Pectin from ripe peels of mango cultivars

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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 & Kharasapat). 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 ‘H₂SO₄-(NH₄)₂SO₄ 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, spectroscopic 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 % (Mesbah 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 hystrix) (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 Kharasapat. Amrapali is a hybrid variety of Dasher and Neelum. Langra is medium size pyramidal shape light green color fruit. Kharasapat is conical shape green with red patches color and Fazlee is Pyramidal green with red patches color. The individual weight of Langra, Kharasapat 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 Kharasapat) were collected from Shaheb Bazar, Rajshahi, Bangladesh.

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**Pectin extraction method**

Pees were removed from mangoes and chopped (1 sq.cm) and dried in oven at 90 °C 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$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ buffer/ pH 1.5/ 80 °C/ 1h , 2) variable extractants (H$_2$SO$_4$-(NH$_4$)$_2$SO$_4$ 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 °C. The yields were 10.92-30.34 % on dry basis.

**Spectroscopical and structural investagation (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 Baird-Parker agar 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°C 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 H₂SO₄-(NH₄)₂SO₄ buffer /pH 1.5 /80 °C /1hour
/Amrapali. With the extraction condition: 'variable sources/
(H₂SO₄-(NH₄)₂SO₄ buffer/ pH 1.5/ 80 °C/ 1h)', the yields
were 30.43, 25.53, 20.62 and 17.31 % (g/g) from Amrapali,
Fazlee, Langra and Kharsapat respectively (Table 1).
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
(Koubula et al. 2008). Using HCl-NaOH buffer instead of
H₂SO₄-(NH₄)₂SO₄ buffer, the yield from Amrapali was
decreased (Table I). This result was consistent to (Rehman
et al., 2004) where the production decreased when HCl used
instead of H₂SO₄. Citric acid and HCl-NaOH buffer

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.

Fig. 1. FTIR analysis of pectin (Langra) (condition: H₂SO₄-(NH₄)₂SO₄ buffer /pH 1.5/80 °C/1h)

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 1). 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 galacturonic 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 1). 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-(NH4)2SO4 buffer/ pH1.5/ 80 °C/ 1h’, Eq.wt. from Amrapali, Fazlee, Langra & Kharsapat were 755.61, 795.61, 815.18 & 686.05 respectively (Table -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: ‘H2SO4/ 1.5/ 80 °C/ 1h’. By changing the extractant, e.g. using HCl-NaOH buffer instead of H2SO4-(NH4)2SO4 buffer, Eq.wt. of Amrapali pectin did not notably decrease (from 755.61-737.51), (Table 1). 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 1) 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 1 and Table 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 ‘H2SO4-(NH4)2SO4 buffer/ pH1.5/ 80°C/ 1h’, where as Fazlee and Kharsapat contained 7.05 and 4.22 % respectively (Table 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 H2SO4-(NH4)2SO4 buffer with constant other conditions, pectin from Amrapali contained maximum MeO content (9.37 %). When using citric acid buffer, it was 8.25 % (Table 1). It was decreased with increasing pH (9.37 % for pH 1.5 and 5.51 % for pH 2.5) (Table 1). 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 1 and Table 5). It was observed that, MeO contents of Amrapali pectin were 9.37-10.64 % for HCl-NaOH buffer and 8.25 % for citric acid buffer. Langra pectin contained 8.94 % for H2SO4-(NH4)2SO4 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 pomello, 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). On the other hand, the lowest AUA content was 39.47 % where protein content was highest (52.33%) (Table I). The AUA content of orange peel pectin was 93.28 % (Devi et al., 2014) and mango peel pectin was 73.16 (Madhav and Pushpalatha, 2002) respectively. With variable pectin sources at ‘H₂SO₄-(NH₄)₂SO₄ buffer/ pH1.5/ 80ºC/ 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 %) 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). These indicated that HCl-NaOH buffer was best according to purity. Changing the pH with the condition: ‘HCl-NaOH buffer/ 80 ºC/ 1h/ Amrapali’, the AUA content increased with decrease of pH (Table I). 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 and Table I 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). 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 Ca²⁺, 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 H₂SO₄-(NH₄)₂SO₄ 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: H₂SO₄-(NH₄)₂SO₄ buffer/ pH1.5/ 80 ºC/ 1h’, the maximum DE was obtained 70.23 % from Langra pectin and the minimum was 40.99 % from Amrapali (Table I). 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 H₂SO₄-(NH₄)₂SO₄ buffer, the obtained pectin from Amrapali was HM pectin where DE content was 68.89 %, but when used H₂SO₄-(NH₄)₂SO₄ buffer, then LM pectin (DE, 40.99 %) was obtained (Table I). 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/ 80ºC/ 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) 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). 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) 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’ (Chen and Choo, 2013).
Table I: Yield, biochemical properties (Eq.wt., MeO%, AUA% and DE%) and protein content of extracted pectin content under different extraction conditions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Extraction conditions</th>
<th>Protein %</th>
<th>DE%</th>
<th>AUA%</th>
<th>MeO%</th>
<th>Eq. wt.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variable extraction time/ HCl- NaOH buffer/80 °C</td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
<td>(Mean±SD)</td>
</tr>
<tr>
<td>HCl-NaOH buffer/ pH 1.5</td>
<td>4.29±0.07</td>
<td>40.09±0.14</td>
<td>9.37±0.01</td>
<td>73.51±1.02</td>
<td>18.49±0.18</td>
<td>70.23±0.07</td>
<td>15.97±0.34</td>
</tr>
<tr>
<td>pH 1.5</td>
<td>4.29±0.07</td>
<td>40.09±0.14</td>
<td>9.37±0.01</td>
<td>73.51±1.02</td>
<td>18.49±0.18</td>
<td>70.23±0.07</td>
<td>15.97±0.34</td>
</tr>
<tr>
<td>pH 2.0</td>
<td>4.45±0.12</td>
<td>63.72±0.11</td>
<td>73.61±0.01</td>
<td>65.41±0.67</td>
<td>18.85±0.13</td>
<td>72.27±0.03</td>
<td>4.22±0.01</td>
</tr>
<tr>
<td>pH 2.5</td>
<td>4.57±0.34</td>
<td>79.16±0.07</td>
<td>39.47±0.015</td>
<td>75.56±2.22</td>
<td>30.43±0.08</td>
<td>48.37±0.13</td>
<td>94.53±0.02</td>
</tr>
<tr>
<td>pH 3</td>
<td>5.36±0.16</td>
<td>78.25±0.16</td>
<td>45.20±0.10</td>
<td>1871.07±2.21</td>
<td>15.15±0.10</td>
<td>77.22±0.13</td>
<td>9.37±0.01</td>
</tr>
<tr>
<td>pH 4</td>
<td>4.93±0.47</td>
<td>64.83±0.39</td>
<td>83.40±0.01</td>
<td>606.87±0.86</td>
<td>14.48±0.06</td>
<td>77.22±0.13</td>
<td>9.37±0.01</td>
</tr>
<tr>
<td>pH 5</td>
<td>3.16±0.06</td>
<td>60.76±0.14</td>
<td>89.10±0.06</td>
<td>506.58±0.66</td>
<td>10.76±0.04</td>
<td>77.22±0.13</td>
<td>9.37±0.01</td>
</tr>
</tbody>
</table>

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 (52.3±0.45 %) with the extractant H2SO4-NH2SO4 buffer and the lowest was (14.09±0.39 %) 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 purity.
pectin) which have DE > 50% and low methoxyl pectin (LM pectin) that are widely utilized in cosmetic industries. Based on the degree of esterification (DE) of pectin, its yields, quality, and functionality (Daniel et al., 2014). The pH of the extraction solution 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 al., 2014). Residual fat and crude fiber content (%), AUA content (%), DE% and Protein% were computed at first.

**Table II. Proximate analyses of extracted pectin**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mixed pectin from Amrapali, Langra and Kharsapat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>9.4±0.05</td>
</tr>
<tr>
<td>Total ash content (%)</td>
<td>5.839±0.01</td>
</tr>
<tr>
<td>Crude fiber content (%)</td>
<td>Absent</td>
</tr>
<tr>
<td>Total fat content (%)</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

Values are given in means of triplicate measurement

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration Metals (ppm)</th>
<th>Reference Concentration Limits for Individual Metal Catalysts and Metal Reagents Oral Exposure</th>
<th>Parenteral Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PDE (µg/day)</td>
<td>Concent. (ppm)</td>
</tr>
<tr>
<td>Na</td>
<td>4.248</td>
<td>(5.75×10⁵-3.45×10⁶)⁶</td>
<td>--</td>
</tr>
<tr>
<td>K</td>
<td>83.16</td>
<td>(31×10⁵)⁷</td>
<td>--</td>
</tr>
<tr>
<td>Ca</td>
<td>12.6534</td>
<td>(7×10⁶)⁸</td>
<td>--</td>
</tr>
<tr>
<td>Mg</td>
<td>1.6426</td>
<td>(1.5×10⁴-5×10⁵)⁹</td>
<td>--</td>
</tr>
<tr>
<td>Mn</td>
<td>0.0205</td>
<td>2500</td>
<td>250</td>
</tr>
<tr>
<td>Fe</td>
<td>BDL</td>
<td>13000</td>
<td>1300</td>
</tr>
<tr>
<td>Cr</td>
<td>0.3874</td>
<td>300</td>
<td>30</td>
</tr>
<tr>
<td>Ni</td>
<td>0.0148</td>
<td>300</td>
<td>30</td>
</tr>
<tr>
<td>Cu</td>
<td>0.0668</td>
<td>2500</td>
<td>250</td>
</tr>
<tr>
<td>Pb</td>
<td>BDL</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Cd</td>
<td>BDL</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>As</td>
<td>BDL</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

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 that 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(0.01486ppm) 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).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Extracted Pectin</th>
<th>Pectin (6 months after preparation)</th>
<th>Recommended (from U.S. Pharmacopeia) (cfu/g or mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Aerobic Microbial Count NMT, (cfu/g)</td>
<td>&lt; 10</td>
<td>35</td>
<td>10⁴</td>
</tr>
<tr>
<td>E. coli, in 10 g, (cfu/g)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absence</td>
</tr>
<tr>
<td>Staphylococcus aureus in 1g, (cfu/g)</td>
<td>Absent</td>
<td>Absent</td>
<td>---</td>
</tr>
<tr>
<td>Salmonella spp. in 10 g, (cfu/g)</td>
<td>Absent</td>
<td>Absent</td>
<td>Absence</td>
</tr>
<tr>
<td>Total combined Yeast and Mold count NMT, (cfu/g)</td>
<td>Absent</td>
<td>Absent</td>
<td>10⁵</td>
</tr>
</tbody>
</table>

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 “H₂SO₄-(NH₄)₂SO₄ /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/ H₂SO₄-(NH₄)₂SO₄ /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, Bangladeshi mango varieties can be considered as the sources of pectin for commercial production along with other sources.

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