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Aegle marmelos fruit pectin for food and pharmaceuticals: Physico-chemical, rheological and functional performance

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ABSTRACT

Pectin is used in a number of foods as a gelling agent, thickener, texturizer, emulsifier and stabilizer. Bael fruit, obtained from *Aegle marmelos*, is a rich source of pectin. Bael fruit pectin (BFP) was extracted from ripe Bael fruits. The process yielded 15% (w/w) pure BFP. The swelling index decreased in the following order: water > pH 7.4 > pH 6.8 > pH 1.2 > HCl (0.1 N). Galacturonic acid content of 87.8%, degree of esterification of 47.2%, 17.3% methoxy groups, 0.29% acetyl groups and equivalent weight of 1209.5, indicate it to be a good gelling agent and easily amenable to derivatization. BFP exhibited a significant concentration-dependent prolongation of prothrombin time. The absence of hemagglutinating activity and antinutritional factors coupled with the activity to confer better emulsion capacity, stability and antimicrobial activity gives BFP a clear edge over commercial citrus pectin (CP) for exploitation as an additive in food and pharmaceuticals.

1. Introduction

Pectins are a specific group of carbohydrate polymers composed largely of a backbone of linked D-galacturonic acid units, many of which are esterified with methyl alcohol at the carboxylic acid, interspersed with a few L-rhamnose residues linked to neutral arabinogalactan side chains. Various amounts of the galacturonic acid regions are present (methyl esterified), and they could greatly influence the physicochemical properties of the pectin (Schols, Huisman, Bakx, & Voragen, 2003). Pectin is a valuable functional food ingredient widely employed as gelling, emulsifying and stabilizing agent. It is used worldwide in jams and jellies, fruit juices, fruit drink concentrates, desserts, baking fruit preparations, dairy and delicatessen products (Koubala et al., 2008). In addition, this polymer is also employed in the formulation of cosmetic and pharmaceutical products (Willats, Knox, & Mikkelsen, 2006), as well as for enzyme immobilization (Gómez, Ramírez, Neira-Carrillo, & Villalonga, 2006).

The primary class of pectin is homogalacturonan (HGA), which consists of linear chains of α-1,4-linked-D-galacturonic acid with some of the carboxyl groups in the methyl ester form. HGA is subdivided according to the degree of esterification (DE): low methoxyl pectins (LMP) have a DE < 50%, whereas high methoxyl pectins (HMP) have a DE > 50%. LMP may be used as a gelling agent in low sugar products, such as low calorie jams and jellies, confectionary jelly products, and other foods applications. The heat reversibility of LMP gels can be utilized in bakery jams and jellies for glazing, retorting, microwaving, baking, and sterilizing or pasteurizing (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007).

Industry, traditionally uses citrus wastes (pulp and peel), apple pomace and sugar-beet pulp as raw material for pectin production. Sugar beet pulp (*Beta vulgaris*), co-product from the sugar industry, owing to its high pectin content (15–30% (w/w)) is also frequently used for pectin production. However, due to poor gelling properties, pectin extracted from sugar beet pulp often does not yield satisfactory performance. These poor gelling properties have been attributed to the presence of large amount of acetyl groups, high neutral sugar content and relatively low average molecular weight (Arslan, 1995).

Aegle marmelos Corr. (Rutaceae) commonly called as 'Bael' in Hindi language is indigenous to India and the fruits are official in The Ayurvedic Pharmacopoeia of India (2007). The fruit is reported to contain important bioactive compounds such as carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids and terpenoids. It contains low amount of total sugars, reducing sugars, non-reducing sugars and tannins and high amount of pectin (Maity, Hansda, Bandyopadhyay, & Mishra, 2009). The fruit is edible and has been recommended for use as antiamoebic, antidiabetic

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and antihistaminic (Baliga, Bhat, Pereira, Mathias, & Venkatesh, 2010).

The abundance of Bael fruit and high content of pectin make it a viable option for exploring its potential in pharmaceuticals and food items. However, its physico-chemical, electrical, mechanical and functional properties have not been explored. Hence, the present investigation was designed for extracting and evaluating physico-chemical, electrical, mechanical and functional properties as well as functional properties of BFP.

2. Materials and methods

2.1. Materials

Fully ripe Bael fruits were collected from Punjabi University, Patiala, India campus. Pectin samples collected were stored in air-tight polypropylene jars in desiccated condition. Commercial citrus pectin (CP) and reagents for estimating prothrombin time APTT assay reagent, PT assay reagent and heparin were purchased from Himedia laboratories Ltd., Mumbai, India. All other chemicals used were of analytical reagent grade. De-ionized (Milli-Q) water was used for all experiments. All the chemical reagents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of BFP

BFP was extracted by modifying the method reported by Virk and Sogi (2004). Briefly, ripe Bael fruits were collected from the *A. marmelos* tree being grown at Punjabi University, Patiala, India campus. Inner ripe mass was washed and extracted with HCl solution (1 N) (Fig. 1). Equal amounts of water and extract were added and boiled for 25 min. The extract was strained through muslin cloth, cooled, mixed with potassium meta-bisulfite (0.5% (w/v)), and kept overnight for clarification. The supernatant was concentrated, precipitated with alcohol, vacuum dried, and powdered. The pectin was further purified by dialysis and purified BFP was obtained by freeze drying.

2.3. Antinutritional factors

Dried pulp was tested for the presence of antinutritional factors. The extracted pectin was tested only for the factors that presented positive results for the dried pulp.

2.3.1. Trypsin and α-amylase inhibitors

The trypsin inhibitory activity was determined using casein as the enzyme substrate. Trypsin inhibitory unit (TIU) was defined as the difference between the units observed in the maximum activity and the activity of the samples containing the inhibitors. The activity of α-amylase inhibitor was determined using starch as the substrate for the enzyme. One unit of α-amylase inhibitor was defined as the amount of inhibitor that inhibits one unit of α-amylase.

2.3.2. Hemagglutinating activity

Hemagglutination assays, using rabbit erythrocytes, were carried out following the method described by Moreira and Perrone (1977) with modifications. The extract (1% (w/v) dried pulp) prepared in 0.05 mol L^{-1} acetate buffer pH 5.0 was diluted in twofold dilution series against a 0.15 mol L^{-1} NaCl solution. One milliliter of a 2% erythrocyte suspension was added to an equal volume of the sample and the mixture incubated at 37 °C for 30 min followed by 30 min of resting at room temperature (25 °C). The tubes were centrifuged at 2000 × *g* for 1 min and the last tube to show visible agglutination was considered the point of equivalence to determination of minimal hemagglutinating concentration.

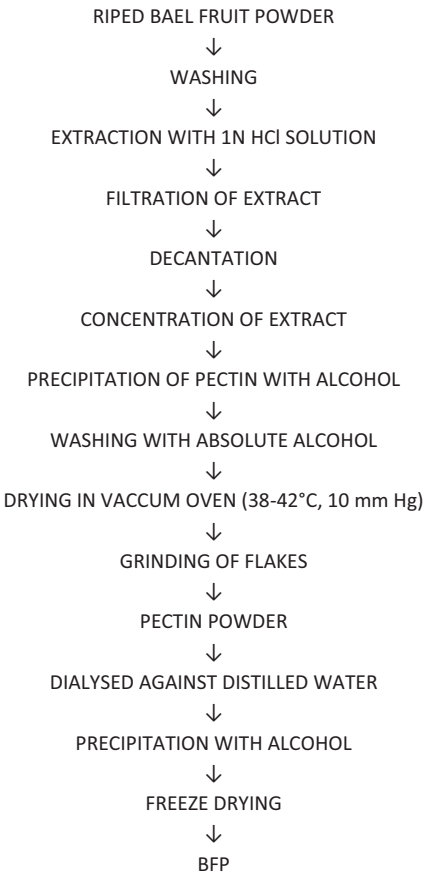

Fig. 1. Extraction process of Bael fruit pectin.

2.3.3. Phytic acid determination

The phytic acid content was determined by the method described by Latta and Eskin (1980) with modifications. A standard curve of phytic acid (Sigma, P8810) was made and the results were expressed as mg g^{-1} of the sample.

2.3.4. Total tannins

The analysis of total tannins in the extract (1% (w/v) dried pulp in 0.05 acetate buffer pH 5.0) was conducted according to Hagerman and Butler (1989). Tannin concentration in the sample was measured using a standard curve of tannic acid.

2.3.5. Saponins determination

Presence of saponins was determined by using the following methodology. 100 mg of dried pulp sample was suspended in 20 mL of distilled water and incubated in boiling water for 5 min. After incubation, the mixture was cooled to room temperature, filtered through a nylon membrane and the volume was adjusted to 100 mL with distilled water. Serial dilutions (10^{-1} to 10^{-5}) were performed using distilled water and the tubes were vortexed for 15 s followed by 15 min of incubation at room temperature (25 °C). The presence of persistent foam after incubation indicates saponin existence.

2.3.6. Alkaloids

The phytochemical analysis to evaluate the presence of alkaloids was carried out by employing the method described by Liener (1994). One gram of *A. marmelos* dried pulp or extracted pectin was dissolved in 10 mL of 1% (v/v) H_2SO_4 solution, and the mixture was

incubated for 2 min in boiling water. The solution was filtered and aliquots of 1 mL were added to tubes containing 40 mL of Dragendorff reagent. The formation of orange-red precipitate indicated the presence of alkaloids. To confirm result, 1 mL of the filtrate was added to tubes containing 40 mL of Mayer reagent. The formation of precipitate confirms the presence of alkaloids.

2.4. Physicochemical characterization of BFP powder

2.4.1. Physical properties

2.4.1.1. Swelling index. The BFP (10 mg) was soaked in distilled water, HCl (0.1 N) or phosphate buffer pH 1.2, 6.8 or 7.4 (100 cm^3) for 24 h. The swollen material was then removed and weighed after superficial drying using a blotting paper. The swelling index (SI) was calculated as:

$$SI = \frac{w_f - w_i}{w_i}$$

where w_f is the weight of swollen material and w_i is the initial weight of the dry material.

2.4.1.2. Effective pore radius. The $R_{eff.p}$ of powder blends was estimated according to the method reported by Goel, Kaur, Tiwary, and Rana (2010). In brief, plastic tip used for micropipette was filled with BFP powder and weighed (W_A). Then *n*-hexane (surface tension, (γ) 18.4 N/m, $\theta = 0^\circ$) was added dropwise to the top of packed bed till the solvent filtered out at the bottom of the tip. The tip was weighed again (W_B). The $R_{eff.p}$ was calculated using formula:

$$R_{eff.p} = \frac{W_B - W_A}{2\pi\gamma}$$

2.4.2. Chemical properties

2.4.2.1. Equivalent weight. BFP (0.5 g), ethanol (5 mL), sodium chloride (1.0 g), carbon dioxide free distilled water (100 mL), and six drops of phenol red indicator were dissolved and titrated against standard NaOH (0.1 M) until the color of indicator changed (pH 7.5) to pink and persisted for at least 30 s (Ranganna, 1986).

$$\text{Equivalent weight} = \frac{\text{Weight of sample (g)} \times 1000}{\text{Volume of alkali (mL)} \times \text{Normailty of alkali}}$$

2.4.2.2. Methoxyl content. NaOH (25 mL of 0.25 M) was added to the neutral solution titrated for equivalent weight containing 0.5 g of pectic substance, shaken thoroughly, and allowed to stand for 30 min at room temperature in a stoppered flask. HCl (25 mL, 0.25 M) was added and titrated with NaOH (0.1 M) to the same end point as before (Ranganna, 1986).

$$\text{Methoxyl content (\%)} = \frac{\text{Volume of alkali (mL)} \times \text{Normailty of alkali} \times 31 \times 100}{\text{Weight of sample (g)} \times 1000}$$

2.4.2.3. Acetyl value. Pectin (0.5 g) and NaOH (25 mL, 0.1 M) were stirred until the pectin dissolved and allowed to stand overnight. The contents were diluted to 250 mL with water and an aliquot (20 mL) was placed into the distillation apparatus. Magnesium sulfate–sulfuric acid (20 mL) solution containing magnesium sulfate (100 g) and sulfuric acid (1.5 g) diluted to 180 mL was also transferred to distillation apparatus, distilled, and about 100 mL of distillate was collected. The distillate was titrated with NaOH (0.05 M) using phenol red indicator. A blank distillation with magnesium sulfate–sulfuric acid solution (20 mL) was carried out and distillate was titrated (Ranganna, 1986).

$$\text{Acetyl value (\%)} = \frac{\text{Volume of alkali (mL)} \times \text{Normailty of alkali} \times 4.3}{\text{Weight of sample in aliquot (g)}}$$

2.4.2.4. Anhydrouronic acid. The alkali milli-equivalents from equivalent weight, methoxyl content and alkalinity of ash were used for calculating anhydrouronic acid (AUA) content (Ranganna, 1986).

$$\text{AUA (\%)} = \frac{176(\text{m.e. for freeacid} + \text{m.e. for saponification} + \text{m.e. for titrable ash}) \times 100}{\text{Weight of sample (mg)}}$$

2.4.2.5. Degree of esterification (DE). The degree of esterification was calculated from methoxyl and anhydrouronic acid content using the following expression:

$$\%DE = \frac{176 \times \text{Methoxyl content (\%)} \times 100}{31 \times \text{Anhydrouronic acid content (\%)}}$$

2.4.2.6. FT-IR spectroscopy. FT-IR spectra of BFP were recorded on a FT-IR–ATR spectrophotometer (Alfa, Bruker, Berlin, Germany). The lyophilized dry powder was mixed with KBr and pressed into pellets. The FT-IR spectra were obtained between wavelengths of 4000 and 400 cm^{-1}.

2.4.2.7. ^1H NMR. ^{1}H NMR spectra were recorded on a Bruker AMX 500 FT spectrometer at 25 °C (Cui, 2005).

2.4.2.8. Thermal analysis. A sample of BFP was hermetically sealed in aluminum pan and heated over temperature range of 40–350 °C in an atmosphere of nitrogen at a constant heating rate of 10 °C/min. The peak transition as well as enthalpy of fusion was estimated from the DSC (Setaram, Lab Sys Evo, France) thermogram.

2.5. Electrical properties

2.5.1. Zeta potential studies

The zeta potential of BFP was measured by using Zetasizer 4 (Malvern Instrument Ltd., UK). The temperature of the samples was maintained at 25 °C. The zeta potential measurements were performed by using an aqueous dip cell in an automatic mode. Samples were diluted with HPLC water (MilliQ Synergy Systems, Millipore) and placed in capillary measurement cell.

2.5.2. Scanning electron microscopy (SEM)

BFP samples were mounted on a clean aluminum stub with silver PAG-915 and coated with gold palladium alloy (160 Å thickness) on a sputter coater. The BFP sample was then photographed using scanning electron microscope (LEO 435VP, Cambridge, UK).

2.6. Rheological behavior

2.6.1. Solution preparation

BFP and CP sample was dissolved in distilled water at varying concentrations (0.5–5.0% (w/v)) using a magnetic stirrer (2MLH, REMI Elektrotechnik Ltd., Vasai, India) for 3 h and then centrifuged (using the C-24 BL, REMI Elektrotechnik Ltd., Vasai, India) for 25 min at 25 °C at a speed of 2500 rpm to remove insoluble matter.

2.6.2. Intrinsic viscosity and determination of molecular weight (M_w)

The intrinsic viscosity of BFP or CP was determined using a Brookfield viscometer (Brookfield DV-1 Prime, Bruker, Berlin, Germany). 100 mg of BFP sample was dispersed in 100 mL of an aqueous solution containing NaCl 0.1 mol L^{-1} to reduce the eletroviscous effect to a minimum. The density of the solutions was measured using Gay–Lussac type pycnometer. All experiments were carried out at 25 °C. The relative viscosity was calculated using the following equation:

$$\eta_r = \frac{\eta}{\eta_s}$$

where η_r is the relative viscosity, η is the viscosity of the pectin solution (mPa s), and η_s is the viscosity of the solvent (mPa s).

The relative viscosity values were converted to intrinsic viscosities using the following equation:

$$\eta_r = 1 + [\eta]C$$

where $[\eta]$ is the intrinsic viscosity and C is the pectin concentration.

The molecular weight of the pectin of BFP or CP was estimated by applying the Mark–Houwink–Sakurada equation, relating $[\eta]$ with M_w (Arslan, 1995):

$$[\eta] = k[M_w]^{\alpha}$$

where k and α are constants. Both, k and α, depend on the solute and solvent characteristics as well as the temperature utilized. In the case of a pectin dissolved in a sodium chloride solution (0.1 mol L^{-1}) they may be assumed to be, $k = 9.55 \times 10^{-2}$ and $\alpha = 0.73$, according to Anger and Berth (1986).

2.7. Functional properties

2.7.1. Emulsion capacity

The emulsification properties of BFP were compared with CP. Aqueous dispersions of BFP were prepared in water. Commercial corn oil (1% (w/v)) was added to BFP or CP dispersion. The amount of BFP or CP in each dispersion was appropriately adjusted so as to yield 0.1, 0.25, 0.50, 0.75 or 1.0% (w/v) concentration in the final mixture. Each mixture was homogenized for 1 min. The dispersions were then centrifuged at 800 × g for 10 min. The emulsifying capacity (EC) was calculated as (Scarini, Maldonado, Ribotta, Perez, & Leon, 2009):

$$\mathrm{EC} = \frac{e_v}{t_v} \times 100$$

where e_v is the emulsion volume and t_v is the total volume.

2.7.2. Emulsion stability

Emulsion stability (ES) of emulsions at high temperature was determined by heating in a water bath at 80 °C for 30 min followed by centrifugation at 800 × g for 10 min. ES was calculated as (Sciarini et al., 2009):

$$\mathrm{ES} = \frac{f_{ev}}{i_{ev}} \times 100$$

where f_{ev} is the final emulsion volume and i_{ev} is the initial emulsion volume.

2.8. Antimicrobial effect

The antimicrobial activities of BFP and CP samples against *Bacillus cereus* and *Escherichia coli* were examined. *B. cereus* and *E. coli* were inoculated in nutrient broth and incubated at 37 °C for 24 h. The pectin solutions (90 μL) with three different concentrations (0.5, 1.0, and 2.0 mg/mL) were added to the culture broth (10 μL) which was incubated at 37 °C for 18 h, then the absorbance of the culture broth was measured at 540 nm. The microbial inhibition effect was calculated as follows:

$$\text{Inhibition effect}\,(\%) = \left[\frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}}\right] \times 100$$

2.9. Anticoagulant activity

The anticoagulant activity of BFP and CP pectin sample was determined by using the method of Matsubara et al. (2001). For the activated partial thromboplastin time (APTT) assay, BFP or CP (50 μL in distilled water at different concentrations of 25, 50, and 100 μg/mL) was mixed with the plasma (50 μL) and incubated at 37 °C for 2 min. Then, APTT assay reagent (100 μL) was added to the resulting solution and further incubated at 37 °C for 6 min. After the addition of 20 mM $CaCl_2$ (100 μL), the clotting time was recorded and compared with that of heparin (Himedia laboratories Ltd., Mumbai, India). In the prothrombin time (PT) assay, PT assay reagent (100 μL) preincubated at 37 °C for 10 min was added to the solution of the BFP or CP (50 μL) and plasma (50 μL) and the clotting time was then measured. The prothrombin time (International Normalized Ratio) was obtained from the clotting time ratio between the sample and control.

3. Results and discussion

The extraction process yielded approximately 57% (w/w) of crude pectin. The purification process of dialysis followed by repeated precipitation and filtration yielded 15% (w/w) pure BFP after freeze drying.

3.1. Antinutritional factors

The results showed the absence of hemagglutinating activity, saponins and trypsin and α-amylase inhibitors in the *A. marmelos* dried pulp. The absence of these antinutritionals improves the nutritional value of the *A. marmelos* and consequently of the pectin. Alkaloids are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores. Results obtained in Dragendorff and Mayer tests evidenced the presence of alkaloids in dried pulp of *A. marmelos* fruit but these compounds were absent in the extracted pectin.

The *A. marmelos* fruit dried pulp presented 1.07 mg g^{-1} of phytic acid. Phytate is considered an antinutritional factor mainly due to its ability to bind essential dietary minerals, proteins and starch, which consequently reduces their bioavailability. Although several authors consider phytic acid an antinutritional factor, recent studies point out that this compound is an important additive, with antioxidant properties that can be exploited in manufacturing bread, pasta and meat products (Oatway, Vasanthan, & Helm, 2001). In addition, several authors have reported beneficial effects of phytic acid against cancer (Vucenik & Shamsuddin, 2006). Tannins are a special group of phenolic compounds that can react with proteins or minerals decreasing their bioavailability (Ferreira, Nogueira, Souza, & Batista, 2004). Although we detected as much as 1.21 mg tannic acid per 100 mg^{-1}, there was no phytic acid or tannins in the extracted pectin, in all tested conditions. These results, associated with the absence of amylase and trypsin inhibitors, hemagglutinating activity suggest improved safety and nutritional quality of the BFP.

Table 1
Physico-chemical properties of Bael fruit pectin.

Parameters		Results
Swelling index	Water	3.8 ± 0.23
	HCl (0.1 N)	1.1 ± 0.12
	Buffer pH 1.2	2.3 ± 0.21
	Buffer pH 6.8	3.2 ± 0.25
	Buffer pH 7.4	3.69 ± 0.31
Effective pore radius (mm)		$3.06 \pm 0.29 \times 10^{-1}$
Equivalent weight		1209.5 ± 6.9
Methoxyl content (%)		17.3 ± 0.64
Acetyl value (%)		0.29 ± 0.08
Anhydrouronic acid content (%)		81.8 ± 1.4
Degree of esterification (%)		47.20 ± 1.2

3.2. Physical properties

3.2.1. Swelling index

The swelling characteristics of BFP were investigated in 0.1 N HCl, pH 1.2, 6.8, 7.4 or water (Table 1). The swelling of BFP in different media was observed to follow the order: water > pH 7.4 > pH 6.8 > pH 1.2 > HCl (0.1 N). It is well established that low swelling in acidic pH restricts the release of drugs from dosage forms. At the same time, high swelling in alkaline pH would be useful for sustaining the drug release as the dosage form travels down the gastrointestinal tract. Therefore, the swelling behavior of BFP can be expected to be useful for modulating the drug release from dosage forms (Rai, Tiwary, & Rana, 2012).

3.2.2. Effective pore radius

Effective pore radius is an indicator of porosity of powders. Rai et al. (2012) reported $R_{eff,p}$ of *Cassia fistula* gum (2.72×10^{-1} mm), carboxymethylated *C. fistula* gum (3.04×10^{-1} mm) and carbamoylethylated *C. fistula* gum (3.42×10^{-1} mm). These were observed to exhibit good wicking properties, which increased with increase in $R_{eff,p}$ suggesting their super disintegration potential. Hence, $R_{eff,p}$ of 3.06×10^{-1} mm suggests high porosity and greater compressibility (Table 1). These properties indicate a potential role of BFP in fast dissolving tablet formulations and food items.

3.3. Chemical properties

3.3.1. Equivalent weight

The equivalent weight of BFP was found to be 1209.5 (Table 1). The equivalent weight of pectins from golden delicious pomace and golden delicious partially ripe apples were 1384 and 1027.7, respectively (Sharma, Lal, Kumar, & Goswami, 1985). Higher equivalent weight of BFP might be responsible for its good emulsion capacity and stability.

3.3.2. Methoxyl content

The methoxylation characteristics of any pectin may affect the gelling conditions and the viscosity of pectin solutions. It is a major factor in the determination of the pectin functionality (Fraeye et al., 2009). The spreading quality and gel grade of pectin are dependent of their methoxyl content. The pectin extracted from *A. marmelos* produced in this study can be categorized as low methoxyl pectin (Table 1).

3.3.3. Acetyl value

The acetyl value of BFP was 0.29% (Table 1). Properties of pectin in cell walls are sometimes modified by low levels of hydroxyl esterification with acetyl groups. The distribution of acetyl groups in pectin is unknown but in sugar beet, pear and apricot pectin, acetyl levels are reported to approach 4% (Ranganna, 1986). Perhaps other pectins may also contain this group. The presence of acetyl groups in pectin inhibits jelly formation. Hence, low acetyl value of BFP, suggests the usefulness of BFP in formulating jams, jellies and other food items.

3.3.4. Anhydrouronic acid content

According to the guidelines laid down by FCC, the pectins containing anhydrouronic acid content higher than the 65% are considered pure (FCC, 2004). Moreover, anhydrouronic acid content of pectin is an important parameter which indicates the suitability of pectin for its use in jams, jellies, etc. The anhydrouronic acid content of BFP was found to be 81.8% (Table 1). Therefore, the pectin obtained from Bael fruits could be considered of a high purity and indicates its suitability for formulating jams, jellies, etc.

3.3.5. Degree of esterification

The degree of esterification of pectin extracted from peel was found to be 47.20% (Table 1). Pectins (with DE < 50%) form rigid gels by the action of calcium or multivalent cations, which cross-link the galacturonic acid chains. This property of pectins is very useful in designing controlled release dosage forms as the lower the DE, the greater the time for drug release (Sungthongjeen, Sriamornsak, Pitaksuteepong, Somsiri, & Puttipipatkhachorn, 2004).

3.3.6. Spectral attributes

In freeze dried BFP (Fig. 2a), a broad band appearing around 3404.99 cm^{-1} corresponds to OH stretching peak, at 1615.95 cm^{-1} depicts the stretching zone of C=O and 1076.11 cm^{-1} depicts the stretching vibration of C—O group, which is characteristic of polysaccharides (Cui, Phillips, Blackwell, & Nikiforuk, 2007). The peak at 2925.84 cm^{-1} results from stretching modes of the C—H bonds of methyl groups (—CH_3). Absorption peaks at 1435.73 cm^{-1} represent CH_2 bending, 1374.06 cm^{-1} represent —OH bending and 1253.80 cm^{-1} represent carboxylic acid moieties of uronic acid units. The wave numbers between 800 and 1200 cm^{-1} represent the finger print region for carbohydrates (Cui et al., 2007).

The ^{1}H NMR spectrum of the BFP sample (Fig. 2b) shows the anomeric H-1 (δ 5.06 ppm) and H-5 (δ 4.82 ppm) proton signals from non-esterified galacturonic acid residues, which are in agreement with previous reports, where the H-5 protons in pectin samples having free carboxylic acid groups were found around δ 4.8–4.9 ppm (Marcon, Carneiro, Wosiacki, Beleski-Carneiro, & Petkowicz, 2005). By other side, the H-1 and H-5 protons from esterified carboxyl groups in the galacturonic acid residues appear as two overlapped signals around δ 4.9 ppm. The H-4 protons signal overlapped with the HOD signal (δ 4.3 ppm), whereas the H-3 and H-2 appeared at δ 3.87 and 3.62 ppm, respectively. The spectra of BFP sample contained a sharp signal at δ 3.67 ppm, which could be assigned to the protons of the methoxyl group from the esterified units of galacturonic acid (Winning, Viereck, Nørgaard, Larsen, & Engelsena, 2007).

3.3.7. Thermal behavior

Fig. 3 shows the thermogram of BFP. Structural and functional group differences in polysaccharides influence the thermal behavior and affect the transition temperature. The endothermic transition of BFP was observed at 85.28 °C. The fact that purified BFP was obtained by freeze drying, the contribution of moisture to this transition can be ruled out. The endothermic transition for gum kondagogu (Vinod et al., 2008) and odina gum (Sinha, Al-Azaki, & Kumar, 2011) have been reported to occur at 34.51 °C and 57.24 °C, respectively. The higher peak temperature of endothermic transition of BFP suggests that the polysaccharide units to be more organized in BFP. In addition, the moderately higher temperature would not pose problems during drying of formulations.

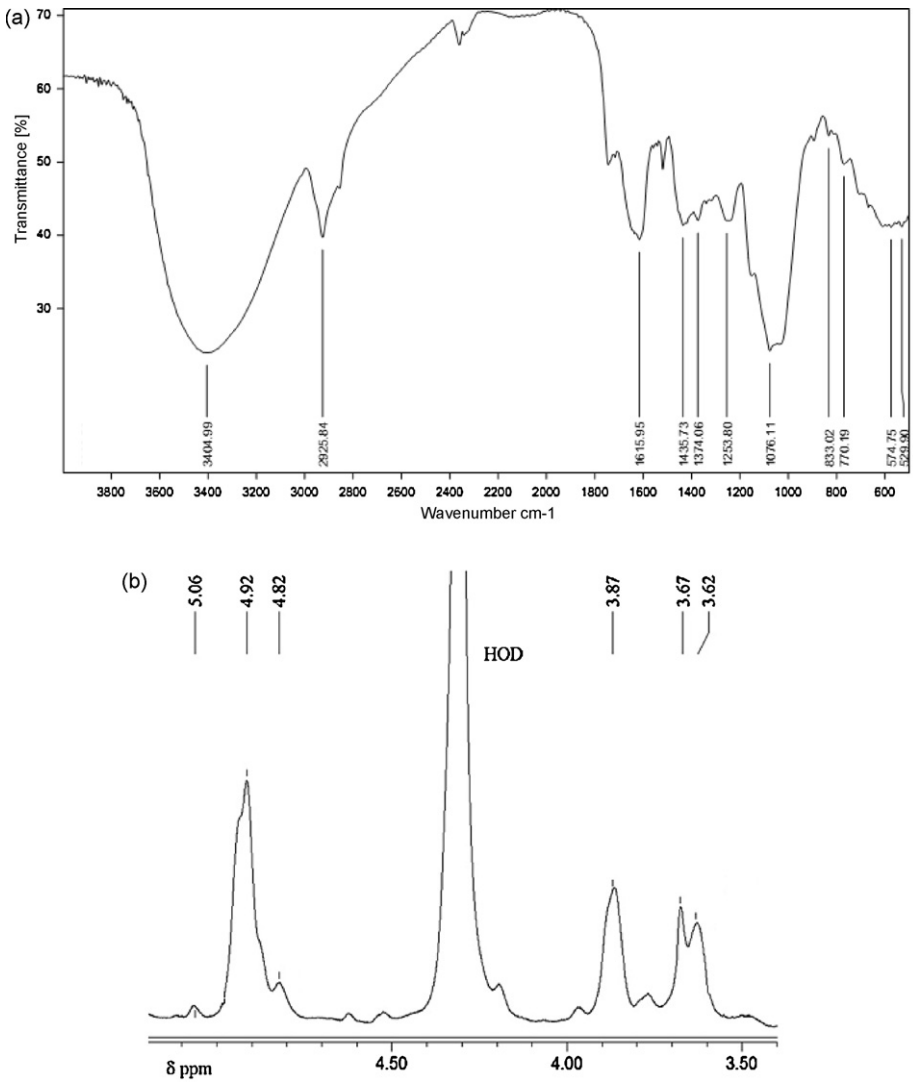

Fig. 2. (a) FT-IR spectrum and (b) NMR spectrum of Bael fruit pectin.

3.4. Electrical properties

3.4.1. Particle size and charge

Aqueous dispersions of BFP gave an acidic pH that could be attributed to presence of uronic acid units in its structure (Cui, 2005). Aqueous dispersions of BFP exhibited zeta potential of −24.2 mV indicating its polyelectric effect in pure water. The negative zeta potential of BFP suggests its utility in enforcing pectin–polymer or pectin–ion interactions for modulating drug release characteristics.

Scanning electron microphotograph (Fig. 4) revealed smooth surface and round shape of BFP particles that would confer good flow properties to the powder.

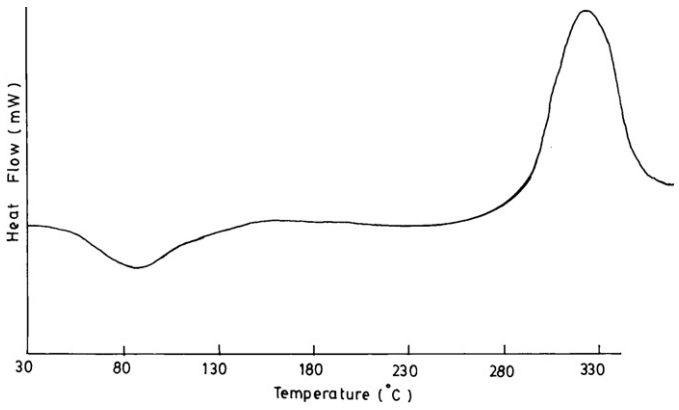

Fig. 3. DSC thermogram of Bael fruit pectin.

3.5. Rheological behavior

The intrinsic viscosity of a polymer solution is a measure of the capacity a polymer molecule to enhance the viscosity of a fluid. The results of the viscosity analysis showed that there were significant differences ($p < 0.05$) between the viscosities of CP and *A. marmelos* pectin solutions. The commercial CP solution presented viscosity ($\eta = 4.97 \pm 0.03$) 4.9% higher than *A. marmelos* pectin ($\eta = 3.97 \pm 0.04$). This difference can be explained by the fact that viscosity is affected by molecular weight, pH, and presence of counter-ions. The molecular weight (M_w) estimated for CP (227.62 ± 3.12) was higher than that of BFP (172.93 ± 2.87). An increase in molecular weight causes an increase in the pectin's viscosity, which alters its properties and applications.

Table 2
Comparison of emulsion capacity and stability of Bael fruit pectin and citrus pectin.

Concentration (w/v, %)	Citrus pectin		Bael fruit pectin	
	Emulsion capacity (%)	Emulsion stability (%)	Emulsion capacity (%)	Emulsion stability (%)
0.1	39.2 ± 2.1	43.6 ± 1.9	46.1 ± 1.3	57.1 ± 2.4
0.25	41.4 ± 3.2	52.4 ± 1.4	52.6 ± 2.8	65.3 ± 3.1
0.5	49.6 ± 3.1	65.3 ± 2.6	68.3 ± 1.7	72.1 ± 3.9
0.75	71.1 ± 3.7	79.9 ± 3.2	80.2 ± 4.2	90.2 ± 4.8
1.0	60.2 ± 2.7	70.1 ± 3.7	72.3 ± 4.1	89.4 ± 4.3

3.6. Functional properties

3.6.1. Emulsion capacity and stability

Surface activity and associated capacity to stabilize emulsions are important functionalities of natural polysaccharides. Many studies have been carried out for assessing the emulsifying potential of different polysaccharides, including sugar beet pectin (Yapo et al., 2007) and non-depolymerized pectin in similar conditions (Huang, Kakuda, & Cui, 2001).

Comparison of emulsification properties of BFP with CP is summarized in Table 2. BFP fractions exhibited better emulsion capacity (80.2%) and stability (90.2%) than CP at 0.75% (w/v) concentration. Earlier investigations on non-depolymerized pectin (Huang et al., 2001) had suggested high molecular weight coupled with high branched substitution to be responsible for the better emulsification properties. Hence, the better emulsion capacity and stability accorded by BFP as compared to CP could be attributed to its branched substitutions of carboxylic acid groups as the molecular weight of the former was less than that of that later.

3.7. Antimicrobial effect

Antimicrobial effects of BFP and CP samples were evaluated using *B. cereus* and *E. coli* as a model system. Overall, the BFP exhibited better antimicrobial effects than CP at all concentrations tested for *B. cereus* (Fig. 5a). Even, dried pulp of *A. marmelos* significantly inhibited the growth up to 10% and 16% at a concentration of 2.0 mg/mL against *B. cereus* and *E. coli*, respectively (Fig. 5a and b). It implies that the higher uronic acid content enhanced the antimicrobial activity of BFP. In addition, the antimicrobial effect had a tendency to increase with increasing concentration of pectin. Cellulose sulfate also exhibited antimicrobial activity against *Neisseria gonorrhoeae* and *Chlamydia trachomatis* which was explained by the interaction between proteoglycan receptors and their target cell ligands (Anderson et al., 2002). Moreover, the growth of *E. coli* was dramatically inhibited by BFP compared to that of *B. cereus*. Thus, the uronic acid rich pectin, BFP, appeared to be more effective against *E. coli* which belongs to the gram-negative bacteria. Previously, Park, Choi, and Chang (1995) reported greater activity of pectin hydrolysate against gram-negative bacteria (*E. coli* and *Acetobacter aceti*) than gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*).

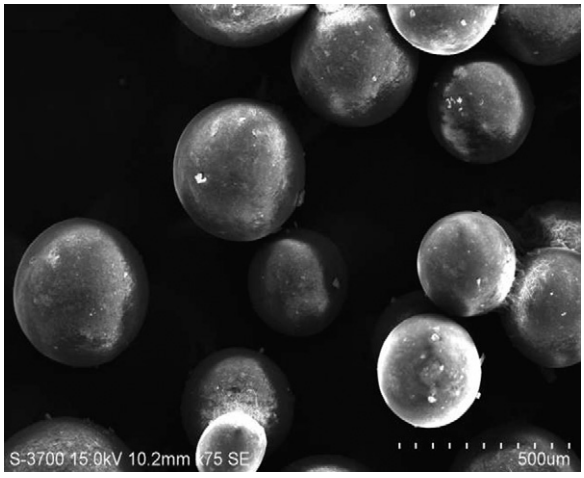

Fig. 4. SEM photomicrograph of Bael fruit pectin.

3.8. Anticoagulant activity

Blood coagulation is a complex process involved in the sequential activation of clotting factors, ultimately leading to insoluble fibrin. The disorders of coagulation can give rise to an increased risk of bleeding and/or clotting. Since the heparin has been clinically used as an effective anticoagulant medicine, the anticoagulant activity of the BFP as well as CP was compared with that of heparin. BFP significantly prolonged the prothrombin time (PT) in a concentration-dependent manner (Fig. 6). Thus, the uronic acid enriched pectin, BFP, appeared to be responsible for greater anticoagulant activity since CP exhibited significantly less anticoagulant effect. In the case of APTT (activated partial thromboplastin time), the BFP showed more than 5 min in the concentration range of 25–100 μg/mL. They could be explained by the anionic characteristics of the pectin that interacts with positively charged coagulant proteins, creating/improving the anticoagulant activity.

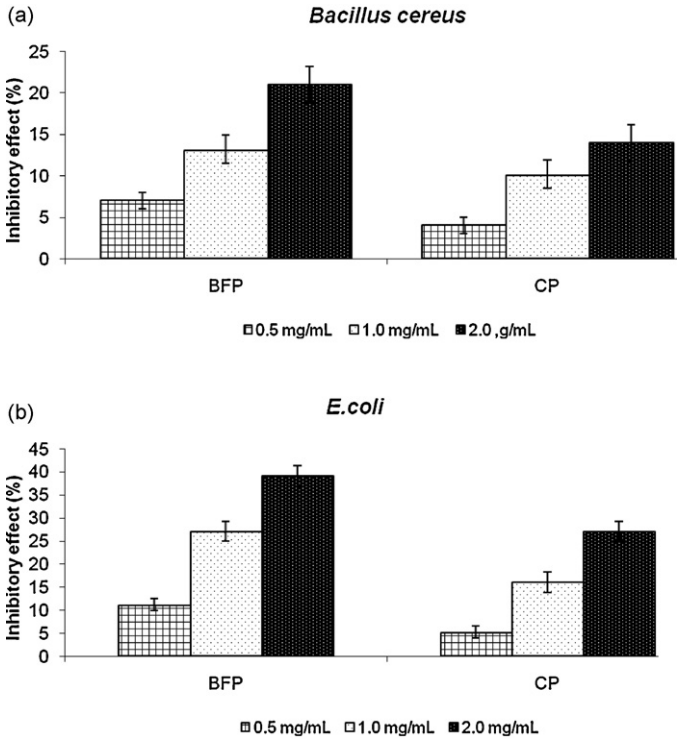

Fig. 5. Antimicrobial activities of BFP and CP against: (a) *Bacillus cereus* and (b) *E. coli*.

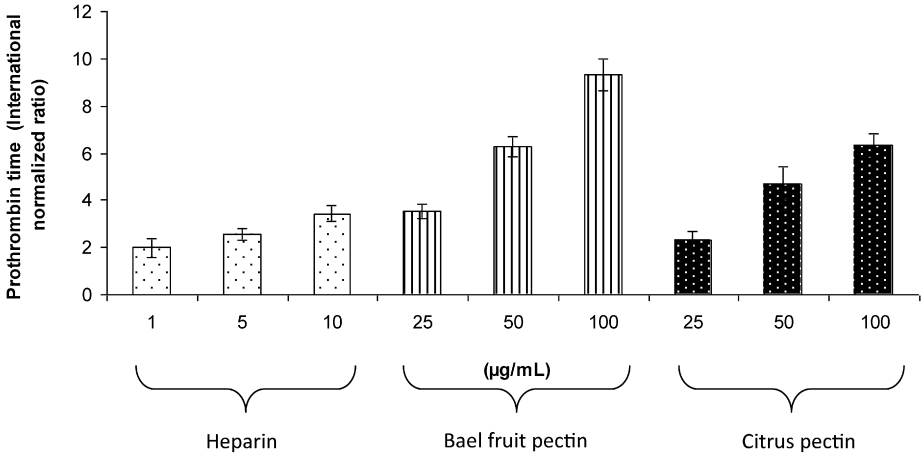

Fig. 6. Comparison of anticoagulant activity of heparin with Bael fruit pectin and citrus pectin.

Similar anticoagulant effects have been observed for other polymers containing anionic groups such as β-glucan (Bae, Chang, Kim, & Lee, 2008), chitosan, galactan (Matsubara et al., 2001) and galactomannan (Mestechkina et al., 2008). The prolonged PT and APTT indicate the inhibition of extrinsic and intrinsic coagulation pathways, respectively.

4. Conclusion

The result of present investigation revealed swelling index and effective pore radius of freeze dried BFP to be suitable for use in pharmaceuticals and food products. Further, low acetyl value, high anhydrouronic acid content and low degree of esterification suggested its usefulness in modifying drug release from pharmaceutical dosage forms and in providing desirable attributes to food products. The absence of hemagglutinating activity and antinutritional factors coupled with the activity to confer better emulsion stability and antimicrobial activity gives BFP a clear edge over commercial CP for exploitation as an additive in food and pharmaceuticals.

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