EXTRACTION AND CHARACTERIZATIONS OF PECTIN FROM POMELO PEEL

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A THESIS

BY

MANIK CHANDRA ROY
Student No: 1305178
Session: 2013-2014
Semester: July-December, 2014

MASTER OF SCIENCE (MS)
IN
FOOD PROCESSING AND PRESERVATION

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR

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DECEMBER, 2014

DEDICATED TO
MY
BELOVED PARENTS

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The Author

ABSTRACT

In this study, we were searching for new pectin sources to meet the regional and domestic demand of pectin. Pectin was extracted under the various extraction conditions of time (30, 60 and 120 min), temperature (80°C, 90°C and 95°C) & pH (1.5, 2.0 and 2.5) from pomelo fruit peel. Extraction time 120 min, temperature 90°C and pH (1.5 and 2.0) were selected for the extraction of pectin from preliminary results on the basis of pectin yield and ash content. The pomelo pectin extracted at pH 2.0 showed that the higher ash content, equivalent weight and total anhydrouronic acid content than extracted at pH 1.5. Extracted pomelo peel pectin was categorized as high methoxyl pectin based on the degree of esterification and methoxyl content value in this study. Therefore, the results concluded that pomelo fruit peel could be used good source of pectin.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Bangladesh produces a large variety of tropical and sub-tropical fruits. During the peak season huge amount of citrus fruits are grown in Bangladesh due to favorable climatic conditions. The most widely cultivated citrus are orange, lemon, lime, mandarin, grape fruit and pomelo. Among all fruits, pomelo is an important citrus crop in Bangladesh which has annual production of 2,000 tones and often leads to excess because of oversupply and low prices when it's in season (BBS, 2010). Citrus fruit pulp consists of 60- 65% peels, 30-35% segment pulp and 0-10% seeds (Afshar and Naser, 2008). Citrus by-products are considered to be rich source of phytochemicals, pharmaceuticals, food products and can also be used as sources of essential oils, pectin and dietary fibers (Hamapitour *et al.,* 2004).

Pectin is the methylated ester of polygalacturonic acid which contains 1, 4-linked α-D-galacturonic acid residues (Levigne *et al.,* 2002). It is generally found in the cell walls and middle lamellae of higher plants. Pectin is a polysaccharide; these polysaccharides consist of 300-1,000 chains of galacturonic acid units (Yeoh *et al.,* 2008). Three major pectic polysaccharides are recognized: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Willats *et al.,* 2006). Pectin can be divided into two types based on the degree of esterification (DE) of the pectin: high methoxyl pectin (DE > 50%) and low methoxyl pectin (DE < 50%) (Mesbahi *et al.,* 2005). According to Canteri-Schemin *et al.,* (2005), there are three classifications of pectin: HM (high ester); LMC (low ester conventional) and LMA (low ester amidated). Usually pectin is widely used in the food industry as a thickener, emulsifier, texturizer and stabilizer (Tsoga *et al.,* 2004).

The main sources for commercial pectin production are apple pomace and citrus peels (Schieber *et al.,* 2003). Usually pectin can be extracted using various acids such as citric acid, oxalic (Koubala *et al.,* 2008), hydrochloric (Chaidedgumjorn *et al.,* 2009; Kulkarni and Vijayanand, 2010), nitric (Constenla *et al.,* 2002) and sulphuric acids (Garna *et al.,* 2007) which are regarded as conventional acid extraction (Yapo, 2009). Sulfuric, hydrochloric and nitric acids are cheaper mineral acids. Among them hydrochloric acid is

recommended as the best solvent to extract pectin (kalapathy and proctor, 2001; Hwang *et al.,* 1998 and Dinu, 2001). Acid has the ability to solubilize the protopectin from the albedo with an increase of acid strength. Lower pectin yield was obtained using water and oxalate chelating agent as compared to acid extraction (Chaidedgumjorn *et al.,* 2009 and Methacanon *et al.,* 2014). Hot acid extraction (HCl) of pectin gave a high anhydroglacturonic acid content and had a low degree of methoxyl esterification (Yapo & Koffi, 2006). Higher galacturonic acid can obtain by chemical methods than the enzymatic method (Jongbin *et al.,* 2012). Kulkarni & Vijayanand (2010) and Methacanon *et al.* (2014) reported that pectin yield was higher but methoxyl content and equivalent weight were lower at high temperature and lower pH than lower temperature and higher pH. It is recognized that an increase in acid strength (that is, decreasing pH) plays an important role in increasing the content of galacturonic acid (Yapo *et al.,* 2007). Pectin quality depends on extraction time, temperature and pH. Pectin quality and purity can also depend on several factors such as degree of esterification, anhydrogalacturonic acid, molecular weight and ash content. Good quality pectin contains 65% anhydrogalacturonic acid and 10% ash.

In recent years the fruits and vegetables production has increased. Therefore, large amount of fruit waste are produced which has not yet been used as value added products. Moreover, huge amount of wastes could be generated pollution of the environment. Hence, pomelo peel can be used as good source of pectin. So that, the objectives of the research work were:

1. To select a suitable method for pectin extraction from pomelo peel.
2. To optimizing the temperature and pH for extraction pectin.
3. To prepare pectin powder and analyze the physico-chemical properties of pomelo peel pectin.

CHAPTER II
REVIEW OF LITERATURE

CHAPTER II

REVIEW AND LITERATURE

2.1 Citrus Wastes

Citrus juice generates a large quantity of waste including fruit pomace peel and seed. Usually, 55-60% peels and 5-10% seed produces which are generally discarded as a wastes (Vikram *et al.,* 2007). Citrus wastes are seasonal and highly perishable, are generally dropout nearby the industrial areas. These wastes are causes problem to the processing industries and pollution monitoring agencies. Citrus wastes have been used as source of ethanol production since 1992 (Grohmann and Baldwin, 1992). In recent years the production of citrus wastes are increased and parts of the citrus wastes are dried and marketed as low-protein cattle feed called "citrus pulp pellets" and the rest are disposed in landfills, constituting severe economic and environmental problems (Tripodo *et al.,* 2004).

Citrus wastes can be used as functional food ingredients such as phytochemicals, pharmaceuticals, food products, essential oils, seed oil, pectin and dietary fibers. Citrus byproducts are also considered to be rich source of edible and heath promoting agents as polymethoxylated flavonoids or hydroxycinnamtes, many of which are found in citrus peels (Vikram *et al.*, 2007; Wang *et al.,* 2007).

2.2 Pectin Background

Vauquelin first discovered the existence of pectin in fruit juices in 1790. Pectin is the complex mixtures of polysaccharides that make up approximately one third of the cell-wall dry substance of most types of plants (Van Buren, 1991). Pectin plays an important role in plants is to give structural integrity to the cell wall and adhesion between cells. Pectin is primarily consists of D-galacturonic acid joined by α-(1-4) glycosidic linkages (Van Buren, 1991).

On other hand, according to the American Chemical Society (Kertesz, 1951), pectic substances have been defined as complex colloidal carbohydrate derivatives that occur in, or are prepared from, plants. They contain a large proportion of anhydrogalacturonic acid units, which are thought to exist in a chain-like combination.

2.2.1. Sources of pectin

Sources of citrus peels and apple pomace are the main sources of commercial pectin(May, 1990) but several other sources exist such as sugar beet, sunflower head, cocoa husk, sweet potato etc. All plant consists of pectin but the content and composition varies depending on the species, variety, maturity, plant part, tissue and growing condition. Pectin content comprises up to 35% depending on the cell wall of most terrestrial (Tombs *et al.,* 1998). Sugar beet, sunflowers and other tropical fruits have been considered as potential sources of pectin but pectin from these sources has poor gelling ability due to the acetylation of some of the hydroxyl groups (Pagan *et al.*, 2001).

Cocoa husks considered as a rich source of pectin. But pectin from cocoa husks was quite low quality as compared to commercial pectin (Ramli & Asmawati, 2011). Other source of pectin includes apple pomace (Chakraborty and Ray, 2011), Orange (Braddock, 2004), sunflower heads (Matora *et al.,* 1995), beet and potato pulp (Turpouis *et al.,* 1999), soy hull (Kalapathy & Proctor 2001) and duckweed (Golovchenko *et al.,* 2002).

2.2.2. Basic Structure of Pectin

Basically pectin is a linear polysaccharide. Pectin consists three major types of chain (Willats *et al.*, 2006), but all three types of pectin chain consist primarily of rings of methylated D-galacturonic acid (Ovodov, 2009). D-galacturonic acid is the oxidized form of D-galactose, in which the sixth carbon in the R-group external to the saccharide ring is converted from an alcohol to a carboxylic acid group through oxidation (Mohnen, 2008). Figure 1 is shown structure of D- galactose and D- galacturonic acid.

While both D-galactose and D-galacturonic acid are present in plant cell walls, D-galacturonic acid which supply charge needed for the strong intermolecular bonding that gives the plant cells, and thus the plants, rigidity and structure (Voragen *et al.,* 2009; Phillips & Williams, 2000).

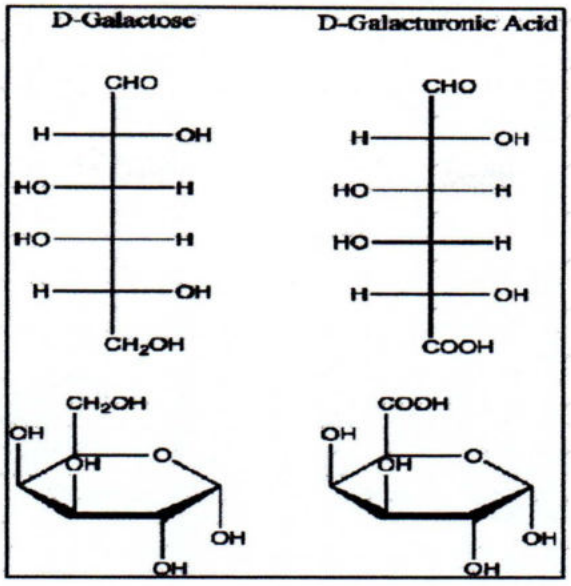

Figure 1: Fischer and Haworth projections of D-Galactose and D-Galacturonic Acid (Mohnen, 2008)

According to the degree and type of substitution pectin chains are classified into three types. The first type called Homogalacturonan (HG) is a simple linear polymer formed by D-galacturonic acid which can be acetylated or methyl esterified with no substitution. Due to its lack of substitution, HG pectin chains are often called as "smooth" chains. According to the HG molecule classification pectin should contains at least 75 % of the carboxyl group methylated (Jayani *et al.*, 2005).

The second type of chain called Rhamnogalacturonan I (RG-I) which is composed of the repeating disaccharide rhamnose- galacturonic acid. The both residues can carry side chains of neutral sugars as galactose, arabinose and xylose and the galacturonic residues can be acetylated (Willats *et al.*, 2006).

The third type is a complex side chains attached to the galacturonic residues is termed as Rhamnogalacturonan II (RG- II). HG and RG- II are long side chains of RG- I shown in figure 2 proposed by Vincken *et al.* (2003). Pectin is found as a water insoluble pectic substance in the unripe fruit, the protopectin, bounded to cellulose microfibrils conferring rigidity on cell walls. The fruit enzymes alter into pectin during ripening structured by breaking the pectin backbone or side chains, resulting in a more soluble molecule (Kashyap *et al.*, 2001).

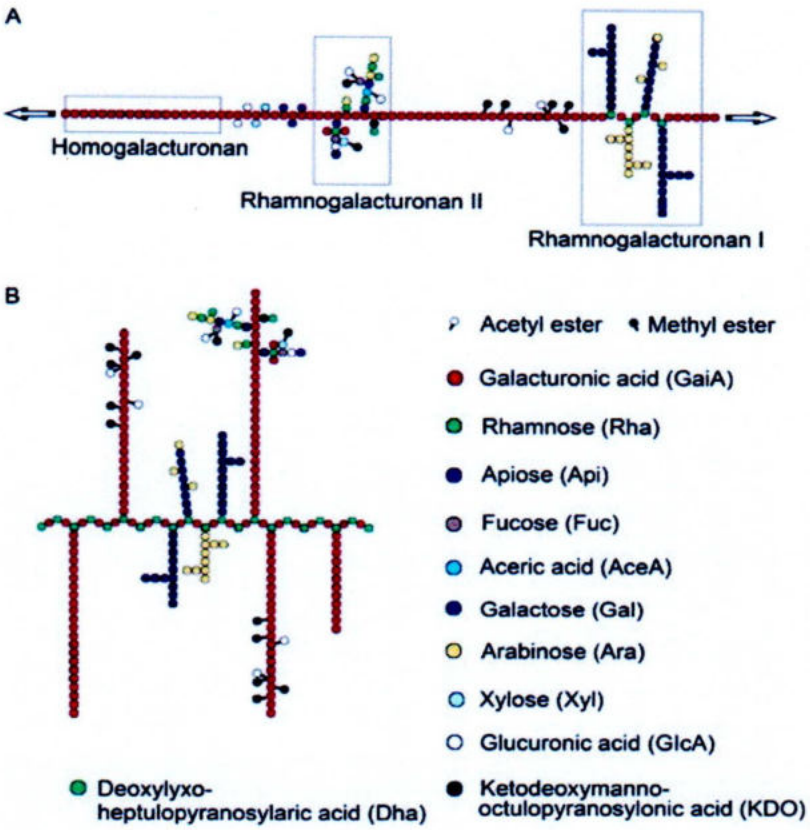

Figure 2: The basic structure of pectin. Schematic representations of the conventional (A) and recently proposed alternative (B) structures of pectin. The polymers shown here are intended only to illustrate the some of the major domains found in most pectins rather than definitive structures (Willats *et al.*, 2006).

2.2.3. Types of Pectin

Early studies researchers are classified the pectin into three classes according to the methods used for their extraction from cell walls. These classes are: water-soluble pectin, chelator-soluble pectin and protopectin. Water-soluble pectin is extracted with water or dilutes salt solutions; chelator-soluble pectin is extracted with solutions of calcium chelating agents such as ethylene diaminetetraacetic acid (EDTA), ammonium oxalate, cyclohexane diaminotetraacetic acid (CDTA) or sodium hexametaphosphate (SHMP) and alkali solutions or hot dilute acid extracted pectin is termed as protopectin (Van Buren, 1991). However, more recently researchers proposed that the extracted pectin can be

categorized into two major categories depending on the percentage of galacturonic acid residues that are esterified with methanol. A degree of esterification (DE) greater than 50% is considered high methoxyl pectin and a DE below 50% is considered low methoxyl pectin (Braddock, 1999). Three major pectic polysaccharides are recognized: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Willats *et al.,* 2006). According to Canteri-Schemin *et al.,* (2005), there are three classifications of pectin: HM (high ester); LMC (low ester conventional) and LMA (low ester amidated).

2.2.4. Pectin Extraction Methods

Quality of pectin depends on the source and extraction conditions. The extraction process of pectin are commonly occurs using a dilute mineral acids such as hydrochloric, sulfuric, nitric, acetic acid etc (Joye and Luzio, 2000). Pectin also extracted by direct boiling and microwave heating method (Yeoh *et al.,* 2008).

The extraction of pectin depends on various factors such as pH, temperature and time (Yapo *et al.,* 2007). Extraction with crossflow microfiltration improves purity of pectin with the increasing of galacturonic acid content compared to extraction with conventional method (Cho *et al.,* 2003).

2.2.5. Uses of Pectin

The consumption of pectin annually in the world is estimated at around 45 million kilograms, with a global market value of at least 444 million Euros (Savary *et al.*, 2003). In the food industry pectin is used as gelling agent, thickener and stabiliser properties. Pectins are generally also used to make various processed products such as jam, jellies, syrups, dietetic fruit juice beverages, flavour emulsions, salad dressings, cream-whipping aids, baker's glazes, melted-milk thickener, milk gels and puddings, barbecue sauce, yogurt and other frozen novelties (Hoefler, 1991). Pectin is also used for stabilising protein in acidic media through conjugation or complexation (Dalev and Simeonova, 1995; Al Hakkak and Karvale, 2002). In non-food products, can also be produced using pectin such as skin-care products (Kertesz, 1951; Okuyama *et al.*, 1981; Bates, 1987) and microbiological culture media (Roth, 1981).

It has already proven that pectin consumption decreased total serum cholesterol, low density lipoprotein (LDL) cholesterol levels (Brown *et al.*, 1999) and suppression of hypertension (Yamaguci *et al.*, 1994).

CHAPTER III
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3. Materials

The fresh mature pomelo was collected from the pomelo orchard adjacent to the Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Chemicals and other reagents used for the study were analytical reagent grade.

3.1. Methods

3.1.1. Preparation of pomelo peel powder

The pomelos were washed carefully with tap water to remove dirt soil from surface, the peel was removed and cut into slices (2-3 mm thickness) with a sharp knife. The slices were blended and dried at 60°C for 24 hours in a cabinet drier (Model- 136-12, Seoul, Korea). Then, the dried slices were ground into powder by using a blender (Jaipan CM/L-7360065, Japan). After that, powder was sieved using stainless steel sieve (Sieve no. MIC- 300) and packed in low density polyethylene bags (thickness of 75μm). The obtained powder was sealed and stored at 6-10°C until used.

3.1.2. Extraction of pectin

Preliminary experimentation to find out optimum conditions

Pectin extraction was performed using 0.1 N HCl solvent, extraction temperature (80°C, 90°C and 100°C) and extraction time (60, 120 min). Extraction pH was 1.5, 2 and 2.5. The solution pH was adjusted with HCl and NaOH. For each condition, pomelo peel powder (30 parts extraction solvent and 1 part pomelo peel powder) was heated in hot water bath. After heating the extractant was filtered with cheese cloth and pressed to recover the extract. The pectin was precipitated by adding absolute ethanol (95-98%) in the ratio of 1:2 (1 part extractant and 2 parts ethanol) and kept at room temperature for overnight. The precipitated pectin was filtered through Whatman No. 1 and washed with 75% ethanol (v/v), 85% ethanol (v/v) and absolute ethanol to remove the soluble impurities. Then pectin was dried at 60°C for 24 hours in a cabinet drier (Model- 136-12, Seoul, Korea). Optimum conditions such as pH, extraction time and extraction temperature were selected based on pectin yield, ash and equivalent weight.

3.2. Characterization of Pectin

3.2.1. Pectin Yield

Pectin yield was calculated as follows:

$$\text{Pectin (g/100g)} = \frac{\text{Weight (g) of dried pectin}}{\text{Weight (g) dried pomace powder taken for extraction}} \times 100$$

3.2.2. Determination of Total Ash Content

AOAC method (2002) was used to determine the total ash content. 0.5 g pectin sample was taken in a dry, clean crucible and weighed accurately. Then sample was kept in hot air oven at 105°C for 4 hr to remove the moisture. After that, the sample was burned in the muffle furnace at 550°C for 4 hours. It was cooled in desiccators and weighed.

Following formula was followed for determination of ash content:

Calculation:

$$\%\text{ of ash} = \frac{\text{Weight of the ash}}{\text{Weight of the raw sample}} \times 100$$

3.2.3. Determination of Equivalent Weight

Equivalent weight was determined by Ranganna method (1995). 0.5g sample was taken in 250 ml conical flask and 5 ml ethanol was added. Then 1 g sodium chloride and 100 ml distilled water were added. After that, 6 drops of phenol red was added and titrated against 0.1 N NaOH. The end point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content.

Equivalent weight was calculated by following formula:

Calculation:

$$\text{Equivalent weight} = \frac{\text{Weight of sample}}{\text{ml of alkali} \times \text{Normality of alkali}} \times 1000$$

3.2.4. Determination of Methoxyl Content (MeO)

Determination of methoxyl content was done by Ranganna (1995). The neutral solution was collected from determination of equivalent weight, and 25 ml of sodium hydroxide (0.25 N) was added. Then stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 ml of 0.25 N hydrochloric was added and titrated against 0.1 N NaOH.

The end point was indicated by purple color. Methoxyl content was calculated by following formula:

Calculation:

$$\text{Methoxyl content (\%)} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample}}$$

3.2.5. Determination of Total Anhydrouronic Acid Content (AUA)

Total Anhydrouronic Acid Content of pectin was obtained by the following formula (Mohamed & Hasan, 1995).

Calculation:

$$\%\text{ of AUA} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

When molecular unit of AUA (1 unit) = 176 g

Where,

z = ml (titre) of NaOH from equivalent weight determination.

y = ml (titre) of NaOH from methoxyl content determination.

w = weight of sample.

3.2.6. Determination of Degree of Esterification (DE)

The DE of pectin was measured on the basis methoxyl and AUA content (Owens *et al.*, 1952) and calculated by flowing formula.

Calculation:

$$\%\text{ DE} = \frac{176 \times \%\text{MeO}}{31 \times \%\text{AUA}} \times 100$$

3.2.7. Determination of Color

The color attributes (Hunter L*, a* and b* values) were measured with a colorimeter (CM-2500d, Minota, Japan).

3.2.8. Determination of Viscosity

Viscosity measurement was carried out by viscometer following the method described by Sotanaphun *et al.* (2012) with some modification. Gel was prepared by adding 65% sucrose, 0.5g pectin in 100 ml distilled water and the pH (about 2.34) was adjusted with

12.5% citric acid. Then, the solution was heated at 100°C for 30 minutes. After that the gel was kept at 4°C temperature for overnight. The gel viscosity was determined using digital viscometer (VR 3000, Vendrell, Spain) with L-1 probe at 25°C and a speed of 200 rpm.

3.2.9. Statistical Analysis

All measurements were carried out in triplicate for each of the sample. Results were expressed as mean values standard deviation. Statistically analysis was performed using the Statistical Package R (version 3.2.2). Duncan test was performed to evaluate the significance of difference between mean values at the level of $P< 0.05$.

CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Effect of pH on extraction yield and ash content of pectin

Table 4.1 shows the extraction yield and ash content of pomelo peel pectin at different pH content. Extraction parameters, pH is considered as one of the most important parameters that affect pectin yield as well as other quality parameters. The yield of pectin from pomelo peel at pH 1.5 and 2 was 16.07%, 16.74% respectively, which was similar with ambarella peel pectin (10 to 13%) and mango peel pectin (4.6 to 18.5%) extracted by deionized water (Koubala *et al.,* 2008). However, yield of pomelo peel pectin was higher than that reported by Yapo (2007) for passion fruit pectin (7.5%) and lower than golden apple pectin (22%, Rha *et al.,* 2011). Pectin yield from pomelo peel was not significantly different for both samples. Mollea *et al.* (2008) obtained higher yield at lower pH than higher pH. Koffi *et al.* (2013) reported that the yield of pectin was strongly pH dependent when the other parameters (time, temperature and solid to liquid ratio) were kept constant. Usually pectin yield is depends on pectin source and extraction conditions (Rha *et al.,* 2011). Usually Pectin extraction at lower pH gave higher yield due to the ability of acid in solubilizing the protopectin during extraction process. However, the yield of pectin can be decreased if the acid strength of extractant further increased. This unexpected result can be explained by degradation of pectin polymers which are ethanol soluble (Koffi *et al.,* 2013).

Table 4.1: Effect of pH on yield and ash content of pomelo peel pectin

Composition%	pH 1.5	pH 2.0
Yield	16.073 ± 0.651^{a}	16.740 ± 0.488^{a}
Ash	5.501 ± 0.027^{b}	5.697 ± 0.005^{a}

Mean ± SD (Three determinations). Mean values in the same row with different letters are significantly different ($p < 0.05$).

The ash content of pomelo peel pectin was 5.501% to 5.697%, for both pH. Pectin extraction at pH 2 gave higher ash content as compared to pectin extraction at pH 1.5. This observation was comparable to that found by Azad *et al.* (2014) who reported ash content of lemon pomace pectin varied from 2.41 to 4.06. Pectin purity and good gel

formation could be depending on low ash content (below 10%). Therefore, the ash content indicates in this study pectin was pure and extracted pectin might have ability to form gel.

4.2. Effect of pH on various physico-chemical properties of pomelo peel pectin

4.2.1. Degree of Esterification (DE)

The DE of pomelo peel pectin was ranged 61.19% to 70.795% (Table 4.2). According to DE our results showed that the both samples pH 1.5 and 2.0 were produced high methoxyl pectin. Methacanon *et al.* (2014) also found high methoxyl pectin from pomelo peel (59.4-70.7%). These results were lower than Sotanaphun *et al.* (2012) who found 76.30% in fruit peel of *Citrus maxima* and 83.41% DE in apple pomace pectin (Wang *et al.*, 2014). The highest value of DE of pomelo peel pectin was found at pH 2 than at pH 1.5. Similar result was obtained by Pagan *et al.* (2001) for peach pomace pectin. This might be due to increase in the de-estrification of methoxyl groups of the chain (Pagan *et al.*, 2001). Degrees of esterification values were significantly different for both pH.

Table 4.2: Effect of pH on various physico-chemical properties of pomelo peel pectin

Composition	pH 1.5	pH 2
Equivalent weight	540.04±11.89 [b]	711.33±13.77 [a]
Methoxyl content (%)	8.76±.62 [b]	10.68±.87 [a]
AUA (%)	84.29±5.83 [a]	85.57±4.96 [a]
Degree of esterification (%)	61.19±2.83 [b]	70.79±1.77 [a]
Viscosity (centipoises)	12±0.0 [a]	12±0.0 [a]

Mean ± SD (Three determinations). Mean values in the same row with different letters are significantly different ($p < 0.05$).

4.2.2. Methoxyl Content

Methoxyl content of analyzed pectin samples varied from 8.76 to 10.68% (Table 4.2). These values were similar than previously reported for peel of mango (7.33%), banana

(7.03%), pumello peel (8.57%), passion (8.81%-9.61%) (Madhav and Pushpalatha, 2002) but higher than dragon fruit pectin (2.98% to 4.34%) (Ismail *et al.,* 2012). The pH 2.0 sample had higher methoxylcontent (10.68%) than pH 1.5 (8.76%). This may be attributed to the partial degradation of pectin (Kulkarni and Vijayanand, 2010). Significant differences were found for both pH. The methoxyl content also depends on spreading quality and sugar binding capacity of pectin (Madhav and Pushpalatha, 2002). Based on the degree of esterification and methoxyl content value in this study indicating that pomelo peel pectin was categorized as high methoxyl pectin.

4.2.3. Equivalent Weight

The equivalent weight of extracted pectin ranged from 540.04 to 711.33 (Table 4.2) which are lower than apple pomace pectin (833.33 to 1666.30) (Kumar & Chauhan, 2010) but higher than cocoa husk pectin (510.68 to 645.19) (Ramli and Asmawati, 2011). The pomelo pectin extracted at pH 2 showed the highest equivalent weight (711.33) than pectin extracted at pH 1.5 (368.0) and had significant differences to each other. Equivalent weight increased or decreased could be depend on amount of free acid (Ramli and Asmawati, 2011). So, in this pectin sample the amount of free acid is lower due to lower equivalent weight than the commercial apple pectin.

4.2.4. Total Anhydrouronic Acid Content (AUA)

In this study, pectin extracted at both pH (1.5 & 2) showed high amount of AUA. The highest AUA content was found in pectin extracted at pH 2 sample (85.57±4.96%) and lowest AUA content in pH 1.5 sample (84.29±5.83%). Although, there were no significant differences in AUA content for both pH. Higher values were found for apple pomace pectin (59.52 to 70.50%, Kumar & Chauhan, 2010), commercial apple pectin (61.72%) and dragon fruit pectin (45.25 to 52.45%, Ismail *et al.,* 2012). Low value of AUA means that extracted pectin had might be high amount of proteins, starch and sugars (Ismail *et al.,* 2012). Purity of pectin or galacturonic acid content depended on pH, rather than temperature (Sotanaphun *et al.,* 2012).

4.2.5. Viscosity of pomelo peel pectin

The viscosity of pomelo peel pectin was 12 centipoises for both pH (1.5 & 2) samples and consistent with the results (2.91 to 13.05 centipoises) was found by Sotanaphun *et al.* (2012) from the fruit peel of pomelo pectin. Even though, the values were not

significantly different. Pectin viscosity can vary depending on extraction pH and extraction temperature. According to Sotanaphun *et al.* (2012) high viscosity products can be obtained when at low pH conditions due to the unknown polymer extracted during extraction. Joye and Luzio (2000) state that viscosity also strongly affected by temperature. Higher temperature gave lower viscosity due to shorten of polymer chain.

Table 4.3: Effect of pH on color values of pomelo peel pectin

Color values	pH 1.5	pH 2
L*	23.99±3.21 [a]	23.88±1.35 [a]
a*	3.76±0.46 [a]	1.77±0.38 [b]
b*	12.3±1.71 [a]	8.99±1.27 [a]

Mean ± SD (Three determinations). Mean values in the same row with different letters are significantly different ($p < 0.05$).

4.2.6. Color of pomelo peel pectin

The color of pomelo peel pectin powder in terms of L*, a*, and b* value were measured where, L*, a* & b* value indicated lightness, redness and yellowness respectively. The visible color of pomelo peel pectin powder for both pH (1.5 & 2) was brown. The L value for pomelo peel pectin powder was recorded 23.99 and 23.88 respectively (Table 4.3), which indicated less lightness property of powder and no significant difference found between pH 1.5 & 2 samples. The L* value was lower (23.88 to 23.99) where as redness (3.76 and 1.77) and yellowness (12.3 and 8.99) were similar with the value observed by Masmoudi *et al.* (2010) for lemon pectin from lemon by-product. The difference in L*, a* & b* value due to Maillard reactions which happened during the extraction process in high temperature and time. It also due to the present of pigment in the precipated extractant.

CHAPTER V
CONCLUSIONS

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In this investigation, pectin was extracted at different pH level (1.5 and 2.0) from pomelo peel at 90°C for 120 min. The results showed that the pectin yield (16.07 to16.74%), ash content (5.50 to 5.69%), equivalent weight (540.04 to 711.33), methoxyl content (8.76 to 10.68%), degree of esterification (61.19 to 70.79%), AUA content (84.29 to 85.57%) and viscosity (12 centipoises) for both samples. The overall results demonstrated that the pomelo peel pectin may be as a rich source of pectin as well as extracted pectin might be used as the functional food ingredients domestically and industrially.

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APPENDICES

APPENDIX I

Effect of pH on extraction yield and ash content of pomelo peel pectin:

Composition (%)	pH 1.5	pH 2.0
Yield	16.12	16.52
	15.4	16.4
	16.7	17.3
Ash	5.502	5.698
	5.473	5.701
	5.527	5.691

APPENDIX II

Effect of pH on various physico-chemical properties of pomelo peel pectin:

Composition	pH 1.5	pH 2
Equivalent weight	544.681 526.53 548.913	715.493 722.535 695.946
Methoxyl content (%)	8.053 9.132 9.104	11.594 9.849 10.594
AUA (%)	78.031 85.271 89.568	90.425 80.52 85.769
Degree of esterification (%)	58.59 60.8 64.202	72.797 69.451 70.127
Viscosity (centipoises)	12 12 12	12 12 12

APPENDIX III

Effect of pH on color values of pomelo peel pectin:

Color values	pH 1.5	pH 2
L*	26.78 24.72 20.49	22.86 25.41 23.39
a*	3.54 4.29 3.46	2.18 1.41 1.74
b*	11.40 14.27 11.23	10.29 7.75 8.93