

Gel formation of pectin from okra (*Abelmoschus esculentus* L. Moench) leaves, pulp and seeds

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Abstract

Okra plant particularly its fruit is highly mucilage which composed of pectin and high content of carbohydrate. Byproducts of okra plant such as leaves and matured fruits will be discarded whenever the young fruits are harvested which eventually leads to environmental pollution. Those byproducts have potential to become plant-based alternative for bovine and pork related gelatin. This study aimed to determine the gel formation of pectin extracted from okra plant byproducts particularly the leaves, pulp (skin without seeds) and seeds. Pectin was extracted using a sequential extraction with the applications of hot buffer (HB) and hot buffer with chelating agents (CH). CH extraction gave the highest pectin yield (>40%) compared to HB and DA. The HB fraction harbored highly purified pectin due to high anhydro uronic acid content and degree of esterification. The highest pectin yield was extracted from seeds with an overall fraction yield of 86%, followed by the leaves (75%) and pulp (71%). The pectin was blended with konjac glucomannan (KG) in 5.0:1.6 ratio to form gel and stored for 16 - 18hr at 4°C ± 1.0. The gel formed using HB extraction was found to have significantly lower ($p < 0.05$) gel strength than HB with CH extraction. This study concluded that HB and CH pectin extracts derived from okra leaves, pulp and seeds have good potential to become gelling agent.

Keywords

Okra
Pectin
Gelatin
Gel formation
Hot buffer extraction

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Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is known as the lady's fingers, 'bhindi' (India), 'gumbo' (Africa), and 'bendi or kacang bendi' (Malaysia). The okra leaves are slightly bigger than tea leaves with longer stem; they alternate at wide space intervals up to the top of the stem and they are deeply divided with toothed margins. Okra is widely known as a viscous mucilaginous plant (Woolfe *et al.*, 1977) and has been used in the production of plasma expander (Nasipuri *et al.*, 1996) by becoming the suspending and emulsifying agents (Nasipuri *et al.*, 1997; Nasipuri *et al.*, 1999). In addition, part of the immature okra fruits have been used in folk medicine as a diuretic agent and dental disease treatment (Ndjouenkeu *et al.*, 1996), fat substitute in chocolate bar cookies (Romanchik-Cerpovicz *et al.*, 2002), egg white substitute and in chocolate frozen dairy desserts (Romanchik-Cerpovicz *et al.*, 2006). Lengsfeld *et al.* (2004) claimed that okra also possesses high carbohydrate content, which eventually hinder adhesion of *H. pylori* during a gastric problem.

Previous studies had reported that okra contained different types of polysaccharides, including pectins, xyloglucans, xylans, and celluloses (Sengkhampan *et al.*, 2009). Pectin is a methylated ester of polygalacturonic acid that contains 1,4-linked α -D-galacturonic acid residues and can be found in many types of plants. For example, heteropolysaccharides derived from the cell wall of higher terrestrial plants and the peels of citrus fruit, guavas and apples (Sengkhampan *et al.*, 2009). It can form gel in the presence of sugar and an acid solution under suitable conditions. It is typically added to jams and jellies as a gelling agent. Moreover, pectin is also widely used as a thickener, texturizer, and stabilizer in the food industry. The suitability of pectin for different purposes depends on the chemical characteristics of the extracted pectin. Pectin from different sources of plants can have different molecular weight, degree of esterification and methoxyl content that contribute to different functional properties. Therefore, more studies are required on pectin from different plant sources (Madhav and Pushpalatha, 2002; Aina *et al.*, 2012; Ismail *et al.*, 2012).

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Several studies have conducted sequential extraction of pectin from okra leaves, pulp, and seeds and identified its functional groups. Besides, there was a study involved optimization extraction and rheological properties of pectin from okra (Chen *et al.*, 2014). However, limited studies have been done upon comparing the gelling formation of animal-based (gelatin) and plant-based (pectin). Therefore, this study aimed to evaluate the gel formation from different extraction conditions of the pectin (plant-based) from okra leaves, pulp, and seeds and compared with gelatin (animal-based). This study applied the concept of “waste into gold” which involved utilization of okra by-product that eventually reduce the waste from post-harvest cultivated into valuable plant-based hydrocolloid. Apart from that, this study provided alternative source of gelatin from animal-based by introducing a potential gelling agent from plant-based.

Materials and Methods

Plant material

Plant materials used were okra fruits and okra leaves. Okra leaves were collected directly from University Agricultural Park, UPM, and Department of Kuala Langat District Agriculture, Selangor, Malaysia whereas the okra fruits were purchased from a commercial market in Putrajaya, Malaysia on August 2012. The plant materials were cleaned and the okra pulp (fruit without seed) was separated manually from the seeds. Then, they were immediately freeze-dried for 96 hr using a freeze drier (VirTis Benchtop K, PA, USA). Commercial pectin (CP) of apple pomace and gelatin (bovine) was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). The solvents and chemicals employed in this study were obtained commercially and were of analytical grade.

Preparation of 6.67% concentration of gelatin and commercial pectin

The gelatin and commercial pectin of 6.67 % concentration were prepared by dissolving 2.66 g of dried gelatin and commercial pectin separately in 40 mL distilled water at 60°C. The dissolved samples were held in a refrigerator (7°C) for 16–18 hr and were used in viscosity analysis.

Fractionation of alcohol-insoluble solids

Leaves, pulp and seeds were homogenized twice with 70% (v/v) aqueous ethanol at room temperature for 1 hr. After filtration, the insoluble residues were pooled together and soaked in chloroform/methanol

(1:1 v/v) with a gentle stirring for 30 min to remove low molecular weight (colored) compounds. Then, the residues were washed with acetone and air dried to obtain alcohol-insoluble solids (AIS) (Sengkhampan *et al.*, 2009).

Sequential extraction of okra AIS

The sequential extraction method was conducted according to Vierhuis *et al.*, (2000) with several modifications. The okra AIS (20 g) were extracted using 600 mL of the following extractants: 0.05 M sodium acetate buffer, pH 5.2 (hot buffer, HB) at 70°C, 0.05 M ethylenediamine tetraacetic acid (EDTA) and 0.05 M sodium acetate in 0.05 M sodium oxalate, pH 5.2 at 70°C (chelating agent, CH) of soluble solids. After 30 min of extraction, the extract was separated from the insoluble residue by centrifugation at 18,500 x g for 25 min. Then, the supernatant was coagulated with isopropanol and lyophilized (Sengkhampan *et al.*, 2010, Ismail *et al.*, 2012).

Determination of equivalent weight, methoxyl content, anhydro uronic acid (AUA) and degree of esterification

The methoxyl and anhydro uronic acid (AUA) contents, equivalent weight, and degree of esterification were determined by following the methods described by Owens *et al.* (1952) and Ismail *et al.* (2012).

Sugar composition

The natural sugar composition of all fractions (HB and CH fractions of okra leaves, pulp, and seeds) were determined by high-performance liquid chromatography (Sengkhampan *et al.*, 2009). Ten milligrams of each fraction were dissolved in 10 mL of 2 M HCl in methanol and hydrolysed initially at 80°C for 16 hr and subsequently increased the temperature to 121°C for 1 hr. The solvent was evaporated using rotary evaporator (Buchi Rotavapor R-200, Essen, Germany). Monosaccharides (rhamnose, xylose, arabinose, mannose, fructose, glucose, and galactose) were determined using a HPLC and refractive index (RI) was used as a detector. The column used was Purospher® star NH₂ (250 mm x 4.6 mm, 5 mm) (Merck, Darmstadt, Germany). The mobile phase used was acetonitrile/water (75:25, v/v), with a flow rate of 1 mL/min. A monosaccharide standards consisting of rhamnose, xylose, arabinose, mannose, fructose, glucose and galactose were used at concentrations between 1 and 5 mg/mL in order to get the equation of calibration curve. The quantification of uronic acid in the fractions was performed using a galacturonic acid

assay (Anthon and Barret, 2008).

Gel formation

Gel formation was carried out by mixing the fractions with konjac glucomannan (KG) in the ratio of 5.00:1.67 (w/w). The fractions and KG were weighed separately and mixed together in a beaker. Then, water was added to solubilize the mixtures and stirred continuously for 15 min in a water bath at 60°C until it dissolved completely. Next, the mixture was stored in the refrigerator for 16-18 hr. The gel mixture was analysed to determine its melting point, gel strength and viscosity at 25°C (Burey *et al.*, 2008).

Determination of melting point

Solution contained a total of 6.67% (w/v) of fractions (5.00%) and KG (1.67%) was prepared in a thin wall (12 mm×75 mm) screw cap test tubes. The test tubes were filled to leave some headspace for gas released during gel formation and closed. The dissolved samples were held in a refrigerator (7°C) for 16–18 hr, and then they were placed into water bath (10°C) in inverted position so that the headspace was at the bottom. The water bath was warmed gradually by adding warm water (~45°C) at intervals of about 60 s. The temperature at which the gel was completely melted, indicated by the flow of the gas in the headspace moving upward was recorded as the melting point. Three replicates were performed for each fraction (Muyonga *et al.*, 2004).

Determination of gel strength

Gel strength was measured according to the method recommended by the manufacturer of the texture measuring instrument (Gómez-Guillén and Montero, 2001). The concentration of pectin gel was 6.67% which made by dissolving 2 g of dried pectin and 0.67 g of KG in 40 mL distilled water at 60°C. The prepared sample will also be used for viscosity determination. The dimension of the sample in the container was 6 cm in diameter and 2 cm in height. The solution was cool down in a refrigerator at 7°C for 16 -18 hr. Measurements were done at 25°C using an Instron model 4501 Universal Testing Machine (Instron Co., Canton, Mass., USA) with a 5 kN of load cell, cross-head speed 1 mm/s, equipped with a 1.27 cm diameter cylindrical Teflon plunger. Maximum force (expressed in g) was determined when the plunger had penetrated 4 mm into the pectin gels. Three replicates were performed for each fraction.

Determination of viscosity

The similar prepared sample (6.67% at 60°C) from

previous gel strength test was used. The viscosity of the sample was determined using an AR-G2 rheometer (TA Instruments, West Sussex, England) equipped with a heating circulator (Julabo, model F12, Germany). A cone and plate geometry (60 mm diameter, 1° angle) was used for the measurements. Approximately 1 mL of each sample was transferred to the Peltier plate and excess material was trimmed off. A platinum resistance thermometer (PRT) sensor was positioned in the middle of the lower sample plate to ensure accurate sample measurement ($25 \pm 0.1^\circ\text{C}$). Flow curves with increasing shear rate ($0.01\text{--}100\text{ s}^{-1}$) were measured at 25°C. The downward curve was analyzed using TA Rheology Advantage Data Analysis Software (version V5.7.0) (Sengkhamparn *et al.*, 2010).

Statistical analysis

All data were expressed as a mean \pm standard deviation. Data were analyzed using one-way ANOVA using SPSS 15.0. Duncan's multiple-range test was used to assess the difference between the means. Pearson's correlation test was used to assess the correlations between the means. A significant difference was considered at the level of $P < 0.05$.

Results and Discussion

Pectin yield

Pectin was obtained with a sequential extraction, where different solvents achieved various fraction yields. HB was the hot buffer solvent used to extract the okra AIS of the seeds, pulp and leaves (Figure 1). Upon comparing the three different parts described above, the seeds contained the highest HB fraction yield from 100 g of AIS, which was 23%, followed by the leaves (19%) and pulp (10%) of the okra AIS. The HB fraction yield was similar to a previous result with okra pulp (Sengkhamparn *et al.*, 2009). The second fraction (CH) indicated that the okra pulp exhibited the highest yield (66%), followed by the seeds (52%) and leaves (43%) of okra AIS. The CH fraction yield of the okra pulp was higher than the CH fraction yield previously reported in okra pulp (Sengkhamparn *et al.* 2009). This CH fraction yield is unusual compared to previous investigations and is likely attributed to differences in the raw materials. The second fraction (CH) exhibited the highest yield in terms of overall parts, followed by the HB (Figure 1). These fractions were used for chemical composition and structural analyses. The okra pulp fraction yield displayed different results likely due to the different okra sources.

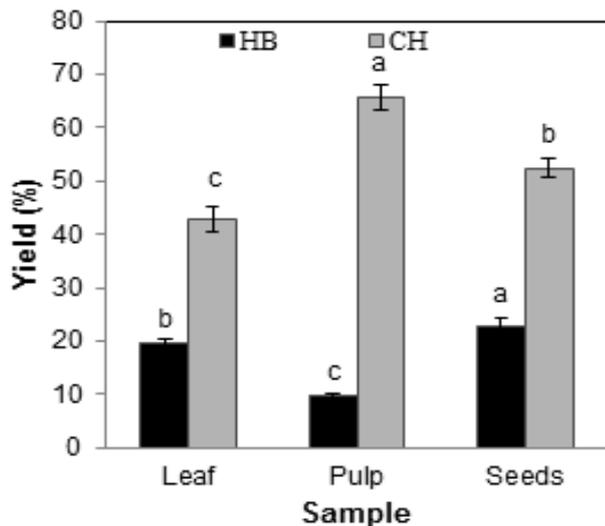


Figure 1. Yield of different fractions from okra leaves, pulp and seeds.

Data were analyzed by using g of sample / 100 g of Alcohol Insoluble Solids (AIS) represented as the mean value from triplicate measurements \pm standard deviation. Values with different superscripts significantly differ ($p < 0.05$) in terms of the same fraction.

HB: Hot buffer; CH: Chelating agent

Source: Abd Rahman *et al.* (2014)

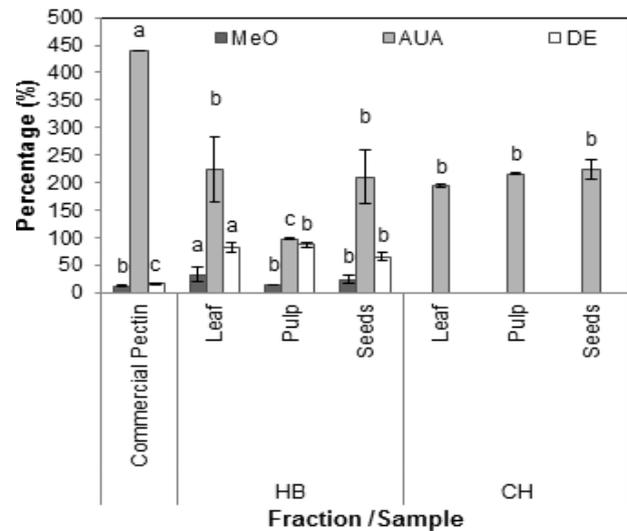


Figure 2. Chemical compositions of okra leaves, pulp and seeds.

Bars represent the standard deviation from three analyses. All results are presented as the means of three independent experiments, and each analysis was determined in triplicate. Values with different superscripts are significantly different ($p < 0.05$) in terms of chemical composition.

MeO, methoxyl; AUA, anhydro uronic acid; DE, degree of esterification

Chemical compositions of fractions from okra leaves, pulp and seeds

The equivalent weight (EW) and methoxyl (MeO) content results are presented in Figure 2. The obtained equivalent weights were used to calculate the anhydro uronic acid (AUA) content and degree of esterification (DE). The highest methoxyl content obtained by okra leaves from the HB fraction was 33%, followed by the seeds (25%) and pulp (15%). The lowest methoxyl content was observed in the commercial pectin (13%), and it did not significantly differ from the okra pulp. In contrast, the okra pulp significantly differed from the okra leaves and seeds, which could be due to the mechanism of deesterification of high methoxyl (HM) pectin in the HB fractions (Thakur *et al.*, 1997). Therefore, the HB fractions of all samples can be categorized as low methoxyl pectin (LMP) due to the low methoxyl content (<50%).

The CH fractions of okra leaves, pulp and seeds displayed an undetectable amount of methoxyl content and degree of esterification compared with HB and commercial pectin due to the extraction at high temperature. Nazaruddin *et al.* (2013) suggested that extraction at a high temperature and low pH decreased the methoxyl content of the extracted pectin. Moreover, Kulkarni and Vijayanand (2010) and Koubala *et al.* (2008) concluded that this observation could be due to hydrolysis of glycosidic

linkages and may results in partial degradation of pectin. As the extraction process involves chelating agents, extracted pectin harbors a low amount of methoxyl and becomes calcium-sensitive (calcium pectate gels) when carboxyl groups are neutralized by calcium ions; moreover, polypectate molecules form dimers in an egg box conformation (Morris *et al.*, 1982; Sengkhamparn *et al.* 2009).

Anhydro uronic acid (AUA) content is essential for determining pectin purity and the degree of esterification (Ranganna 1986; Madhav and Pushpalata 2002). According to Ismail *et al.* (2012), the AUA content indicates the purity of the extracted pectin and should not be less than 65% (Food Chemicals Codex 1996). Okra leaves, pulp and seeds of both (HB and CH) fractions contained more than 65% AUA and thus can be considered highly purified pectin.

The degree of esterification (DE) is known as the mechanism of galacturonide units that are esterified and become methyl galacturonate (Seymour and Knox 2002; Nazaruddin *et al.* 2013). The degree of esterification (DE) can be used as a parameter to determine whether pectin is high methoxyl pectin ($DE > 50\%$) or low methoxyl pectin ($DE < 50\%$) (Thakur *et al.*, 1997; Mesbahi *et al.*, 2005; Ismail *et al.* 2012). We categorized the commercial pectin in this study as low methoxyl pectin (LMP) because its DE percentage was lower than 50%. The types of pectin determine the mechanism of gel formation.

Table 1. Monosaccharide compositions of HB, CH and DA of okra leaves, pulp and seeds

Sample	Fractions (mg/10mg)	Rhamnose (mg/10mg)	Xylose (mg/10mg)	Arabinose (mg/10mg)	Fructose (mg/10mg)	Mannose (mg/10mg)	Glucose (mg/10mg)	Galactose (mg/10mg)	Galacturonic acid (mg/10mg)
Leaves	HB	nd	0.02±0.01	nd	3.85±0.12	0.57±0.06	nd	nd	5.13±0.23
	CH	5.76±0.18	nd	nd	1.54±0.02	1.14±0.04	nd	nd	0.86±0.13
Pulp	HB	0.47±0.02	nd	nd	1.68±0.08	0.91±0.02	1.22±0.06	1.78±0.10	3.46±0.21
	CH	1.38±0.07	1.75±0.10	1.03±0.04	nd	2.28±0.12	nd	2.34±0.15	0.63±0.05
Seeds	HB	0.38±0.01	0.37±0.02	nd	1.40±0.07	0.62±0.06	nd	nd	6.42±0.23
	CH	0.94±0.05	2.01±0.08	0.51±0.02	2.79±0.10	1.79±0.07	0.60±0.03	nd	0.48±0.03

All results are presented as means ± SD of three independent experiments, and each analysis was determined in triplicate.

HB: Hot buffered; CH: Chelating agent

*nd =not detected

Limit of detection: 0.001mg

LMP can form gels in the presence of divalent cations and with the addition of a low amount of sugar or without sugar. LMP is highly calcium-sensitive and useful in low-sugar applications, such as in diet jams and jellies. In contrast, the HB fraction of okra pulp displayed the highest DE (88%), followed by the leaves (83%) and seeds (66%).

The HB fraction harbored a low methoxyl content, although the degree of esterification was higher than 50%, which differed from the commercial pectin. This can be considered as a high DE percentage compared to commercial pectin due to the high degree of esterification (DE > 50%). Nazaruddin *et al.* (2013) demonstrated a similar result for pectin extraction from roselle (*Hibiscus sabdariffa* L.) calyces. This HB fraction from okra leaves, pulp and seeds could be used as an alternative source of pectin due to the good quality of the gelling properties. To form gels for high DE pectin, it should consist of a high concentration of sugar (>55%) and perform well under acidic conditions (pH 2.0-2.5) (Mishra *et al.*, 2001).

Sugar composition and uronic acid composition

The monosaccharides composition of okra leaves, pulp and seeds were shown in Table 1. HB fraction was composed of xylose, fructose and mannose meanwhile; CH fraction was composed of rhamnose, fructose, and mannose. The polysaccharide was found as the galacturonic acid in HB and CH fraction. It could relate to the pectin, an acidic polysaccharide, found in mango (Iagher *et al.*, 2002; Al-Sheraji *et al.*, 2012) and okra fruits (Sengkhampan *et al.*, 2009). Sengkhampan *et al.* (2009) showed similar monosaccharides composition in okra pulp. These monosaccharides indicate the presence of large proportions of pectic polysaccharides (Hokputsa *et al.*, 2004; Al-Sheraji *et al.*, 2012).

Galactose was found as the main neutral sugar in

the HB fraction of okra pulp. The ratio of the major sugars present in the HB fraction was 1.8:0.5:3.5 for galactose: rhamnose: galacturonic acid respectively, which was closely similar to literature (Woofle *et al.*, 1977; Tomoda *et al.*, 1980; Sengkhampan *et al.*, 2009). HB fraction contained slightly more galactose, which was similar to the acidified fraction of okra polysaccharides (Tomoda *et al.*, 1980). A comparison between the extraction of okra polysaccharides using water and acid showed lower amount of galacturonic acid content by water extraction (Lengsfeld *et al.*, 2004; Deters *et al.*, 2005).

The CH fraction contained higher amounts of rhamnose and galactose, but the lower content of galacturonic acid when compared to the HB fraction. The CH fraction also contained arabinose and xylose while these sugars were not found in the HB fraction. Thus, the presence of xylogalacturonan can be attributed in CH fraction (Sengkhampan *et al.*, 2009). In addition, the CH fraction contained higher ratio of rhamnose to galacturonic acid (3.0) than the HB fraction (0.18).

Gel formation, gel strength and melting point of okra leaves, pulp and seeds fractions

The strength of mixed gel made from HB and CH fractions of okra leaves, pulp, and seeds with KG were shown in Table 2. Blending of fraction with KG is important as KG helped to improve the rheological properties of the gel by forming specific junction couple between gel networks (Turquois *et al.*, 1999). Most of the CH fractions showed higher gel strength which significantly different ($p < 0.05$) compared to HB fractions and commercial pectin (CP) regardless of different parts of okra plant. CH fraction from leaves showed highest gel strength (31.28 ± 1.80) which approximately two times higher than CP. In contrast, HB fraction from leaves showed the lowest gel strength (10.14 ± 0.43).

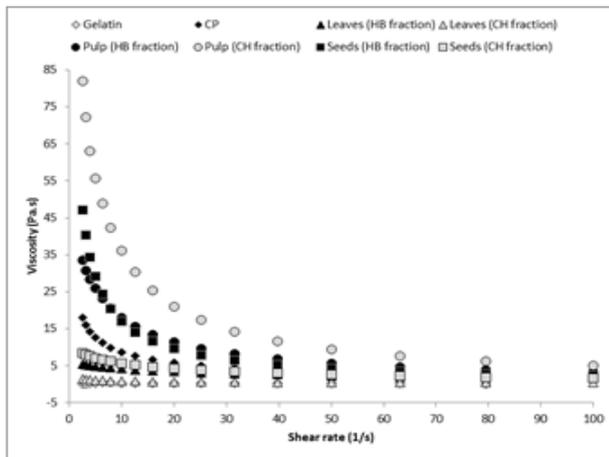


Figure 3. Viscosity of CP, HB and CH fraction of okra leaves, pulp and seeds.

All results presented as means \pm standard deviations from triplicate determinations at 25°C with concentration 6.67% for commercial pectin and gelatin. The other fraction was blended with konjac glucomannan (KG) in 5.0:1.6(w/w) ratio.

CP: commercial pectin; HB: hot buffered; CH: chelating agent

Lower gel strength of HB fraction was due to the presence of cations in the HB fraction. They reduced the electric charge of macromolecule of HB fraction by expanding the distance of free carboxyl groups (Tibenský, 1968). In contrast, CH fraction comprised of counter ions such as potassium, sodium and calcium which promote the gelation process to occur (Hermansson *et al.*, 1991; Yamazaki *et al.*, 2008).

In addition, HB fraction showed a positive presence of methoxyl (MeO) in contrast with CH fraction (Abd Rahman *et al.*, 2014). MeO content of pectin is important to control the gel strength, setting time and sensitivity to metal ions (Ramli and Asmawati, 2011). Therefore, it could be concluded that MeO content affects the gel strength of the HB and CH fractions of okra leaves, pulp, and seeds.

As for commercial gelatin (CG), the gel strength lies in the range of 30-500, meanwhile, CH fraction of okra leaves show no significant different ($p > 0.05$) with gel strength of CG showed is Bloom 31 grams. The results shown were coherent with commercial gelatin. These could be due to the solubilization of calcium compound attached with pectin during extraction process using chelating agent solvent. Previous studies proved that calcium is necessary to stabilize boron cross-linked rhamnogalacturonan II (Hilz *et al.*, 2005). However, in terms of melting point, CH fraction of okra leaves showed significantly higher ($p < 0.05$) than gelatin and CP. In this situation, it could be due to the presence of KG which was blended with CH pectin in gel formation. KG is highly complex and involves α -coil-helix transition followed by aggregation and network formation. It requires a higher temperature to melt down the gel

formed (Rees *et al.*, 1982).

The melting point of CG, CP, HB and CH fraction of okra leaves, pulp and seeds were summarized in Table 2. HB fraction of okra pulp showed the highest melting point compared to the other fractions. Fractions that have comparable melting point with CP were the HB fraction of okra leaves, and CH fraction of okra seeds. Meanwhile, the melting point of CH fraction of okra leaves and pulp were slightly higher than CP around 49°C and 47°C respectively. Both were slightly similar to the melting point of amyloamylase modified starch gels which melted at 50°C (Karim and Bhat, 2008). As the temperature or pH increased, elimination process starts and results in chain cleavage and very rapid loss of viscosity and gelling properties (Rolin, 1993).

Unlike gelatin, most of polysaccharides and proteins were not thermoreversible gels (Karim and Bhat, 2008). Even though it was not really comparable with commercial pectin and could not be regarded as a replacement for gelatin, it can be treated as an extension of available gelling products with their own specific applications (Kaper *et al.*, 2005).

Moreover, the viscosity is one of the significant parameters to determine the characteristic of gel formation. The result of viscosity can be referred to Figure 3. Commercial pectin (6.67%) which was chosen as the control in this analysis has viscosity around 60 Pa.s. Similar viscosity of CP was found by Sharma *et al.* (2013). In contrast, HB and CH fractions of pulp and HB fraction of seeds showed higher viscosity than CP (> 100 Pa.s). These results could be explained by looking on the physical properties of both fractions. The factors that could influence the viscosity of polysaccharides are molecular mass, stiffness, molecular charges (Williams and Phillips, 2000) and molecular structures (Sengkhamparn *et al.*, 2010). Second reason is the presence of carboxyl groups of galacturonic acid in both fractions that contribute to high viscosity (Sengkhamparn *et al.*, 2010, Abd Rahman *et al.*, 2014).

The presence of methyl ester in HB fraction between the ranges of 1412-1413 cm^{-1} could also influence the viscosity due to entanglement of the okra pectin molecules (Abd Rahman *et al.*, 2014; Sengkhamparn *et al.*, 2010). This can be explained by the nature of the substituents of the pectin backbone. HB fraction of okra fruit composed of highly branched of rhamnogalacturonan I compound. It contains rhamnose and galacturonic acid as substituents residues which promotes hydrophobic associations through acetylated rhamnose. In contrast, CH fraction contains relatively much more homogalacturonan (Sengkhamparn *et al.*, 2009; Abd

Rahman *et al.*, 2014)

HB and CH fractions of leaves and CH fraction of seeds showed lower viscosity than CP and fractions from okra pulp. These results showed that different parts of the plant could have different level of viscosity. Various physicochemical properties could be attained due to different parts of the plant have different properties (El-Mahdy and El-Sebaiy, 1984). Besides, it could be due to the molecular weight, degree of esterification (DE), and the concentration of preparation, pH and the presence of counter ions in the solution (Srivastava and Malviya, 2011).

Briefly, viscosity starts to decrease as the shear rate increases because it would lead to a greater molecular structure breakdown. The shear thinning behavior of the blended HB and CH fractions might due to modifications in the macromolecular arrangement which correspond to irreversible structural breakdown. Burey *et al.* (2008) reported that in an entangled network system, the rate of disruption of existing intermolecular entanglement becomes greater than the rate of reformation of new entanglement with increasing shear rate. Thus, the overall content of junctions in three-dimensional networks decreased, resulting in less resistance to flow and a low apparent viscosity. Similar shear thinning effects have been reported by pectin from mango (Iagher *et al.*, 2002) and pumpkin (Evageliou *et al.*, 2005).

This is supported by previous studies where non-Newtonian shear thinning behavior was observed and lead to decrease in viscosity property with an increase in shear rate (Min *et al.*, 2011; Vriesmann and Petkowicz, 2013). In addition, a similar character of viscosity by which high concentration of okra pectin exhibited distinct pseudoplastic flow behavior, along with the increment of shear rate viscosity starts to decrease rapidly under steady-shear condition (Chen *et al.*, 2014). Moreover, the results obtained might due to different extraction approaches that give significant variations in their composition and flow behaviour properties (Kontogiorgos *et al.*, 2012).

Conclusion

Lady's finger also known as okra is a plant with high natural viscous mucilage particularly in the fruits. Previous studies reported that mucilage from okra possessed good properties to be used as gelling agent in many fields. With the issues reported on gelatin derived from bovine and pork, okra is one of the potential plants in Malaysia to provide plant-based gelatin alternative. Okra was selected in this study, due to the high availability, easy to get all

year round at low cost. Vegetarians, for instance, could not accept animal based ingredient. In this case, pectin which is plant based is the most suitable alternative for gelling agent. In this study, a gel could not be formed by okra pectin itself; therefore, another polysaccharide known as konjac glucomannan (KG) was used. The concentration used was weight basis; 5.0% of okra pectin and 1.6% of KG. Gel properties were analyzed based on gel strength, viscosity and melting point analysis. Okra pulp showed higher viscosity than okra leaves and seeds, meanwhile, okra leaves resulted in highest gel strength compared to others. To conclude, based on the gel properties, HB and CH fraction derived from okra leaves, pulp, and seeds have good potential to become plant-based gelling agent.

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