
Isolation and characterization of pectin extracted from lemon pomace during ripening

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Abstract: The research was conducted to find out the various extraction conditions of pectin from lemon pomace under different of solvents (6M HCl, 1N H₂SO₄, 1N HNO₃, 6.2g/100g citric acid, 1N acetic acid, combination with acetic acid and ammonium oxalate and distilled water), temperatures (70, 80, 90 and 100°C), times (30, 60, 90 and 120 min) and maturity stages (premature, mature and over ripen). Preliminary results showed that optimum conditions for extraction of pectin were found at a temperature of 100°C by 60 min on the basis of pectin yield and equivalent weight extracted with distilled water. Pectin extracted with distilled water was characterized in terms of yield, moisture content, ash content, equivalent weight, methoxyl content, degree of esterification and neutral sugar contents. There were significant differences ($p < 0.05$) in yields, equivalent weights, degree of esterifications and neutral sugars among the lemon pomace pectin extracted from premature, mature and over ripen maturity stages. The degree of esterification and methoxyl contents were varying depending upon the maturity stages. Therefore, the premature lemon pomace can be considered as rich source of pectin in terms of yield, methoxyl content, degree of esterification and anhydrouronic acid content.

Keywords: Lemon Pomace, Pectin, Maturity Stages, Solvents, Temperature

1. Introduction

During the peak season huge amount of citrus fruits such as orange, lemon, lime, mandarin, grape fruit and pomelo are grown in Bangladesh due to favorable climatic conditions. Among all fruits, lemon is an important citrus crop in Bangladesh which has annual production of 20,000 tones and often leads to excess because of oversupply and low prices when it's in season (BBS, 2010). Usually fruits are processed into juice, beverage, squash and syrups. During processing, peel is contributes almost 5-20% of the total fruit. Therefore, huge amount of by-products 55-60% peel and 5-10% seed produces which is generated pollution of the environment. However by-products can be used as functional food ingredients such as phytochemicals, pharmaceuticals, food products, essential oils, seed oil, pectin and dietary fibers. These by-products are also considered to be rich sources of edible and health promoting agents like polymethoxylated flavonoids or hydroxycinnamates, many of which are found in citrus peels

(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. These polysaccharides consist of 300-1,000 chains of galacturonic acid units (Yeoh et al., 2008). Pectin is widely used in the food industry as a thickener, emulsifier, texturizer and stabilizer. Pectin is usually used in jams and jellies as a gelling agent and also used for fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products (Tsoga et al., 2004). In terms of nutrition, pectin has been shown to lower blood cholesterol levels specially low-density lipoprotein cholesterol fractions and thus reduces the risk for coronary heart diseases (Liu et al., 2006). In addition for the prevention of hyperlipidemia as well as bowel cancer various dietary products are used which are usually prepared from pectin fiber (Iglesias and Lozano, 2004). According to the FAO (1969) pectin is considered to be a safe additive that can be taken daily

without limits.

Pectin is one of the most valuable products which can be obtained from various sources. Commercial pectins are primarily extracted from citrus peel such as lime peel, guava extract, apple pomace (Chakraborty and Ray, 2011), and orange (Braddock 2004). Other source of pectin includes cocoa husk (Mollea et al., 2008), sunflower heads (Matora et al., 1995), beet and potato pulp (Turquoise et al., 1999), soy hull (Kalapathy & Proctor, 2001) and duckweed (Golovchenko et al., 2002). Pectin extraction is usually accomplished with water, mineral acids, hydrochloric acid, nitric acids, sulphuric acids and phosphoric acid. In a broad sense two types of pectin are available in nature including high methoxyl pectin (greater than 50% degree of esterification) and low methoxyl pectin (below 50% degree of esterification). High methoxyl can be extracted using water or mineral acids whereas various extraction solvents such as hydrochloric, nitric, sulphuric and phosphoric acids (Zhang and Taihua, 2011) are generally used for low methoxyl pectins. Besides, extraction of pectin also depends on various factors such as extraction time, temperature, pH and types of extraction solvent (Koubala et al. 2008b). Extraction time 20 to 60 minutes, extraction temperature 80 to 100° C and pH 1.4 to 2.6 are used for extraction of pectin from various sources (Chakraborty and Ray 2011). Pectin quality and purity can vary depending on an hydrogalacturonic acid, degree of estrification, ash content and molecular weight among low ash (below 10%) content and high anhydrogalacturonic acid (above 65%) are called good quality pectin (Chakraborty and Ray, 2011).

In recent years the production of fruits and vegetables has been increased. Therefore, large amount of fruits and vegetables could be used value added products as well as good source of pectin. With regard to the present condition a research was conducted to find out the various extraction conditions such as extraction solvent, extraction time, extraction temperature and maturation stages for pectin extraction from lemon pomace with a good yield, low ash content, higher galacturonic acid as well as equivalent weight, value of methoxyl content and degree of esterification.

2. Materials and Methods

2.1. Sample and Materials Collection

The fresh lemons at different stages (premature, mature and over ripen) were collected from the lemon orchard adjacent to the Hajee Mohammad Danesh Science and Technology University, Dinajpur; Bangladesh. Maturity of the lemons was selected on the basis of color. Chemicals and other reagents used for the study were analytical reagent grade.

2.2. Sample Preparation

The lemons were washed carefully with tap water to remove dirt soil from surface and cut into slices (2-3 mm

thickness) with a sharp knife. The juice was extracted by juice extractor (Nova, JC-805, Bangladesh). After juice extraction the residue was dried at 60°C for 24 hours in a cabinet drier (Model- 136-12, Seoul, Korea) followed by grinding into powder by using a blender (Jaipan CM/L-7360065, Japan). The powder was sieved using 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 for further study.

3. Extraction of Pectin

3.1. Optimized Conditions

Pectin extraction was performed using various conditions such as (6M HCl, 1N H₂SO₄, 1N HNO₃, 6.2g/100g citric acid, 1N acetic acid, combination with acetic acid and ammonium oxalate and distilled water), extraction temperature (70, 80, 90 and 100° C) and extraction time (30, 60, 90 and 120 min). The pH for all acids solutions was maintained at 2 while the pH of the acetic acid and ammonium oxalate combined acid solution was set at 4.6. The pH of water was almost 7.0. For each condition, 30 parts extraction solvent with 1 part lemon pomace powder was heated in hot water bath. After heating the extractant was filtered with cheese cloth and pressed the extract. The pectin was precipitated by adding absolute ethanol (95-98%) in the ratio of 1 part extractant to 2 parts ethanol and kept at room temperature for overnight. The precipitated pectin was filtered through the filter paper what man No. 4 and washed with 75% ethanol (v/v), 85% ethanol (v/v) and absolute ethanol to remove the soluble impurities. The pectin was dried at 40°C for 24 hours in a cabinet drier (Model- 136-12, Seoul, Korea). Optimum conditions such as distilled water solution, extraction time 1h, and extraction temperature 100°C were selected based on pectin yield and Equivalent weight.

4. Characterization of Pectin

4.1. Pectin Yield

Pectin yield was calculated as follows:

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

4.2. Determination of Moisture and Ash Content

Moisture and ash content of pectin was determined by the method of AOAC (1995) and AOAC (1975) respectively.

4.3. Determination of Equivalent Weight

Equivalent weight was determined by Ranganna's method (1995). 0.5 g sample was taken in a 250 ml conical flask and 5 ml ethanol was added. 1 g of sodium chloride and 100 ml of distilled water were added. Finally 6 drops of

phenol red was added and titrated against 0.1 N NaOH. Titration point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content.

Equivalent weight was calculated by following formula:

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

4.4. Determination of Methoxyl Content (MeO)

Determination of MeO was done by using the Ranganna's method (1995). The neutral solution was collected from determination of equivalent weight, and 25 ml of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 ml of 0.25 N hydrochloric acid was added and titrated against 0.1 N NaOH. Methoxyl content was calculated by following formula:

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

4.5. Determination of Total Anhydrouronic Acid Content (AUA)

Total AUA of pectin was obtained by the following formula (mohamed & Hasan, 1995).

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

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

4.6. 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 following formula.

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

4.7. Determination of Neutral Sugars

Neutral sugars such as glucose, galactose, rhamnose, mannose, arabinose and xylose contents were determined by Miller (1959) method with some modification. Pectin powder (0.5 g) was taken into a volumetric flask and made up 10 ml by adding distilled water. It was kept for 20 min at room temperature and filtrate through Whatman filter paper No 540. 1 ml filtrate was transferred into a test tube and

added 3 ml of dinitrosalicylic acid (DNS) solution was added to it. The test tube was vortex and then heated at boiling temperature for 15 min. After boiling it was cooled with tap water. Then the absorbance was taken at 550 nm. The neutral sugar contents were determined using different standard curve and were expressed as mg/ml.

5. Statistical Analysis

All measurements were carried out in triplicate for each of the sample. Results are expressed as mean values standard deviation. Data were statistically analyzed using MSTAT-C for windows version 2.10.

6. Results and Discussion

6.1. Extraction of Yield, Moisture and Ash Content of Pectin

Table 1 shows the extraction yield, moisture and ash contents of lemon pomace pectin at different maturity stages. The yield of pectin from lemon pomace at premature, mature and over ripen stage was 13.13%, 10.83% and 10.33%, respectively (Table 1) 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.*, 2008a; Koubala *et al.*, 2008b). However, yield of lemon pomace pectin was higher than that as reported by Yapo (2007) for passion fruit (7.5%) and lower than golden apple (22%) (Rha *et al.*, 2011). The yields of lemon pomace pectin significantly decrease with the increase of maturation. The highest yield of pectin was 13.13±0.17% obtained from premature lemon pomace whereas lowest yield obtained from over mature sample (10.33±0.15%). The pectin yield was the lower at ripening because the pectin in fruits is usually converted into protopectin, sugar and others constituent increase during ripening. Usually pectin yield is also depends on pectin source and extraction conditions. (Rha *et al.*, 2011).

The moisture content of all samples was varied 11.49±0.55 to 13.40±0.79% at different mature stages. This observation was comparable to that found by Ismail *et al.* (2012) who reported moisture content of dragon fruit pectin varied from 11.13 to 11.33%. Premature sample showed the lower moisture content than that of over ripen one. It is necessary to keep in mind that high moisture content could enhance the growth of microorganisms and production of pectinase enzymes that can further affect the pectin quality (Muhamadzadeh *et al.*, 2010).

The ash content was ranged from 2.41±0.51 to 4.06±0.29% depending on different maturity of lemon pomace samples. The premature sample gave the lower ash content than mature and over ripens. The ash content increases as the pectin yield decreases, indicating that the sugar content and others constituent increases significantly due to ripening of the fruit. Low ash content (below 10%) and maximum limit of ash content 10% are one of the good

criteria for gel formation (Ismail et al., 2012). Therefore, the ash content found in this experiment indicates the purity of the pectin.

7. Characterization of Pectin

7.1. Degree of Esterification (DE)

The DE of lemon pomace pectin was ranged from 33.59 ± 0.17 to $79.51 \pm 0.36\%$ (Table 2). Based on DE pectin can be classified as low methoxyl pectin with $\leq 50\%$ DE and high methoxyl pectin with $>50\%$ DE. The premature and mature samples were produced high methoxyl pectin with the degree of esterification ($79.51 \pm 0.36\%$) and ($70.39 \pm 4.20\%$), respectively and over mature sample produced low methoxyl pectin with the degree of esterification $33.59 \pm 0.17\%$. These results were consistent with previous measurement of 76.30 % DE in *Citrus maxima* (Sotanaphun, et al., 2012) and 31 to 52% DE in dragon fruit (Ismail et al. 2012). Degree of esterification decreased with the increase of maturity. The lower DE might be attributed to the conversion of pectins into proto-pectin which increases the sugars and makes the fruit softer (Bartley and Knee, 1981; Redgwell et al., 1997) during the maturation. According to Sundar Raj et al. (2012) DE actually depends on species, tissue and stages of maturity.

7.2. Methoxyl Content

Methoxyl content is an important factor in controlling the setting time of pectins and the ability of the pectin to form gels (Constenla and Lozano, 2003). Table 2 shows that the premature sample had higher methoxyl content ($10.25 \pm$) followed by mature (4.24%) and over ripe (4.26%) ones. These values were approximately similar to those as found for peel of mango (7.33%), banana (7.03%), pomelo peel (8.57%), Lime (9.92%), (Madhav and Pushpalatha, 2002), passion (8.81%-9.61%) but higher than dragon fruit pectin (2.98% to 4.34%) (Ismail et al., 2012). The results showed that the methoxyl content significantly decreased with increase of maturity. Due to ripening the sugar content of the fruits are increased and the methoxyl content decreased (Sirisakulwat et al., 2008). Spreading quality and sugar binding capacity of pectin are increased with increase methoxyl content (Madhav and Pushpalatha, 2002). Based on methoxyl content value in this study indicates that lemon pomace pectin was categorized as high and low methoxyl pectin.

7.3. Equivalent Weight

The equivalent weight of extracted pectin ranged from 368 ± 3 to 1632 ± 137 (Table 2) which was 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). Equivalent weight was significantly different ($p < 0.05$) at the maturity stage. The over ripens extracted pectin showed lower equivalent weight (368 ± 3) while the mature extracted sample showed

the highest equivalent weight (1632 ± 137). The lower equivalent weight could be higher partial degradation of pectin. The increased or decreased of the equivalent weight might be also dependent upon the amount of free acid (Ramli and Asmawati, 2011).

7.4. Total Anhydrouronic Acid Content (AUA)

The AUA indicates the purity of the extracted pectin and its value should not be less than $< 65\%$ (Food Chemical Codex, 1996). In this study the highest AUA content was found in premature sample ($73.22 \pm 3.92\%$) and the lowest AUA was in mature sample ($34.12 \pm 2.06\%$). Resembled values were found in apple pomace pectin, commercial apple pectin and dragon fruit pectin which was 59.52 to 70.50%, (Kumar & Chauhan, 2010), 61.72% and 45.25 to 52.45% (Ismail et al., 2012) respectively. Low value of AUA means that the extracted pectin might have a high amount of protein (Ismail et al., 2012).

7.5. Composition of Neutral Sugar in Pectin

Individual neutral sugars are summarized in Table 3. The main neutral sugars were arabinose ($3.54 \pm 0.09\%$), galactose ($3.54 \pm 0.09\%$), glucose ($4.12 \pm 0.09\%$), mannose ($4.13 \pm 0.09\%$), rhamnose ($9.64 \pm 0.24\%$) at various maturity stages. It seems that the studied pectin samples contained higher amount of rhamnagalacturonic regions (Masmoudi et al. 2010). The small amount of xylose (0.03%) could come from hemicelluloses. These values were consistent with that as reported for chicory roots (Panouille' et al., 2006), carrots (Massiot et al., 1988) and sugar beet pectin (Oosterveld et al., 2000). The mature and over ripen samples showed higher amounts of neutral sugar contents than that of premature sample. There were no significant difference between mature and over ripe samples and the total neutral sugar ranged from 14.20 ± 0.47 to $23.99 \pm 0.57\%$. The similar result of neutral sugar contents from citrus pectin ranged 15.45 to 19.40% was reported by Georgiev et al. (2012). Sugar contents may also vary based on the plant source and different extraction procedures (Hwang et al., 1993). Lower neutral sugar contents and higher molecular weight (similar to equivalent weight) showed greater gelling ability than that of higher neutral sugar contents and lower molecular weight (Rombouts & Thibault, 1986; Phatak et al., 1988).

8. Conclusions

In the present study, pectin was extracted successfully from lemon pomace using different extraction conditions and maturity stages (premature, mature and over ripen). Pectin extracted with distilled water showed high yield and low ash content as compared to other solvents. On the other hand, the premature stage of lemon gave the highest yield with the higher methoxyl content, anhydrouronic acid content (AUA) and greater degree of esterification, and lower neutral sugar content. When the fruits goes to

premature to mature conditions the yield was decreased along with anhydrouronic acid content (AUA), methoxyl content and degree of esterification, and increased the neutral sugar content. The overall results demonstrated that the premature lemon pomace 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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Table 1. Yield, moisture and ash contents of lemon pomace pectin.

Composition	Premature	Mature	Over Ripen
Yield (%)	13.13±0.17 ^a	10.83±0.02 ^b	10.33±0.15 ^c
Moisture content (%)	10.92±0.17 ^b	11.49±0.55 ^b	13.40±0.79 ^a
Ash content (%)	2.41±0.51 ^b	2.46±0.21 ^b	4.06±0.29 ^a

Mean ± SD (Three determinations).

Mean followed by different superscript letters in each column are significantly different ($p < 0.05$).

Table 2. Physicochemical constituents of lemon pomace pectin.

Composition	Premature	Mature	Over Ripen
Degree of esterification (%)	79.51±0.36 ^a	70.39±4.20 ^b	33.59±0.17 ^c
Methoxyl content (%)	10.25±0.50 ^a	4.24±0.46 ^b	4.26±0.01 ^b
Equivalent weight	1175±82 ^a	1632±137 ^b	368±3 ^c
AUA (%)	73.22±3.92 ^a	34.12±2.06 ^b	71.99±0.44 ^a

Mean ± SD (Three determinations).

Mean followed by different superscript letters in each column are significantly different ($p < 0.05$).

Table 3. Neutral sugar contents of lemon pomace pectin.

Composition (% w/w)	Premature	Mature	Over Ripen
Arabinose	2.05±0.07 ^b	3.36±0.13 ^a	3.54±0.09 ^a
Galactose	1.51±0.05 ^b	2.40±0.09 ^a	3.54±0.09 ^a
Glucose	2.57±0.007 ^b	3.90±0.15 ^a	4.12±0.09 ^a
Xylose	0.02±0.00 ^a	0.03±0.00 ^a	0.03±0.00 ^a
Mannose	2.54±0.08 ^b	3.93±0.15 ^a	4.13±0.09 ^a
Rhamnose	5.49±0.20 ^b	9.12±0.37 ^a	9.64±0.24 ^a
Total	14.20±0.47 ^b	21.46±2.80 ^a	23.99±0.57 ^a

Mean ± SD (Three determinations).

Mean followed by different superscript letters in each column are significantly different ($p < 0.05$).

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