Extraction, Characterization and Application of Three Varieties of *Citrus limon* L. Pectin in Jelly Product

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Abstract

In this study, pectin was extracted from the local varieties of *Citrus limon* L. including ginger lemon, cardamom lemon and China lemon by using organic tartaric acid and 95% ethanol and characterized regarding yield, moisture content, ash content, pH, equivalent weight, methoxyl content, anhydrouronic acid (AUA) content, degree of esterification, solubility and Fourier transform infrared (FT-IR) spectroscopy. The extracted pectin was also applied to produce pineapple jelly in a laboratory scale and analyzed. The highest yield of pectin was extracted from China lemon and the lowest yield was from ginger lemon and the yield of cardamom lemon was in between. The results also indicated that only cardamom lemon produced high methoxyl pectin, while the other two produced low methoxyl pectin. They can be also classified as highly pure pectin according to their high AUA content and low ash content. Moreover, FT-IR spectroscopy was used for the confirmation of structural characterization of pectin and no significant difference was found in the pectin structure. The extracted pectin samples were applied in pineapple jelly and that was analyzed by comparing with a commercial pineapple jelly. Although commercial pineapple jelly obtained the highest overall acceptability, pineapple jelly made from cardamom lemon pectin also obtained a good score. Therefore, pectin which was extracted from the local varieties of *Citrus limon* L. can be used as an effective food additive in jelly product.

Keywords: Pectin, *Citrus limon* L., Extraction, FT-IR, Jelly

1. Introduction

Pectin can be interpreted as a family of hetero-polysaccharides in the cell wall of plant tissues (Bagherian *et al*, 2011; Prakash Maran *et al*., 2014). It mainly consists of esterified D-galacturonic acid and the acid groups are largely along with methoxyl groups in the natural product. There can also be acetyl groups present on the free hydroxyl groups (Maran and Priya, 2015). Pectin is often classified by means of the method of extraction from cell walls namely water-soluble pectin, chelator-soluble pectin and proto-pectin (Roberts, 1990). Also, based on degree of esterification (DE) pectin is divided into two groups: high methoxyl pectin (HMP) with DE higher than 50% and low methoxyl pectin (LMP) with DE lower than 50% (Thakur *et al*., 1997).

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Received: 31 July 2018/ Revised: 30 October 2018/ Accepted: 5 November 2018

Citrus limon L. is a member of Rutaceae family widely found in Asia (likely India and Pakistan) and is consumed for its dietary functions (Zhao *et al*., 2015). *Citrus limon* peel is a rich source of pectin and is discarded as a by-product in food industries and therefore using this waste can be important for obtaining a value–added product and in helping to protect the environment (Shen *et al*., 2013).

Pectin is generally extracted through hot diluted mineral acid and alcoholic precipitation (Kalapathy and Proctor, 2001) but the use of mineral acid causes increased costs and can be harmful to the environment by producing toxic elements (Chan and Choo, 2013). So, an organic acid, such as tartaric acid is used for extraction of polysaccharides such as pectin (Bartolomeu *et al*., 2012).

Pectin is widely used as a gelling and stabilizing agent in the food industry (Maran and Priya, 2015). Also pectin has multiple positive effects on human health including lowering cholesterol and serum glucose (Inngjerdingen *et al*., 2007), inhibiting growth and metastasis (Jackson *et al*., 2007), and stimulating the immune response (Santos *et al*., 2013). Nowadays, researchers are searching for find out new sources of this polysaccharide due to the high demand of pectin in the global market (Yeoh *et al*., 2008).

Therefore, the objective of this study was to extract pectin from the peels of three local varieties of *Citrus limon* L. by using organic tartaric acid and to characterize the extracted pectin and to apply it in a laboratory-scale jelly production.

2. Materials and methods

2.1 Sample preparation

The three different varieties of *Citrus limon* L. which are locally called ginger lemon, cardamom lemon and China lemon were collected from a local market in Sylhet, Bangladesh. The lemons were washed carefully to remove dirt soil from surface and each of the lemons were split or cut into four parts. The peels were removed and cut into smaller pieces for easy drying. Then, the peels were air dried at 60 °C for 24 h. After that the dried peels were ground by a blender (MJ-M176P, Panasonic, Japan) and the obtained powders were sealed and stored in a refrigerator for further use.

2.2 Organic extraction of pectin

At first a total of 10 g fruit lemon peel powder from different varieties (ginger lemon, cardamom lemon, China lemon) was measured on an analytical balance. After that the powder was blended with 250 mL distilled water in a 1000 mL beaker and acidified with 5% tartaric acid. The pH of the solution was maintained around 2.0. The mixture was then homogenized using a homogenizer (Wise Tis HG-15A, Witeg, Germany) until all the fruit peel powder was

evenly wetted by acidified water in homogenous form. The extraction procedure was continued treating the acidified samples at 70 °C for a duration of 90 min in an unstirred water bath (WB-1000D, Digisystem Laboratory Instruments Inc., Taiwan). The mixture was kept at room temperature for 24 h. Then the mixture was filtrated and the filtrate was further purified by using a benchtop centrifuge (416g, Gyrozen, Korea) at 4,000 rpm for 25 min. After measuring the volume of the filtrate, it was taken into a beaker and double volume of 95% ethanol (1:2 v/v) was added to precipitate pectin and stored in dark condition at room temperature of 25 °C for 24 h to allow pectin flotation. The pectin was then separated by filtration and subsequently washed twice with 70% ethanol. The resulted pectin substance was dried in a conventional oven (OF-21E, Jeio Tech, Korea) at 65 °C for 16 h and weighed by an analytical balance (AY220, Shimadzu, Japan). The percentage yield of the lemon peel pectin was determined as g of product obtained per g of lemon peel powder used and was calculated as follows:

$$\text{Pectin yield} = \frac{\text{Weight of dried pectin (g)}}{\text{Weight of lemon peel powder (g)}} \times 100$$

2.3 Characterization of extracted pectin

The extracted pectin was analyzed for moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid (AUA) content, degree of esterification (DE), solubility in cold and hot water, solubility in cold and hot alkali (NaOH), determination of pH and FT-IR spectroscopy.

2.3.1 Determination of moisture and ash content

The determination of moisture and ash contents of the extracted pectin was done by the methods of AOAC (2000).

2.3.2 Determination of equivalent weight

Equivalent weight was determined by the method described by Ranganna (1995). 0.5 g of a sample was taken into a 250 mL conical flask and 5 mL of ethanol was added. 1 g of sodium chloride and 100 mL of distilled water were added. Finally 6 drops of 0.4% phenol red indicator were added. The sample was then titrated against standardized 0.1 N NaOH. Titration end point was indicated by the appearance of pink color. This neutralized solution was stored for determination of methoxyl content. The following equation was used to calculate equivalent weight:

$$\text{Equivalent weight} = \frac{\text{Weight of the sample (g)} \times 100}{\text{mL of alkali} \times \text{Normality of alkali}}$$

2.3.3 Determination of methoxyl content

The neutral solution was collected from determination of equivalent weight, and 10 mL of standardized 0.1 N NaOH was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min, 10 mL of 0.1 N HCl was added and titrated against standardized 0.1 N NaOH (Ranganna, 1995). Methoxyl content was calculated by following formula:

$$\text{MeO (\%)} = \frac{\text{mL of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample (g)}}$$

2.3.4 Determination of anhydrouronic acid (AUA)

Estimation of anhydrouronic acid is essential to determine the purity and degree of esterification. Total AUA of pectin was obtained by the following formula (Suhaila and Zahariah, 1995):

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

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

2.3.5 Determination of degree of esterification (DE)

The DE of pectin was measured on the basis of methoxyl and AUA contents (Owens, 1952) and calculated by following formula:

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

2.3.6 Solubility of pectin in cold and hot water

0.2 g of the pectin samples were separately placed in a conical flask and 10 mL of 95% ethanol was added, followed by 50 mL of distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 85–95 °C for 15 min (Fishman *et al*., 1984).

2.3.7 Solubility of pectin solution in cold and hot alkali (NaOH)

1 mL of 0.1 N NaOH was added to 5ml of pectin solution and then heated at 85–90 °C for 15 min (Fishman *et al*., 1984).

2.3.8 Determination of pH

Pectin solution was prepared by the method stated by Fishman *et al*. (1984). The pH of the solution was determined by using a standard pH 7 buffer solution and the temperature was adjusted to room temperature. The glass electrode of the pH meter (Model 744, Metrohm, Switzerland) was standardized with standard buffer solutions and rinsed with distilled water before inserting into the pectin solution and pH of the pectin solution was read off (Aina *et al*., 2012).

2.3.9 Fourier transform infrared (FT-IR) spectroscopy for structural analysis of pectin

FT-IR spectroscopy of pectin was determined by using a FT-IR spectrometer (IR Prestige21, Shimadzu, Japan) using the KBr pellet method in 4000–400 cm^{-1}. Sample was incorporated with KBr (spectroscopic grade) and pressed into a 3 mm pellet. The samples were scanned at wavenumbers ranging from 4000 to 400 cm^{-1} and corrected against the background spectrum of air. The spectrum of each sample was obtained by taking the average of 50 scans.

2.4 Application of extracted pectin

The application of extracted pectin was done by producing pineapple jelly with the extracted pectin from the three different local varieties of *Citrus limon* L. Fresh, fully matured and ripe pineapples were obtained from local market. Sugar, citric acid and other required materials were used from the laboratory stock. Pectin used in the jelly preparation was extracted from the three different local varieties of *Citrus limon* L. and this experiment was conducted in comparison with pineapple jellies from a single commercial brand also made by pineapple. For analyzing the parameters, these products were coded as A, B, C and D. The code also indicated a single commercial brand jelly along with different types of product. Specification was done as follows: A = Pineapple jelly made with pectin extracted from ginger lemon peel, B = Pineapple jelly made with pectin extracted from cardamom lemon peel, C = Pineapple jelly made with pectin extracted from China lemon peel, D = Commercial pineapple jelly.

At first, the pineapples were washed thoroughly and then peeled. Pulp was collected by cutting the edible portion (flesh). Before juice extraction, the core and eyes of pineapple were removed carefully. Only minimum quantity of water was added to the flesh for extracting the juice. Juicer machine (MJ-M176P, Panasonic, Japan) was used to extract juice from the flesh. After proper clarification, the juice was pasteurized at a temperature of 75 °C for 2 min. The pasteurized juice was stored in a deep freeze at a temperature of -20 °C for future use. The formulation of the jelly is given on Table 1. At first, the frozen pineapple juice was

defrosted one day before cooking. Sugar and juice were weighed according to the formulation (Table 1) and heated to boiling for 5–10 min on the following day. Citric acid was dissolved in water and added at this stage. Calculated amounts of extracted pectin and sugar were mixed together and added to the cooking pot. The mixture was allowed to boil for further 5 min to ensure complete dissolution of pectin. Soluble solids were determined by refractometer (Eclipse Hand-held Refractometer: 45–80 °Brix, Global Water, U.S.A) at a desired value before pouring the hot jellies in to desired glass jars. Then the jars were sealedand stored at ambient temperature. No preservatives were used in the pineapple jellies (Ahmmed *et al*., 2017).

Table 1 Formulation of Jelly

Ingredients	Percentage (%)
Juice	45
Sugar	55
Citric acid	0.6
Extracted pectin	1

2.5 Analysis of pineapple jelly

2.5.1 Determination of moisture and ash content

The determination of moisture and ash contents of the jellies were done by the method of AOAC (2000).

2.5.2 Total soluble solids (TSS) content

TSS was measured directly from the refractometer (Eclipse Hand-held Refractometer: 45–80 °Brix, Global Water, U.S.A).

2.5.3 Determination of pH

The determination of the pH of the pineapple jelly measured at room temperature with a digital glass electrode pH meter (Model 744, Metrohm, Switzerland), which was calibrated prior to sample pH measurement using standard buffer solutions of pH value 7.0.

2.5.4 Titratable acidity

1 g of jelly was taken and dissolved in 20 mL of distilled water in a conical flask, two to three drops of 2% phenolphthalein indicator were added then titrated with standardized 0.1 N NaOH till pink colour appears (Patil *et al.*, 2013). Titratable acidity can be calculated as follows:

$$T_a = \frac{B \times 0.1 \times 0.064 \times 100}{W}$$

Where,

T_a = titratable acidity

B = ml of 0.1 N NaOH

W = weight of sample

2.5.5 Sensory evaluation of pineapple jellies

The sensory evaluation of prepared pineapple jellies and commercial jelly was conducted for color, flavor, texture and overall acceptability by a panel of 10 untrained tasters whom were selected from the teachers, students and employees of Shahjalal University of Science and Technology, Sylhet and were briefed before evaluating sensory quality of the jellies. For statistical analysis of sensory data, a 9-point hedonic rating test (Singh, 2002) was performed to assess the degree of acceptability of jellies. The panelists were asked to rate the sample on a 9-point hedonic scale for color, flavor, texture and overall acceptability with the ratings of: 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3= Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely.

2.6 Statistical analysis

Data (3 replications for all experiment and analysis except sensory evaluation which had 10 replications) were expressed as the mean ± SD and comparisons of data were carried out using the one-way analysis of variance. $p < 0.05$ was considered statistically significant. It should be noted that all computation in this study were performed using the statistical software IBM SPSS Statistics 21.0.

3. Results and discussion

3.1 Yield of extracted pectin

As shown in Table 2, the yields of extracted pectin ranged from 5.72 ± 0.17% to 12.73 ± 0.23%. Pectin extracted from ginger lemon was similar to the pectin extracted from orange peel (6%) (Bagde *et al*., 2017). The yield of pectin extracted from ginger lemon peel is higher than that of pectin extracted from sweet orange (1.68%) (Aina *et al*., 2012) but lower than pectin extracted from apple flour (9.73%) (Canteri-Schemin *et al*., 2005). The amount of pectin extracted from China lemon peel is similar to ambarella peel pectin (10 to 13%) *(*Koubala *et al*., 2008). Although lower than that of golden apple (22%) (Rha *et al*., 2011), it is greater than the pectin extracted from passion fruit (7.5%) (Yapo et al., 2007). The yield of pectin extracted from cardamom lemon is similar to the pectin extracted from passion fruit (7.5%) (Yapo *et al*., 2007). However, it is lower than jackfruit rind pectin (14.81 ± 1.02%) (Leong *et al*, 2016) but

higher than the pectin extracted from orange peel (6%) (Bagde *et al*., 2017). Although pectin yield depends on pectin source and extraction conditions, eco-friendly organic acids such as tartaric acid showed significant results influencing the yield of pectin than mineral acids. The yield of the extracted pectin of each lemon variety was significantly different ($p < 0.05$) (Table 2).

Table 2 Yield, moisture and ash content from extracted pectin

Composition	Ginger lemon	Cardamom lemon	China lemon
Yield (%)	5.71 ± 0.17^{a}	8.08 ± 0.07^{b}	12.73 ± 0.23^{c}
Moisture content (%)	12.94 ± 0.39^{b}	14.16 ± 0.23^{c}	10.43 ± 0.21^{a}
Ash content (%)	4.15 ± 0.56^{b}	2.61 ± 0.37^{a}	4.02 ± 0.43^{b}

Note: [1] Results are presented as mean ± SD (Three determinations). Mean followed by different superscript letters in each row are significantly different ($p < 0.05$)

[2] Yield (%) is expressed in dry basis and moisture content (%) is expressed in wet basis

3.2 Moisture and ash content

The moisture contents of the samples were significantly different than each other ($p < 0.05$), ranging from 10.43 ± 0.21% to 14.16 ± 0.22% (Table 2). This observation was comparable with the moisture content of dragon fruit pectin (11.13 to 11.33%) (Ismail *et al*., 2012). It can be also noted that high moisture content could enhance the growth of microorganisms and produce pectinase enzymes that can affect the pectin quality (Mohamadzadeh *et al*., 2010). The moisture contents found in the pectin samples were low, therefore, microorganism growth and enzyme production will be minimum.

The ash contents ranged from 2.61 ± 0.37% to 4.15 ± 0.56% (Table 2). The pectin extracted from cardamom lemon peels showed lower ash content than ginger lemon and China lemon. The quality of the pectin yield decreases as the ash content increases (Ahmmed *et al*., 2017). Maximum limit of ash content which is 10% are one of the good requirements for gel formation (Ismail *et al*., 2012). Therefore, the ash content found in the extracted pectin samples indicates the purity of the pectin.

3.3 Equivalent weight

The values of equivalent weight are used in the calculations for Anhydrouronic acid (AUA) content and Degree of esterification (DE). The equivalent weights of extracted pectin was ranged from 298 ± 21 to 532 ± 24 (Table 3), which was lower than apple pomace pectin ranging 833.33 to 1666.30 (Kumar and Chauhan, 2010) but higher than lemon (100) and orange (86.87) pectin (Bagde *et al*., 2017). The lower equivalent weight could be higher partial

degradation of pectin (Azad, 2014). The increased or decreased of the equivalent weight might be also dependent upon the amount of free acid (Nazaruddin, 2011). Therefore, it can be also stated that tartaric acid could also be an influencing factor for the low equivalent weight of the pectin samples.

Table 3 Physicochemical constituents of extracted pectin

Composition	Ginger lemon	Cardamom lemon	China lemon
Degree of esterification (%)	27.38 ± 0.32^{b}	62.12 ± 1.46^{c}	21.96 ± 0.84^{a}
Methoxyl content (%)	3.97 ± 0.56^{b}	9.35 ± 0.39^{c}	2.86 ± 0.29^{a}
Equivalent weight	298 ± 21^{a}	532 ± 24^{b}	301 ± 12^{a}
AUA (%)	82.12 ± 2.93^{b}	85.45 ± 4.12^{b}	74 ± 1.39^{a}
pH	3.41 ± 0.39^{a}	3.63 ± 0.49^{a}	3.37 ± 0.26^{a}

Note: [1] Results are presented as mean ± SD (Three determinations). Mean followed by different superscript letters in each row are significantly different ($p < 0.05$)

3.4 Methoxyl content

Table 3 shows that the methoxyl content ranged from 2.86 ± 0.29% to 9.35 ± 0.39%, where cardamom lemon pectin contained higher methoxyl content followed by pectin from other two varieties. Only the methoxyl content of cardamom lemon pectin was approximately similar to that found for peel of lime (9.92%) (Madhav and Pushpalatha, 2002). The methoxyl contents of the other two samples were similar to those of dragon fruit pectin (2.98% to 4.34%) (Ismail *et al*., 2012). The methoxyl content could be decreased due to some degree of ripening of the lemons collected for this study. From this study, the extracted pectin samples can be divided into high and low methoxyl pectin.

3.5 Anhydrouronic acid (AUA) content

The AUA content indicates the purity of the extracted pectin and its value should not be less than < 65% (Food Chemical Codex, 1996). In this study it was found that the highest AUA content was found in cardamom lemon (85.45 ± 4.12%) and the lowest AUA was in China lemon (74.06 ± 1.39%). Ginger lemon was in between (82.12 ± 2.94%) (Table 3). Approximate values were found in pomelo pectin ranged 84.29 ± 5.83% for pH 1.5 and 85.57 ± 4.96% for pH 2.0 (Roy *et al.*, 2018), apple pomace pectin and commercial apple pectin which were 59.52 to 70.50%, (Kumar and Chauhan, 2010) and 61.72% (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). As organic tartaric acid was used in this study, it significantly increased AUA recovery for the extracted pectin as stated by Devi *et al*. (2014).

3.6 Degree of esterification (DE)

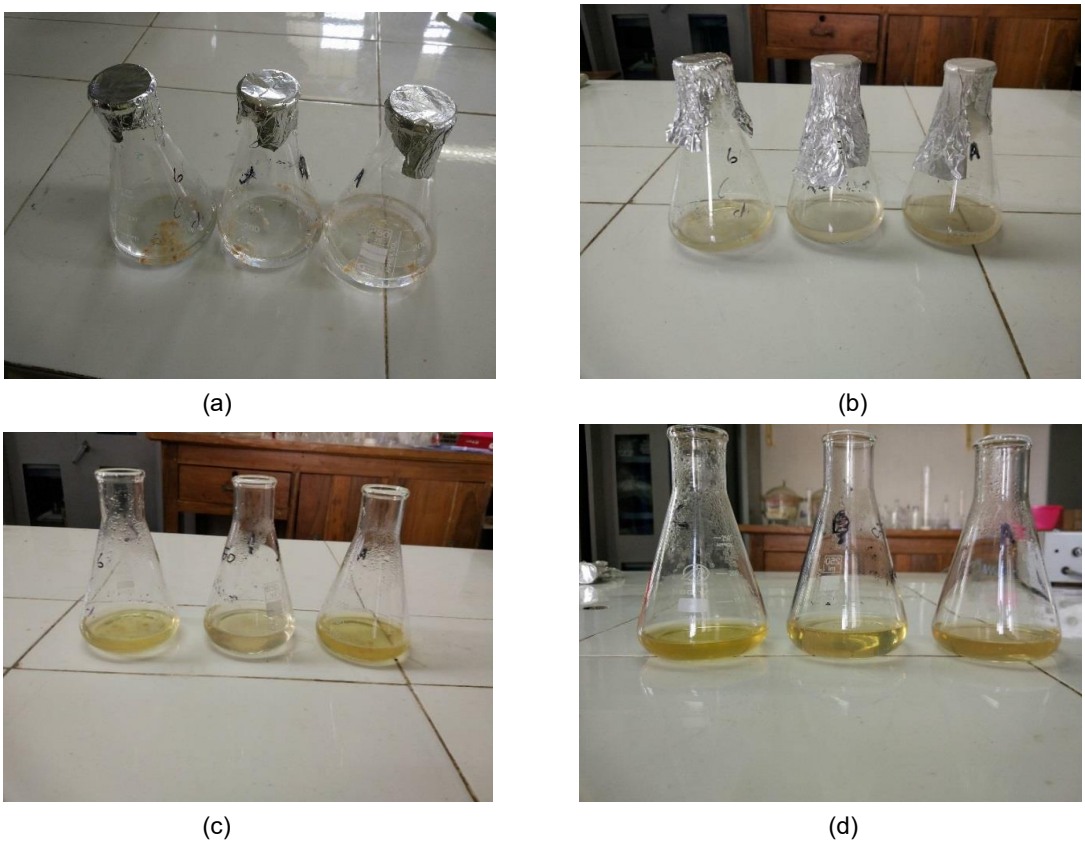
The DE of pectin extracted from the different local varieties of *Citrus limon* L. was ranged between 21.96 ± 0.84% to 62.12 ± 1.46%, (Table 3). Based on DE, pectin can be classified as low methoxyl pectin with ≤50% DE and high methoxyl pectin with >50% DE. Therefore, it can be stated that ginger lemon and China lemon produced low methoxyl pectin and cardamom lemon produced high methoxyl pectin. These results were consistent with previous measurement of 76.60% DE in *Citrus maxima* as stated by 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 although it actually depends on species, tissue and stages of maturity (Sundar *et al*., 2012).

3.7 pH determination

The pH of the pectin extracted from the different local varieties of *Citrus limon* L. was ranged between 3.41 ± 0.39 to 3.63 ± 0.49 (Table 3) which is similar to that of lemon (4.1), grape (4.0) and sweet orange (3.6) as stated by Aina *et al*. (2012).

3.8 Solubility in cold and hot water

Pectin extracted from the different local varieties of *Citrus limon* L. by organic tartaric acid were insoluble in cold water (Figure 1(a)). Furthermore, the pectin swelled after much shaking. On the other hand, the mixtures were dissolved in hot water (Figure 1(b)).



(a) (b)

(c) (d)

Figure 1 Solubility of pectin in (a) cold water (b) hot water (c) cold alkali (d) hot alkali

3.9 Solubility in cold and hot alkali

All of the pectin suspensions were insoluble in cold alkali and gave a yellow gelatinous liquid which turned yellowish when heated at 85–90 °C for 15 min (Figure 1(c, d)). However, it should be stated that pectin is unstable under alkaline solution (Fishman *et al*., 1984) which corresponded with what was obtained from this experiment.

3.10 Structural analysis by FT-IR spectroscopy

FT-IR spectroscopy was used to confirm the isolated pectin from the cell wall material of ginger lemon, cardamom lemon and China lemon. Figure 2, Figure 3 and Figure 4 represents the spectrum of studied pectin of ginger lemon, cardamom lemon and China lemon respectively. Also Table 4 represents the assignments of FT-IR wavenumbers in the range 4000–400 cm^{-1} of the extracted pectin samples. Several absorption peaks were found between 3100 and 3600 cm^{-1} was due to OH stretching. The peak around 2947.23 cm^{-1} was related to C-H stretching vibrational modes including CH, CH_2 and CH_3 groups (Wang *et al*., 2014). The peak around 1700 to 1800 cm^{-1} is due to the vibrating of the CO group of O-CH_3. Carboxylate groups have two peaks, one peak is related to asymmetrical vibrating around 1600 to 1650 cm^{-1}, and another one is due to weaker symmetric vibrating at 1450 to 1490 cm^{-1} as stated by Pasandide *et al*., (2017). The two strong absorption ranged around 1022.27 to 1099.43 cm^{-1} and 1219.01 to 1282.66 cm^{-1} are attributed to glycosidic linkage between sugar units (Gnanasambandam and Proctor, 2000). In general, the total peak area between 800 and 1200 cm^{-1} is expressed “Finger print” region that is individual and it is difficult to interpret (Kozarski *et al*., 2012). It can be said that the obtained precipitate is rich in polygalacturonic acid according to the statements mentioned above.

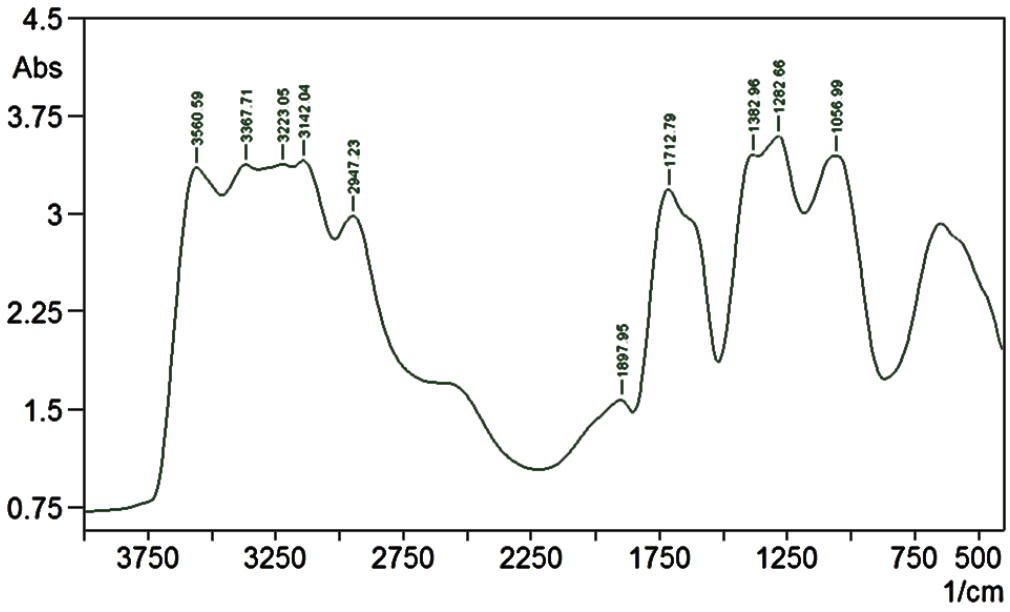


Figure 2 FT-IR spectrum of ginger lemon peel pectin

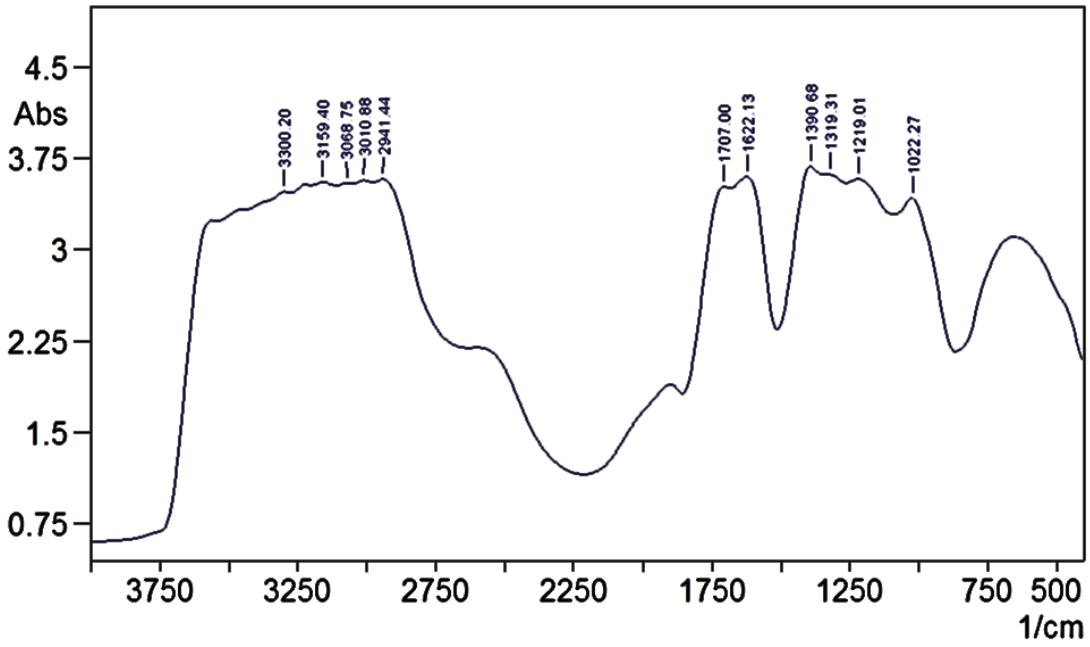


Figure 3 FT-IR spectrum of cardamom lemon peel pectin

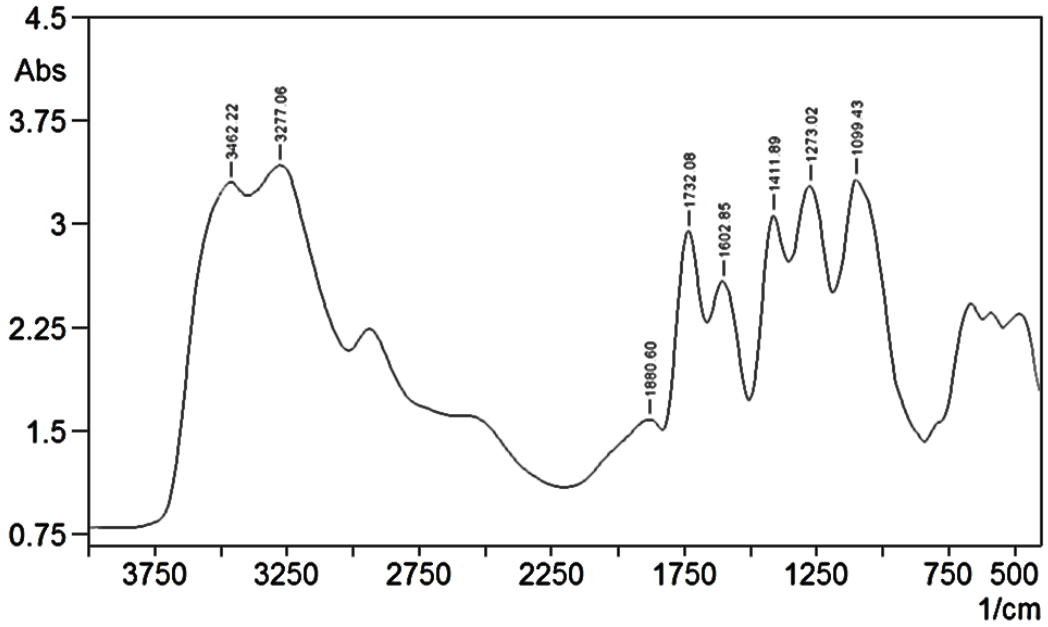


Figure 4 FT-IR spectrum of China lemon peel pectin

Table 4 Assignments of FT-IR wavenumbers in the range 4000–400 cm^{-1} of pectin samples

FTIR wave numbers (cm^{-1})			Assignments
Ginger lemon pectin	**Cardamom lemon pectin**	**China lemon pectin**	
3142.04 to 3560.56	3159.40 to 3300.20	3277.06 to 3462.22	O – H
2947.43	2941.44	2952.37	C – H
1712.79	1707	1732.08	C = O from methyl esterified carboxyl groups
1640.23	1622.13	1602.85	C = O from free carboxyl groups
800 to 1200	800 to 1200	800 to 1200	Finger print

3.11 Analysis of pineapple jelly

The physicochemical compositions of pineapple jellies were analyzed and results are reported in Table 5.

Table 5 Physicochemical constituents of pineapple jelly

Parameter	A	B	C	D
Moisture content (%)	13.34 ± 0.38^{a}	13.37 ± 0.41^{a}	14.06 ± 0.23^{a}	18.49 ± 0.45^{b}
Ash content (%)	1.5 ± 0.11^{c}	2.63 ± 0.56^{d}	1.19 ± 0.22^{b}	0.81 ± 0.01^{a}
TSS (%)	66 ± 0.5^{a}	67 ± 1^{b}	71 ± 0.5^{c}	68 ± 0.5^{b}
pH	3.40 ± 0.31^{a}	3.29 ± 0.17^{a}	3.46 ± 0.23^{a}	3.22 ± 0.15^{a}
Titratable acidity (%)	1.09 ± 0.06^{c}	1.02 ± 0.09^{b}	0.91 ± 0.03^{b}	0.79 ± 0.07^{a}

Note: [1] A = Pineapple jelly from ginger lemon pectin, B = Pineapple jelly from cardamom lemon pectin, C = Pineapple jelly from china lemon pectin, D = Commercial Pineapple jelly

[2] Results are presented as mean ± SD (Three determinations). Mean followed by different superscript letters in each row are significantly different ($p < 0.05$)

[3] Moisture content (%) is expressed in wet basis

3.11.1 Moisture and ash content

The moisture contents of pineapple jellies from three pectin samples and commercial jelly was ranged between 13.34 ± 0.38% to 18.49 ± 0.45% and the moisture content of commercial jelly was significantly higher than other pineapple jellies ($p < 0.05$) (Table 5). High moisture content was important factor affecting the flavor of product (Akubor, 1996). Low moisture contents of the jelly samples indicate the high amount of pulp used in jelly preparation.

The ash contents of pineapple jellies from the pectin samples and commercial pineapple jelly were between 1.5 ± 0.11% to 2.61 ± 0.08% (Table 5). Here, the ash content of each pineapple jellies was significantly different ($p < 0.05$) (Table 5). Ash content indicate cumulative amount of mineral present in food (Ahmmed *et al*., 2017). The high amount ash content was indicating higher amount of pulp used in jelly preparation. Although the jellies were made from the same juice the ash content were different for each sample. This might be due to unequal distribution of pulp during jelly preparation.

3.11.2 TSS content

TSS values of pineapple and commercial jellies were ranged between 66 ± 0.5% to 71 ± 0.5% (Table 5). Total soluble solids (TSS) content was directly related to both the sugars and fruit acids as these were the main contributors (Ahmmed *et al*., 2017). It was possible to had gel formation at 60% solids, by increasing the pectin and acid levels but an optimum TSS was found 67.5% (Desrosier, 1977).

3.11.3 pH determination

The pH of pineapple jellies were ranged between 3.22 ± 0.15 to 3.40 ± 0.31 (Table 5) and the values are approximate to the standard value of 3.4 (Ahmmed *et al*., 2017). The pH was low due to use of acidic fruit and citric acid. The low pH value of commercial jelly was due to the high amount of citric acid used during preparation. An optimum pH condition was found near 3.2 for gel formation (Desrosier, 1977). The overall range of pH was 2.0 to 5.0 for common fruits with the most frequent figures being between 3.0 and 4.0 (Ahmmed *et al*., 2017).

3.11.4 Titratable acidity

The titratable acidity of the produced pineapple jellies and commercial pineapple jelly were ranged between 0.79 ± 0.07% to 1.09 ± 0.06% (Table 5). These values are comparable to the values of orange jelly (1.0752%) and pineapple jelly (1.12%) (Ahmmed *et al*., 2017).

3.11.5 Sensory evaluation of pineapple jellies

The mean scores for color, flavor, texture and overall acceptability of pineapple jellies and commercial pineapple jelly were given in Table 6. The first judgment was done by visual inspection. Table 6 represented the mean scores obtained for color for all samples. Among these samples, sample B and D (commercial pineapple jelly) scored subsequently, which were aesthetically best. Sample A and C got low scores, which was aesthetically bad.

Samples B and D had pleasant aroma and characteristic flavor of the product, free from any objectionable smell or odor. Sample A and C had a low score and sample B and D got high scores (Table 6), which make samples B and D aesthetically best. All scores except sample C and D were within acceptable limit. Several factors could influence the flavor of the jelly samples such as cooking period, fruit maturity, fruit variety and fruit content (Ahmmed *et al*., 2017).

The texture of jellies were depended upon the quality of pectin used and sugar content (Ahmmed *et al*., 2017). Table 6 represented that, sample D got the highest score with sample B having an acceptable score. But sample A and C scored low compared to sample B and D. The overall acceptability of sample D was most preferred although sample B got a good overall acceptability and they are significantly better than samples A and C ($p < 0.05$). Sample C had the lowest acceptability among all samples because of the color, flavor and texture was not as good as sample D (Table 6).

Table 6 Sensory evaluation hedonic scores of pineapple jelly

Samples	Sensory attributes			
	Color	**Flavor**	**Texture**	**Overall acceptability**
A[1]	4.4 ± 1.08[b]	4.0 ± 0.94[a]	4.0 ± 0.94[b]	4.2 ± 0.79[a]
B	6.8 ± 0.79[c]	6.9 ± 0.88[b]	6.4 ± 0.84[c]	6.6 ± 0.84[b]
C	3.6 ± 0.84[a]	4.1 ± 1.2[a]	3.1 ± 0.74[a]	3.8 ± 0.79[a]
D	7.3 ± 0.48[c]	6.6 ± 0.7[b]	7.4 ± 0.97[d]	7.1 ± 0.84[b]

Note: [1] A = Pineapple jelly from ginger lemon pectin, B = Pineapple jelly from cardamom lemon pectin, C = Pineapple jelly from china lemon pectin, D = Commercial Pineapple jelly.

[2] Results are presented as mean ± SD (Ten determinations). Mean followed by different superscript letters in each row are significantly different ($p < 0.05$).

4. Conclusion

In this research pectin has been extracted from the peels of three different varieties of *Citrus limon* L. by means of environment friendly organic tartaric acid through alcoholic precipitation in an easily applicable way. The yield of extracted pectin from the different local varieties of *Citrus limon* L. showed that China lemon had highest amount of pectin yield while ginger lemon had the lowest. However, cardamom lemon had the highest esterification value. Therefore, cardamom lemon pectin can be categorized as high methoxyl pectin, while ginger lemon pectin and China lemon pectin can be categorized as low methoxyl pectin. They can also be classified as highly pure pectin according to their high AUA content and low ash content. Moreover, FT-IR spectroscopy was used for the confirmation of structural characterization of pectin and also no significant difference was found in the pectin structure. A sensory evaluation of pineapple jellies developed from the extracted pectin samples and commercial pineapple jelly was also performed. The sensory evaluation shows that the commercial jelly was highly acceptable along with pineapple jelly produced from cardamom lemon pectin. However, the pH values of all samples were satisfactory which is essential for the feasibility of the jellies. Therefore, from the above discussion it can be stated that the cardamom lemon peel may be a valuable source of pectin and furthermore the extracted pectin could be used as a functional food ingredient in the food industry.

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