$_2$SO$_4$-(NH$_4$)2SO$_4$ buffer with constant other conditions, pectin from *Amrapali* contained maximum MeO content (9.37 %). When using citric acid buffer, it was 8.25 % (Table I[2]). It was decreased with increasing pH (9.37 % for pH 1.5 and 5.51 % for pH 2.5) (Table I[3]). This decreasing tendency was observed to the study by (Hussain *et al*., 1991) who showed MeO content decreased from 8.35-8.13 at pH 2.0-4.0 respectively. Only increasing temperature (80-90 °C) and time (1-2.3 h), the MeO content increased (9.37-9.59 % and 9.37-10.64 % respectively) (Table I[4] and Table I[5]). It was observed that, MeO contents of *Amrupali* pectin were 9.37-10.64 % for HCl-NaOH buffer and 8.25 % for citric acid buffer. *Langra* pectin contained 8.94 % for H$_2$SO$_4$-(NH$_4$)**2**SO$_4$ buffer. According to the content of MeO (8.25-10.64 %), the gel grade was 200-213. This work was similar to the work of pectin from pumello, lime and mangosteen where MeO range was 8-11% and the gel grade was 200-213 (Madhav and Pushpalatha, 2002).

Effect of extraction conditions on AUA

The AUA content indicates the purity of pectin and the value of it should not be less than 65% (Food Chemicals Codex, 1996). In this study, the highest AUA content was obtained 89.10 % at 'HCl-NaOH buffer/ pH 1.5/ 99.8 °C/ 1h/ *Amrapali*' which was the best condition on the basis of pectin's purity that was also indicated by its lowest impurity content, (protein content, 3.16 %) (Table I[4]). On the other hand, the lowest AUA content was 39.47 % where protein content was highest (52.33%) (Table I[1]). The AUA content of orange peel pectin was 93.28 % (Devi *et al.,* 2014) and mangosteen rind pectin was 73.16 (Madhav and Pushpalatha, 2002) respectively. With variable pectin sources at 'H$_2$SO$_4$-(NH$_4$)2SO$_4$ buffer/ pH1.5/ 80°C/ 1h', pectin from *Langra, Fazlee, Kharsapat* and *Amrapali* showed that the AUA contents were decreased gradually (72.27, 62.20, 49.53 and 39.47 % respectively) whereas the protein content were increased in the same way (12.61, 14.09, 15.97 and 52.33 % respectively) (Fig: 3) where Langra showed the best source among four. Changing the extractant e.g. using HCl-NaOH buffer with other conditions constant, the AUA content was 77.22 % and using citric acid buffer, it was 73.61% (Table I[2]). These indicated that HCl-NaOH buffer was best according to purity. Changing the pH with the condition: 'HCl-NaOH buffer/ 80 °C/ 1h/ Amrapali', the AUA content increased with decrease of pH (Table I[3]). The cause of this may be degrading sugar chain from pectin polymer with increasing of hydrogen ion concentration present in the extraction medium. In this condition, the highest AUA was 77.22 % at pH 1.5. In another work, the AUA was 66.73-68.72 % for mango peel pectin at the same pH (Rehman *et al.,* 2004). The lowest value was 39.52 % at pH 2.5. Low AUA value indicates a high amount of protein, starch and sugars in pectins (Ismail *et al.,* 2012). The AUA content was increased with increasing of temperature and time at 'HCl-NaOH buffer / pH 1.5 /*Amrapali*' (Table I[4] and Table I[5] respectively). Protein was denatured at 100°C or nearer to that temperature with prolongs heating while extracting, the purity of extracting solution might have increased. In this study, the maximum AUA content was 89.10 % and the lowest protein content was 3.16 at 99.8 °C (Table I[4]). Under a constant pH and temperature, prolong extraction time will lead to higher pectin quality (Faravash and Ashtiani, 2008).

Effect of extraction conditions on DE

DE is the identification parameter for categorizing of pectins where DE > 50% are known as HM pectins and a DE < 50% are LM pectins (Walter, 1991). HM pectin forms gels under acidic conditions (pH < 4.0) with sucrose (> 55%) (Morris *et al.,* 2000) whereas LM forms gels by the interaction of divalent cations, especially Ca^{2+}, between free carboxyl groups (Cardoso *et al.,* 2003). In this study, it was observed that pectin from *Amrapali* contained both HM pectin and LM pectin, because it has a wide range of DE (40.99-79.16 %), depending on the extraction condition used. When using H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer, LM pectin (DE, 40.99 %) was observed, but using HCl-NaOH buffer HM pectin (DE, 60.76-79.16 %) was noticed. The results were relevant to the values of DE from lemon pomace pectin where DE found 33.59 -79.51 % (Azad et al, 2014). Using four varieties with the condition: 'H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer/ pH1.5/ 80 °C/ 1h', the maximum DE was obtained 70.23 % from Langra pectin and the minimum was 40.99 % from Amrapali (Table I[1]). Pectins from Langra and Fazlee were HM pectin and from Amrapali and Kharsapat were LM pectin. But changing extractant such as using HCl-NaOH buffer instead of H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer, the obtained pectin from Amrapali was HM pectin where DE content was 68.89 % , but when used H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer, then LM pectin (DE, 40.99 %) was obtained (Table I[2]). This result was consistent to another study where it was observed that the DE was influenced by extractant (DE ; 76% using HCl and DE, 73%; using deionized water for Ceni mango peels) (Kratchanova *et al.,* 1991). From the result of this study, it could be said that DE is not only depend on species, tissue, stages of maturity (Sundar Raj *et al.,* 2012) but also depend on the extraction condition. Hence, the extraction conditions have a considerable impact on pectins' biochemical characteristics. Only changing pH at 'variable pH/ HCl-NaOH buffer/ 80°C/ 1h/ Amrapali', DE increased with increasing pH and the result was relevant to the study by Woo *et al.,* (2010) who found that by increasing pH, higher DE was demonstrated in the pectin from red dragon fruit. In this condition, pectins with DE; 79.16 % and 78.25 % (at pH 2.5 and 2.0 respectively, Table I[3]) were rapid-set pectins because it was categorized that pectins which have more than >72% DE are rapid-set pectins and pectins which have a DE of 58-65% are slow-set pectins (Shaha *et al.,* 2013). By keeping all other extraction conditions constant other than temperature, the DE decreased with increasing temperature (Table I[4]). Because, the high acid concentration and temperature together influence the pectin extraction and de-esterification process simultaneously. Only enhancing heat treatment from 1-2.3 h, the DEs were not significantly changed (Table I[5]) and this study was relevant to the other study of cocoa husks pectin where no significant effect of DE was observed on '1.5-3.0 h/ HCl/ pH 2.5-4.0' (Chan and Choo, 2013).
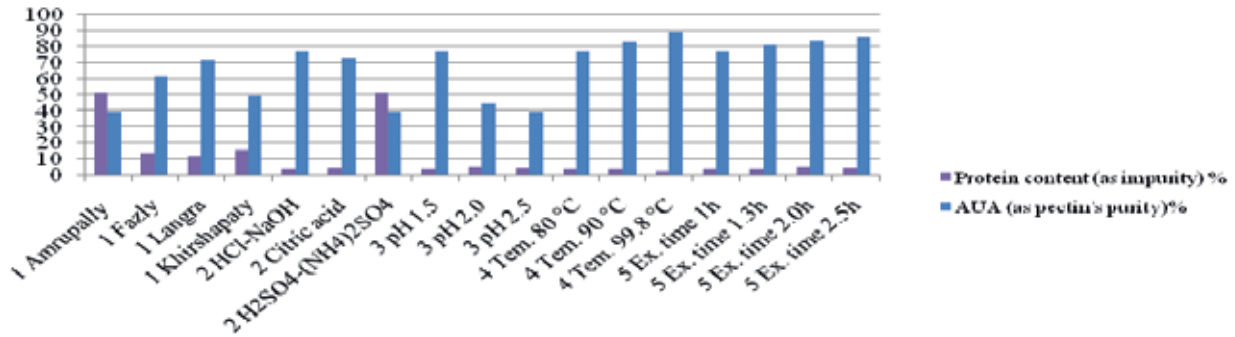
Effect of extraction conditions on protein content

In this study, the protein content significantly ($p < 0.05$) varies with the variation of extractant and mango variety. In this study, the highest protein content was (52.33±0.45 %) with the extractant 'H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer' and the lowest was (4.29±0.67 %) for 'HCl-NaOH buffer'. On the other hand, different pHs, temperatures and extraction times with HCl-NaOH buffer did not influence notably on protein content. It has been observed remarkably that protein content was decreased with increasing pectin's purity (Table I and Fig. 3).

Table I. Yield, biochemical properties (Eq.wt., MeO, AUA and DE) and protein content of extracted pectin content under different extraction conditions

Characteristic																			
					Extraction conditions														
		[1]Variable source/ H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer / pH 1.5 80°C/1h				[2]Variable extractant / pH1.5 / 80 °C /1h /Amrapali			[3]Variable pH/ HCl-NaOH buffer /80 °C /1h /Amrapali			[4]Variable Tem. /HCl-NaOH buffer/pH1.5/ 1h / Amrapali			[5]Variable extraction time/HCl-NaOH buffer/pH1.5/80 °C/1h/Amrapali			
		Amrapali	Fazlee	Langra	Kharsapat	HCl-NaOH	Citric acid	H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$	pH 1.5	pH 2.0	pH 2.5	Tem. 80 °C	Tem. 90 °C	Tem. 99.8 °C	Ex. time 1h	Ex. time 1.3h	Ex. time 2h	Ex. time 2.3h
Yield%	(Meam±SD)	30. 43±0.08[a]	25.53±0.33[b]	20.62±0.29[c]	17.31±0.11[d]	18.49±0.18[c]	18.85±0.13[b]	30.43±0.08[a]	18.49±0.19[a]	15.15±0.10[b]	10.99±0.09[c]	18.49±0.19[a]	14.48±0.06[b]	10.76±0.04[c]	18.49±0.19[a]	14.55±0.06[d]	15.94±0.03[c]	16.91±0.05[b]
Eq. wt.	(Meam±SD)	755.61±2.22[c]	795.61±1.19[b]	815.18±0.98[a]	686.05±0.015	737.51±1.02[b]	654.14±0.67[c]	755.61±2.22[a]	737.51±1.02[c]	1871.07±2.21	2140.70±1.13	737.51±1.02[a]	606.87±0.86[b]	506.58±0.66[c]	737.51±1.02[a]	695.49±0.83[b]	661.41±0.90[c]	695.70±1.01[b]
MeO%	(Meam±SD)	2. 85±0.01[d]	7.05±0.01[b]	8.94±0.01[a]	4.22±0.01[c]	9.37±0.01[a]	8.25±0.02[b]	2.85±0.01[c]	9.37±0.01[a]	6.23±0.01[b]	5.51±0.01[c]	9.37±0.01[c]	9.59±0.01[a]	9.57±0.02[b]	9.37±0.01[d]	9.95±0.02[c]	10.38±0.03[b]	10.6±0.01[a]
AUA%	(Meam±SD)	39.47±0.015[d]	62.20±0.01[b]	72.27±0.03[a]	49.53±0.02[c]	77.22±0.13[a]	73.61±0.01[b]	39.47±0.015[c]	77.22±0.13[a]	45.20±0.10[b]	39.52±0.11[c]	77.22±0.13[c]	83.40±0.01[b]	89.10±0.06[a]	77.22±0.13[d]	81.13±0.01[c]	83.91±0.03[b]	86.60±0.10[a]
DE%	(Meam±SD)	40.99±0.14[d]	64.35±0.10[b]	70.23±0.07[a]	48.37±0.13[c]	68.89±0.18[a]	63.72±0.11[b]	40.99±0.14[c]	68.89±0.19[c]	78.25±0.16[b]	79. 16±0.07[a]	68.89±0.19[a]	64.83±0.39[b]	60.76±0.14[c]	68.89±0.19[c]	69.65±0.10[b]	70.20±0.17[a]	69.85±0.15[b]
Protein %	(Meam±SD)	52..33±0.45[a]	14.09±0.39[c]	12.61±0.91[d]	15.97±0.34[b]	4.29±0.67[b]	4.45±0.12[b]	52.33±0.45[a]	4.29±0.67[b]	5.36±0.16[a]	4.57±0.34[b]	4.29±0.67[a]	4.33±0.47[a]	3.16±0.06[b]	4.29±0.67[b]	4.33±0.09[b]	5.30±0.04[a]	4.79±0.35[ab]

1, 2, 3, 4, 5 symbols indicate the different extraction conditions. Bold values indicate the maximum and minimum values in a certain condition. (Eq.wt. = Equivalent Weight, MeO= Methoxyl, AUA= Anhydrouronic acid, DE= Degree of Esterification). a, b,c,d symbols indicate the Means containing same letters do not differ significantly at 5% level of significance.

Fig. 3. Pectin's purity-impurity with different extraction condition (1, 2, 3, 4 , 5 indicate different extraction conditions)

Proximate and Elementary Analysis

The obtained moisture content was 9.4% (Table II) which is within the limit of commercial grade (9.4-11.3%) (Devi *et al*., 2014). Pectin is very hygroscopic, for this reseason, it must be preserved in closed dry atmosphere. The good quality pectin contains up to 10% ash content on the view point of gel formation (Devi *et al*., 2014).Therefore, isolated pectin may be considered to satisfactory good quality, supported by (Azad *et at*., 2014). Residual fat and crude fiber

Table II. Proximate analyses of extracted pectin

Parameters	Mixed pectin from Amrapali, Langra and Kharsapat
Moisture content (%)	9. 4±0.05
Total ash content (%)	5.839±0.01
Crude fiber content (%)	Absent
Total fat content (%)	Negligible

Values are given in means of triplicate measurement

Table III. Elementary analysis of extracted pectin and comparison with reference limit (EMEA, 2008)

Parameters	Concentration Metals (ppm)	Reference Concentration Limits for Individual Metal Catalysts and Metal Reagents				
		Oral Exposure		Parenteral Exposure		
		PDE (µg/day)	Conc. (ppm)	PDE (µg/day)	Conc. (ppm)	
Na	4.248	$(5.75 \times 10^5 - 3.45 \times 10^6)^6$	--	--	--	
K	83.16	$(31 \times 10^5)^7$	--	--	--	
Ca	12.6534	$(7 \times 10^5)^8$	--	--	--	
Mg	1.6426	$(15 \times 10^4 - 5 \times 10^5)^9$	--	--	--	
Mn	0.0205	2500	250	250	25	
Fe	BDL	13000	1300	1300	130	
Cr	0.3874	300	30	30	3	
Ni	0.0148	300	30	30	3	
Cu	0.0668	2500	250	250	25	
Pb	BDL	100	10	10	1	
Cd	BDL	--	--	--	--	
As	BDL	--	0.0008[*]	--	--	

BDL= Bellow detection limit (As = 1.0 ppb, Cd = 0.03 ppm, Pb = 0.10ppm, Fe = 0.30ppm). PDE=permitted daily exposure limit.
6 indicate that, the acceptable range of sodium intake for adults according to (European Food Safety Authority, 2006).
7 indicate the average dietary intake of potassium according to (European Food Safety Authority, 2009).
8 indicate that acceptable range of calcium intake for adults according to (European Food Safety Authority, 2009).
9 indicate that acceptable range of magnesium intake according to (European Food Safety Authority, 2009).
*indicate that acceptable range of As.

was also observed negligible amount. From the analysis it has been shown (Table III) that the heavy metals like As, Cd, Fe and Pb were in below the detection limit (BDL) and Cr(0.3874ppm), Ni(0.0148ppm) and Cu(0.0668ppm) were in acceptable limit comparing with given reference data (Table III) . On the other hand, the nutrient inorganic components like Na, K, Ca, Mn and Mg are shown in acceptable limit.

Microbiological analysis

Microbiological test clearly indicated that the product is microbiologically acceptable up to 6 months compared with 'Recommended Microbial Limit (U.S. Pharmacopia)' (Table IV).

Bangladeshi mango varieties can be considered as the sources of pectin for commercial production along with other sources.

Table IV. Microbial shelf-life studies of extracted pectin and microbial Limit (U.S. Pharmacopia)

Parameters	Extracted Pectin	Pectin (6 months after preparation)	Recommended (from U.S. Pharmacopeia) (cfu/g or mL)
Total Aerobic Microbial Count NMT, (cfu/g)	< 10	35	10^4
E. coli, in 10 g, (cfu/g)	Absent	Absent	Absence
Staphylococcus aureus in 1g, (cfu/g)	Absent	Absent	---
Salmonella spp. in 10 g, (cfu/g)	Absent	Absent	Absence
Total combined Yeast and Mold count NMT, (cfu/g)	Absent	Absent	10^3

NMT= Not more than

Conclusion

The optimum condition identified for highest purity (AUA; 89.10±0.06%) and lowest impurity (protein content; 3.10±0.06%) is "Tem. 99.8° C /HCl-NaOH /pH1.5/ 1h" for Amrapali. The condition for highest yield (30.43±0.08%) is "H_2SO_4-$(NH_4)_2SO_4$ /pH 1.5 /80 °C /1h /Amrapali". A correlation between AUA and protein content (fig: 3) was observed sharply in this study. Langra is the best source among four as its pectin contain highest purity (AUA, 72.27±0.03%) and lowest protein content (12.61±0.91) with the condition of "variable source/ H_2SO_4-$(NH_4)_2SO_4$/ pH 1.5 80°C/1h". Two kinds of pectin (HM and LM) were observed; HM pectin from Langra and Fazlee and LM pectins from Amrapali and Kharsapat. It was also noticed that both HM and LM pectins were obtained from Amrapali only for the change of extractants. Using HCl-NaOH for Amrapali pectin, the highest Eq.wt. was 2140.70±1.13 at pH 2.5 and the highest MeO content was 10.6±0.01% (at pH 1.5/2.3 h). From this investigation, it can be concluded that a suitable extraction procedure for high yield and high quality pectin from Bangladeshi mango varieties was established. Thus,

References

Albersheim P, Neukom H and Deuel H (1960), Splitting of pectin chain molecules in neutral solutions, *Arch Biochem Biophys*. **90:** 46–51.

Andrews WH and Hammack TS (1998), Bacteriological Analytical Manual, 8th Ed., Chapter 5 (*Salmonella*).

AOAC (2005), Official Methods of Analysis of AOAC International, 18th Ed., Association of Official Analytical Chemists, Gaithersberg, MD, USA, Official method (978.10), (2003.06), (976.05), (968.08).

Azad AKM , Ali MA, Aktar MS, Rahman MJ and Ahmed M (2014), Isolation and Characterization of Pectin Extracted from Lemon Pomace during Ripening, *Journal of Food and Nutrition Sciences* **2:** 30-35.

Barua H, Patwary MMA and Rahman MH (2013), Performance of BARI mango (*Mangifera indica* L.) varieties in Chitagong region, *Bangladesh j. Agril*. **38:** 203-209.

Bennett RW and Lancette GA (1998), Bacteriological Analytical Manual, 8th Ed., Revision A, Chapter 12 *(Staphylococcus aureus)*.

Cardoso SM, Coimbra MA and Lopes da Silva JA (2003), Temperature dependence of the formation and melting of pectin–Ca2+ networks: a rheological study, *Food Hydrocolloid*. **17:** 801–807.

Chan SY and Choo WS (2013), Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks, *Food Chem*. **141:** 3752–3758.

Constenla D and Lozano JE (2003), Kinetic model of pectin demethylation, *Latin Am Appl Res*. **33:** 91–95.

Devi WE, Shukla RN, Abraham A, Jarpula S and Kaushik U (2014), Optimized Extraction Condition and Characterization o f Pectin from Orange Peel, *IJREAT*. **2:** 1–9.

Daniel, Ikenna Udenwobele (2014), Extraction, Partial Purification and Characterization of Pectinases Isolated from Aspergillus Species Cultured on Mango *(Mangifera Indica)* Peels, *African Journal of Biotechnology* **13**(24): 2445–54.

EMEA (2008), Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents, *Emea/Chmp/Swp/4446/2000*, (January), 1–34.

European Food Safety Authority (2006), Tolerable Upper Intake Levels Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies.

European Food Safety Authority (2009), Chromium (III) , iron (II) and selenium-humic acid / fulvic acid chelate and supplemented humifulvate added for nutritional purposes to food Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (Questions No EFSA-Q, 1147, 1–36).

Faravash RS and Ashtiani FZ (2008), The influence of acid volume, ethanol-to-extract ratio and acid-washing time on the yield of pectic substances extraction from peach pomace, *Food Hydrocolloid*. **22:** 196–202.

Fertonani HCR, Scabio A, Canteri MH, Carneiro EBB, Nogueira A and Wosiacki G (2006), Influence of acid concentration on extraction and quality of apple pomace pectin, *Semin-Ciências Agrár Londrina* **27:** 599–612.

Food Chemical Codex (1996), 4th Ed., National Academy Press, Washington D.C.

Hitchins AD, Feng P, Watkins WD, Rippey SR and Chandler LA (1998), Bacteriological Analytical Manual, 8th Edition, Revision A, Chapter 4 (*Escherichia coli and the Coliform Bacteria*).

Hussain T, Baig MQ and Haroon R (1991), Extraction and evaluation of pectin from mango peel, *Pakistan J Agric Res*. **12:** 213 – 216.

Ismail NSM, Ramli N, Hani NM and Meon Z (2012), Extraction and characterization of pectin from dragon fruit (Hylocereus polyrhizus) using various extraction conditions, *Sains Malays*. **41:** 41-45.

Kansci G, Koubala BB and Lape IM (2003), Effect of ripening on the composition and the suitability for jam processing of different varieties of mango *(Mangifera indica)*, *Afr j Biotechnol*. **2:** 301-306.

Kobra K, Hossain MA, Talukder MAH and Bhuyan MAJ (2012), Performance of twelve mango cultivars grown in different agro-ecological zones of Bangladesh, Bangladesh *J. Agril. Res*. **37:** 691-710.

Koubala BB, Kansci G, Mbome LI, Crépeau MJ, Thibault JF and Ralet MC (2008), Effect of extraction conditions on some physicochemical characteristics of pectins from "Améliorée" and "Mango" mango peels, *Food Hydrocolloid* **22:** 1345–1351.

Kratchanova M, Benemou C and Kratchanov C (1991), On the pectic substances of mango fruits, *Carbohyd Polym*. **15:** 271–282.

Madhav A and Pushpalatha PB (2002), Characterization of Pectin Extracted From Different Fruit Wastes, *Journal of Tropical Agriculture* **40:** 53–55.

Maturin LJ and Peeler JT (1998), Bacteriological Analytical Manual, 8th Ed., Revision A, Chapter 3 (Aerobic Plate Count).

Mohamed S and Hasan Z (1995), Extraction and characterisation of pectin from various tropical agrowastes, *AFJ*. **10:** 143–150.

Mesbahi G, Jamalian J and Farahnaky A (2005), A comparative study on functional properties of beet and citrus pectins in food systems, *Food Hydrocolloid*. **19:** 731–738.

Morris GA, Foster TJ and Harding SE (2000), The effect of the degree of esterification on the hydrodynamic properties of citrus pectin, *Food Hydrocolloid* **14:** 227–235.

May CD (1990), Industrial pectins:Sources, production and applications, *Carbohyd Polym*. **12:** 79-99.

Nazaruddin R (2011), Effect of ammonium oxalate and acetic acid at several extraction time and pH on some physicochemical properties of pectin from cocoa husks *(Theobroma cacao)*, *African Journal of Food Scienc*. **5:** 790–798.

Toxicological Review (2010), *Review Literature And Arts Of The Americas* **39**(110): 759–786.

Ralet MC and Thibault JF (2002), Interchain heterogeneity of enzymatically deesterified lime pectins, *Biomacromolecules* **3:** 917–925.

Rehman Z, Salariya A, Habib F and Shah WH (2004), Utilization of Mango peels as a source of Pectin, *Jour. Chem. Soc. Pak*. **26:** 73-76.

Robert C, Devillers T, Wathelet B, Van Herck JC and Paquot M (2006), Use of a Plackett-Burman experimental design to examine the impact of extraction parameters on yields and compositions of pectins extracted from chicory roots *(Chicorium intybus* L.), *J Agric Food Chem*. **54:** 7167–7174.

Rombouts FM and Thibault JF (1986), Feruloylated pectic substances from sugar-beet pulp, *Carbohyd Res*. **154:** 177–187.

Sahari MA, Akbarian MA and Hamedi M (2003), Effect of variety and acid washing method on extraction yield and quality of sunflower head pectin, *Food Chem*. **83:** 43-47.

Shaha RK, Punichelvana YNAP and Afandi A (2013), Optimized Extraction Condition and Characterization of Pectin from Kaffir Lime (Citrus hystrix), *Res J Agriculture & Forestry Sci*. **1:** 1-11.

Sirisakulwat S, Nagel A, Sruamsiri P, Carle R and Neidhart S (2008), Yield and quality of pectins extractable from the peels of Thai mango cultivars depending on fruit ripeness, *J Agric Food Chem*. **56:** 10727–10738.

Sundar Raj AA, Rubila S, Jayabalan R and Ranganathan TV (2012), A review on pectin: Chemistry due to general properties of pectin and pharmaceutical uses, *Scientific reports* **1**(2): 1-4.

Tournas V, Stack ME, Mislivec PB, Koch HA and Bandler R (1998), Bacteriological Analytical Manual, 8th Ed., Revision A, Chapter 18 (Yeasts, Molds and Mycotoxins).

Voragen ACJ, Rolin C and Marr BU (2003), Pectins ***In***: Polysaccharides, Ed., Voragen ACJ, Rolin C, Marr BU, Challen I, Riad A and Lebabar R, Wiley-VCH., New York, pp 189-195.

Walter RH (1991), The chemistry and technology of pectin, Food Science and Technology-A series of monographs, Academic Press, New York.

Woo K, Chong YY, Li Hiong SK and Tang PY (2010), Pectin Extraction and Characterization from Red dragon Fruit *(Hylocerus polyrhyzuz)*: A Preliminary Study, *J Biol Sci*. **10:** 631-636.

Yapo B and Koffi K (2013), Extraction and Characterization of Highly Gelling Low Methoxy Pectin from Cashew Apple Pomace, *Foods* **3:** 1–12.