



## Optimized Extraction Condition and Characterization of Pectin from Kaffir Lime (*Citrus hystrix*)

Ranajit Kumar Shaha\*, Yoga Nayagi A.P. Punichelvana, and Asrul Afandi

Faculty of Agro Based Industry, University Malaysia Kelantan, Beg Berkunci No.100, 17600 Jeli, Kelantan, MALAYSIA

Available online at: [www.isca.in](http://www.isca.in)

Received 25<sup>th</sup> September 2012, revised 1<sup>st</sup> December 2012, accepted 3<sup>rd</sup> January 2013

### Abstract

The present study was focused on the potential of Kaffir lime (*Citrus hystrix*) peel to be a source of pectin. Kaffir lime belongs to the citrus family and has a thicker skin which can be used as a source of pectin. Pectin was extracted from Kaffir lime peel using three different acids (citric, hydrochloric or nitric) and at three different temperatures (45, 65 & 90°C). Also, sun-dried and microwave-dried peels were studied at the same extraction time 1 hour at 1.5 pH. Pectin yields varied from 10.4% to 59.30% for sun dried peel while for microwave dried peel was 25.9% to 61.80%. The best extraction condition is using citric acid at 90°C using microwave-dried peels. The isolated pectin contained 12.40% moisture, 85.07% anhydrouronic acid (AUA), equivalent weight at 234.742, 1.78% methoxyl content and 11.86% degree of esterification. Purification using alcohol precipitation procedure (APP) was sufficient to yield pectin of high purity. This is supported by the content of AUA which is higher than 65% for most of the pectin isolates. According to the values of methoxyl content and degree of esterification, pectin isolated from Kaffir lime peels can be classified as low methoxyl pectin and are of potential use in manufacture of low sugar products such as low sugar jam and jellies.

**Keywords:** Kaffir lime peel, pectin, sundried, microwave dried.

### Introduction

Pectin's are biopolymers found in the primary cell walls of numerous living plant cells and have been widely studied<sup>1-3</sup>. Pectin is found in most plants, but is most concentrated in citrus fruits (oranges, lemons, grapefruits) and apples. Currently, commercial pectin's are almost utterly derived from citrus peel or apple pomace, both by-products from juice (or cider) manufacturing. According to the literature, fresh weight of plant material accomplishes 0.5-4.0% of pectin substances<sup>4,5</sup>. The structure of pectin is very difficult to determine because pectin can change during isolation from plants, storage, and processing of plant material<sup>6</sup>. According to Levigne *et al.*<sup>7</sup>, pectins are methylated ester of polygalacturonic acid that contains 1,4-linked  $\alpha$ -D-galacturonic acid residues. Yeoh *et al.*<sup>8</sup> had reported that these polysaccharides consist of 300-1000 chains of galacturonic acid units. High methoxyl (HM) pectins have more than half the carboxyl groups as methyl esters and form gels in the presence of high sugar concentrations and acid (e.g. jams, jellies, marmalades). Low methoxyl (LM) pectins have less than half the carboxyl groups as methyl esters<sup>9</sup>.

Lemons, oranges and grapefruits are pectin rich fruits that may help decrease cancer tumor formation<sup>10</sup>. According to Memorial Sloan-Kattering Cancer Centre, the citrus pectin acts as a ligand for galectin-3, a protein involved in cell growth and cell cycles. Elevated galectin-3 is associated with inflammation of the heart and cancer tumours. Institute, N.B.<sup>11</sup> and Walding<sup>10</sup> had reported that pectin supplementation in the diet forbids excess galectin-3 from binding to receptors that

might result in the spread of cancer cells through angiogenesis or blood vessel growth. Pectin is usually added in jams and jellies as a gelling agent. It has also been used as a fat substitute in spreads, ice-cream and salad dressings. Liu *et al.*<sup>12</sup> reported that in terms of nutrition, pectin has been shown to lower blood cholesterol levels and low-density lipoprotein cholesterol fractions, which is beneficial for human health. Studies have shown that the content and degree of etherification of mango peel pectin's to range from 12% to 21% and from 56% to 66% respectively<sup>13</sup>. Furthermore, mango peels have been shown to be a rich source of flavonol and benzophenone derivatives<sup>13</sup>. Rehmann *et al.*<sup>14</sup> extracted pectin from mango peels with sulphuric acid, and the maximum yield was obtained at 80°C and pH 2.5 with an extraction time of 120 minutes. Virk and Sogi<sup>15</sup> studied pectin extraction and characterization from apple peel waste and revealed that citric acid was more effective than hydrochloric acid. Scemin *et al.*<sup>16</sup> carried out a practical follow-up to pectin extraction from apple pomace and observed that the pectin yield was higher with 6.2g per 100mL of citric acid and a reaction time around 150 min.

Kaffir lime (*Citrus hystrix*) fruit is about the size of a western lime. The fruit is dark green and round, with a distinct nipple on the stem end. It has a thick rind, knobby and wrinkled, and one of its common names is 'porcupine orange'. As the fruit becomes older, the colour fades to a lighter, yellowish green. All parts of the plant are strongly aromatic. Especially the sometimes pungent aroma of the leaves is appreciated.



Picture-1  
Kaffir lime

Though the juice is infrequently used in cooking, the zest of the rind is often used for making curry pastes. The lime belongs to the family *Rutaceae* and genus *Citrus*. Originally the Kaffir Lime was considered inferior to other limes until the unique flavour of its oil in the leaves and in the zest was discovered. Since it belongs to the genus *Citrus*, it is believed that the peel contains a wholesome amount of pectin which fewer studies have reported. Kaffir lime peel could be included in the list of fruits naturally possessing relatively large amount of pectin provided that the best extraction method is known. Kaffir lime being one of the notable citrus fruit has limited research reporting of the pectin content in its peel. It is highly notable that the peel, twig and leaves of the Kaffir lime are used to extract citrus oil which is used majorly by the food industry. The Kaffir lime peels, if treated as waste materials, may create environmental problems, particularly water pollution, since the presence of biomaterials in Kaffir lime peels such as peel oil, pectin, as well as sugar, stimulate aerobic bacteria to decompose the biodegradable organic matters into products such as carbon dioxide, nitrates, sulfates and phosphates in water. Suitable methods have to be adopted to utilize them for the conversion of the problem into an asset. This is achievable if potentially marketable bioproducts such as pectin could be extracted from these peels. Now in the present study a method of extraction of pectin from citrus fruit wastes-Kaffir lime under different processing conditions viz. drying procedure, temperature and different acidic extracting solvent which are evaluated to achieve potential economic value of pectin.

## Material and Methods

**Sample collection:** Mature Kaffir limes were collected from the Kota Bharu, fruits market, Kelantan, Malaysia. The Kaffir limes were peeled to discard the pulp. They were cut into small pieces and were treated in absolute ethanol for 30 minutes. Then, they were washed with water and were pressed under hand pressure (made in wood) to remove excess water. The pressed peels were subjected to two types of drying namely sun drying and microwave. For sun drying, the peels were exposed in the sunlight for approximately 8 hours in an open place. For the peels subject to microwave drying, they were exposed at 0.45kW for 10 minutes. Before drying, the peels were weighed and readings were again taken after drying. The dried peels

were grinded and packages in air tight polyethylene bags and stored in a dedicator.

**Extraction of pectin:** The extraction procedure was based on method given by Kratchanova M. et al<sup>17</sup>, considering several variables. 5g of the peel of the dried Kaffir lime ground was weighed into a trade 1000mL beaker which contains 200mL of distilled water. Then, 1.2g of ground sodium hexametaphosphate was added and the pH was adjusted to 1.5 with hydrochloric acid (0.5N), nitric acid (0.5N) or citric acid respectively. Thereafter, the mixture was heated while stirred continuously to 45, 65 or 90°C in a stirring hot plate for 60 minutes. Next, the pH was checked at intervals of 15 minutes and the water loss was replaced except for the last 20 minutes. The hot acid extract was filtered through filter funnel equipped with two-layer of muslin cloth. For each acid, three different ranges of temperature extract collected separately for further experiments. The filtrate was cooled to 4°C.

**Purification Determination Procedure**<sup>18</sup>: Pectin-containing aqueous extract was coagulated by using an equal volume of 95% ethanol at 4°C and was left for 1 hour. The precipitate (ethanol-insoluble fraction) formed was recovered through centrifugation and filtration, was washed with 55% and then with 70% ethanol. The pectin yield was calculated using equation 1.

$$y_{pec}(\%) = Y = 100 \times \left[ \frac{P}{B_i} \right] \quad (1)$$

Where  $y_{pec}$  is the extracted pectin yield in percent (%), P is the amount of extracted pectin in g and  $B_i$  is the initial amount of ground lime peel (5g).

**Analysis and Characterization of Pectin: Moisture:** 1 g of sample was weighed in desiccators and was then dried in oven 4 hour at 100°C. Then, the sample in desiccators cooled over silica gel. Percent moisture observed is added (1%) to obtain agreement with the Fischer method<sup>19</sup>.

**Equivalent Weight:** Equivalent weight is used to calculate the anhydrouronic acis content and the degree of esterification. It is determined by titration with sodium hydroxide to pH 7.5 using either phenol indicator<sup>20</sup>. 0.5g of pectin substances (ammonia- and ash-free) was weighed into a 250-ml conical flask. Then, it was diluted with 5ml ethanol. 1g of sodium chloride was added to sharpen the end point. 100ml of deionised water was added together with six drops of phenol red or Hinton's indicator. All the pectin substances are made sure to have dissolved. Then, the solution was titrated slowly with 0.1N NaOH until the colour of the indicator changes (pH: 7.5)<sup>20</sup>. The neutralized solution was used for methoxyl determination.

**Calculation:** Equivalent weight

$$\frac{\text{Weight of sample} \times 1000}{\text{ml of alkali} \times \text{Normality of alkali}} \quad (2)$$

**Methoxyl Content:** The methoxyl (MeO) contents were determined by adding 25 ml of 0.25 N NaOH to the neutral solution, mixed thoroughly, and was allowed to stand for 30 minutes at room temperature in a Stoppard flask. 25 ml of 0.25N HCl was added and titrated with 0.1N NaOH to the same end point as before<sup>21</sup>.

$$\text{MeO \%} = \frac{Z}{31} \quad (3)$$

Where 31 is the molecular weight of methoxyl

**Anhydrouronic acid (AUA):** When the equivalent weight and methoxyl content of pectin is known, its AUA was calculated as follows<sup>20</sup>.

$$\% \text{ AUA} = \frac{\text{meq of Sodium Hydroxide} \times 31 \times 100}{176 \times \frac{\text{weight of sample (mg)}}{Z}} \quad (4)$$

Where, 176 is the molecular weight of AUA and

$$Z = \frac{\text{weight of sample (mg)}}{\text{Mequivalent of alkali for free acid} + \text{meq of alkali for methoxyl}} \quad (5)$$

**Degree of esterification:** The degree of esterification (DE) of pectin was determined according to the formula given below<sup>22</sup>.

$$\% \text{ DE} = \frac{176 \times \text{CH}_3\text{O \%} \times 100}{31 \times \text{AUA \%}} \quad (6)$$

Where CH<sub>3</sub>O is % methoxyl content.

## Result and Discussion

**Preparation of peel:** The peel was pre-treated with absolute ethanol for 30 minutes. The reason is because ethanol peel pre-treatment improves purity by the removal of ethanol-soluble components, including sugars, and inactivates enzymes without leaching soluble pectin fractions. The peel was cut into the smallest size as the effect of size of the particles of the raw material had been studied by Canteri-Schemin M.H. et al<sup>23</sup>. The pectin yield was reported to be significantly higher with the use of flour as raw material (9.73 %); when the extraction was made from the pomace the yield was 6.13%. It proved that protopectin is more available in small particles than in large ones.

**Moisture:** Table 1: contains the wet and dry weight of the Kaffir lime dried under sun and microwave respectively. It turns to be that sun drying gave a significant moisture loss compared to microwave drying (table-1).

**Table-1**  
**Weight distribution of peel**

Type of drying	Wet weight (g)	Dry weight (g)	% Dry weight
Sun drying	27.008	3.891	85.589 ± 0.0935
Microwave drying	27.572	4.697	82.962 ± 0.0568

The loss is directly influenced with the exposure intensity (37-45°C for sun drying and 0.45kW for MW) and time (8 hours for sun drying and 10 minutes for MW). The standard deviation value of MW drying (0.058) is lesser compared to the sun

drying (0.0935). The consistency in drying is much higher in MW drying compared to sun drying. This could be due to the fact that the peels are exposed to a fixed exposure intensity and time compared to sun drying.

Apart from that, the size of the peel exposed to the drying have to be made sure to be at the smallest size to allow quicker water loss. For that, the size of the cut peel was ensured to be at the same size for both sun drying and MW drying.

**Characterization of pectin: Colour:** Colour of pectin is important as it affects the appearance of the gel produced. The lighter colours having little effect on the final appearance of a food product would be preferred. The dried pectin derived from sun dried peel had light brown colour whereas the dried pectin derived from the MW drying had brown colour (refer Picture-2 and 3).



**Picture-2**

**Dried pectin from sun dried peel**



**Picture-3**

**Dried pectin from microwave dried peel**

The pectin's from some sources investigated by Mohamed S. et al<sup>24</sup> showed that musk lime had an off white colour pectin. Highly coloured pectin may have the presence of polyphenols or other water soluble pigments trapped inside the pectin during precipitation. It was suggested that better filtration techniques using a filter aid, activated carbon, diatomaceous earth, etc may remove these pigments to improve the colour of pectin extracted.

**Moisture:** Table-2 shows the considerable amount of moisture available in the pectin before they were subjected to drying at 50°C to a constant weight. For sun dried peel, the pectin has moisture content which ranges from 94.64% to 97.55%. In contrary, pectin from the microwave dried peel had a more stable moisture loss which ranges at 94.35% to 96.90%.

For sun dried peel, the moisture content of the pectin ranges from 9.6% to 17.1%. However, for microwave dried peel the moisture content of the pectin ranges from 10.0% to 19.3% which is higher than the sun dried peel (figure -1).

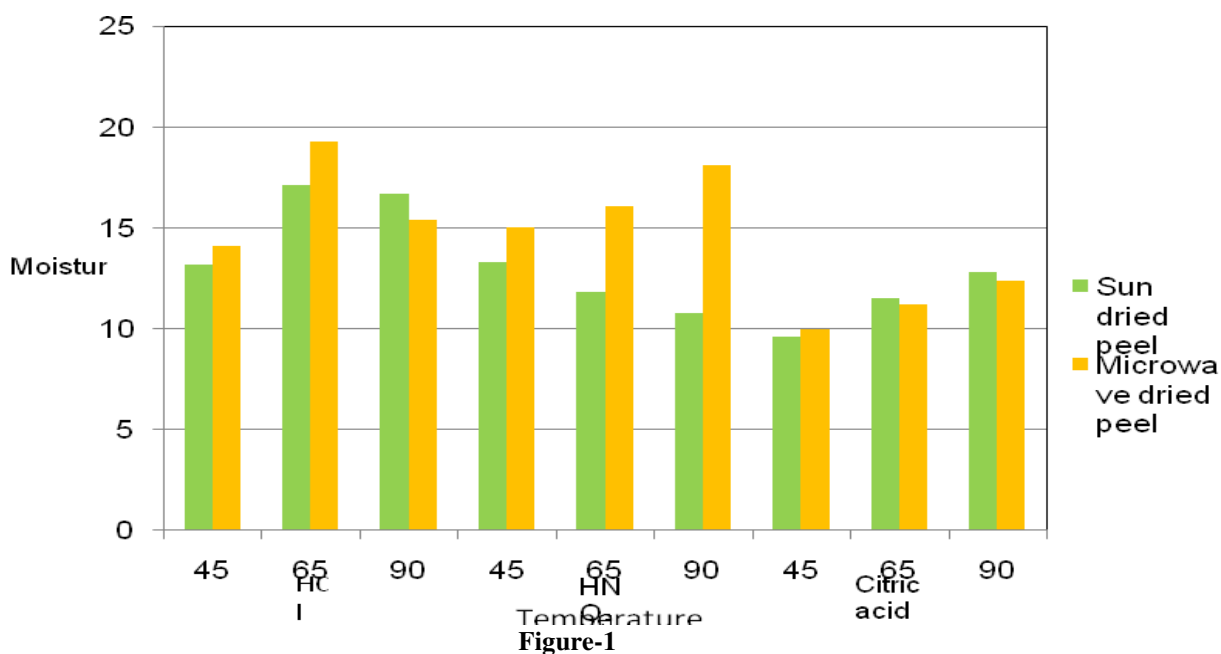
1% to the percent moisture observed was added to obtain agreement with the Fischer method<sup>19</sup>. Pectin should have as low moisture content for safe storage and to inhibit the growth of microorganisms that can affect the pectin quality due to the production of pectinase enzymes.

The peel treated under microwave show more yield in comparison to the sun dried peel. The highest yield (61.80%) is obtained from microwave dried peel using citric acid and heated at 90°C. For sun dried peel, the highest yield (41.20%) is

obtained using citric acid and heated at 90°C (figure-2). Kratchanova *et al.*,<sup>17</sup> had reported that during microwave heating, considerable pressure builds up inside the material. The high pressure then modifies the physical properties of the orange peel tissues-their subject matter, breaking down the cell structure and improving the capillary-porous structure of orange peel tissues. This feature allows better penetration of extracting solvent into the tissues, thus improving the subsequent extraction of pectin and confirming the findings of Fishman *et al.*<sup>25</sup> and Liu *et al.*<sup>26</sup>. It is obvious that the main reason for the increased yield in the samples subjected to MW-heating was the improved capillary-porous characteristics of the raw material. This ensured better and faster permeation of the extracting agent.

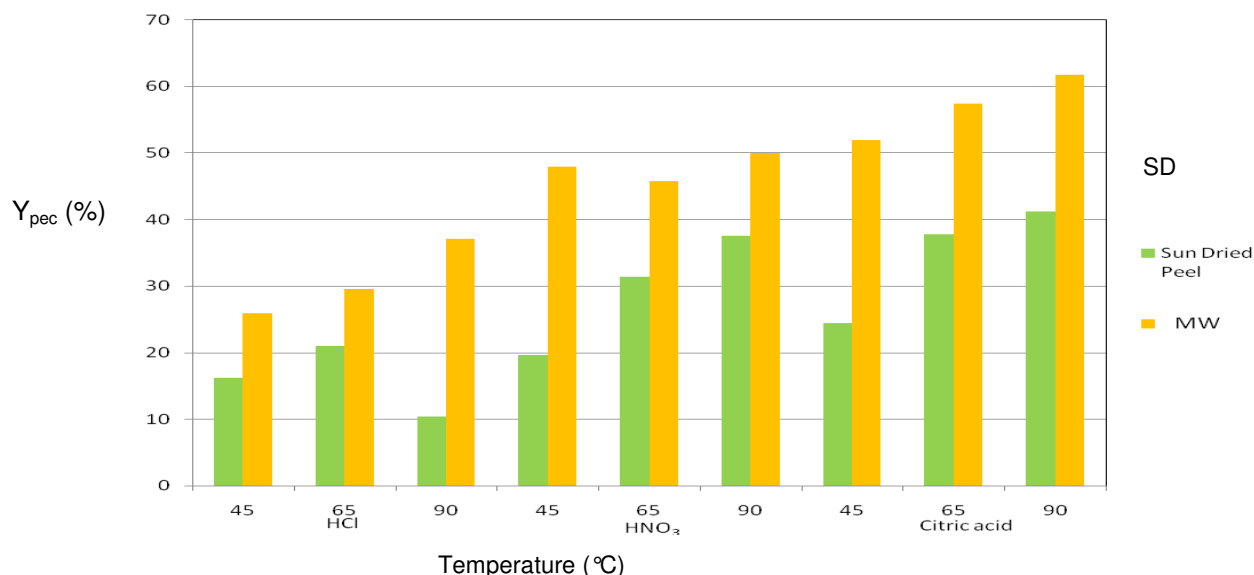
**Table-2**  
**Moisture content of pectin upon drying to constant weight**

		Sun dried peel Moisture (%)	Microwave dried peel Moisture (%)
HCl	45	96.77	96.77
	65	97.41	97.41
	90	97.23	97.23
HNO <sub>3</sub>	45	94.64	94.64
	65	95.11	95.11
	90	96.63	96.63
Citric acid	45	97.55	97.55
	65	95.26	95.26
		97.09	97.09



**Figure-1**  
**Comparison of moisture content in pectin between different extracting conditions**





**Pectinyield**

**Figure-2**  
**Comparison between peel drying, different temperature and extracting acid to pectin yield**

Data from oranges of the Navel 1996 and Navel 1997 type confirm the observation in previous studies<sup>27</sup> that there was an inverse correlation between the field power and duration of exposure mainly expressed for 0.45 and 0.63 kW power. In case of weaker field, longer microwave treatment is needed. Duration of acceptable microwave heating was reduced for the higher values of the field power because of burning of the material. Kratchanova *et al.*<sup>27</sup> had reported that the pretreatment of the fruit material by microwave heating led to a considerable increase in the yield and quality of pectin. These results were confirmed by experimental data obtained by Ilina *et al.*<sup>28</sup>, Kohg *et al.*<sup>29</sup> and Fishman *et al.*<sup>30</sup> during investigations into the microwave treatment of citrus peels and apple pomace.

It was determined by Kratchanova *et al.*<sup>17</sup>, that with an increase of the intensity of the field and duration, the pectin yield increased between 180 and 240% compared with the control. At higher field intensities, however, prolonged period of microwave heating led to a decrease in gel strength. That implied that optimization of the microwave heating conditions is needed for each particular case of microwave treatment of the pectin material. A study carried out by Kratchanova *et al.*<sup>17</sup> revealed that the measurement of the pectinesterase activity in the dry orange peels showed that a 10-minute heating in a MW field with a power of 0.45 kW led to a considerable inactivation. Also, prolonged exposure in microwave leads to a complete suppression of the pectolitic activity in the orange peel under study. Thus, the peels were dried under MW at optimum condition-0.45kW for 10 minutes.

Citric acid was the best for the extraction of pectin. This is in agreement with the results reported by Virk and Sogi<sup>15</sup> and

Schemin *et al.*<sup>16</sup> who had compared the yields of pectin extracted from apple with different acids-hydrochloric acid, nitric acid and citric acid. Between the two strong acids, it was observed that there was no great difference in pectin yield regardless of the effects of nitric acid being slightly greater than those of hydrochloric acid. Even though a low pH is necessary to improve the yield, the strong acid solution could lead to smaller pectin particles owing to partial hydrolysis. Consequently, pectin solubility would increase to the point that no precipitate was formed by the addition of alcohol. As reported by Kalapathy and Proctor<sup>31</sup> this could be the reason why the use of a stronger acid resulted in a lower pectin yield. Yap<sup>18</sup> had reported that by using citric acid, nitric or sulphuric acid extractant, it has been shown that acid type strongly influences the macromolecular and gelling properties of isolated pectins; with citric acid being the least pectin degrading (depolymerising and deesterifying) extracting agent. Therefore, it leads to pectin isolates with the best gelling properties.

Extreme of high temperature and extraction time would lead to decomposition of pectin since pectin is composed of  $\alpha$ -(1, 4) linked units of galacturonic acid or methyl ester. Yujaroen *et al.*<sup>2</sup> had reported that the glycosidic bond is an ether bond that can go through hydrolysis reaction at the right conditions (80°C at pH 2, or at pH 8 for two hours). In this case, it is considered that by hydrolysis of high polymer of pectin molecules to low polymer leads to an increase of solubility in water, which makes it more difficult to separate pectin as a solid compound by the addition of ethanol. In the study carried out by Woo *et al.*<sup>3</sup>, pectin yield increased initially but declined after 60 minutes of extraction. The decrease in pectin yield by the increase in extraction period may be due to the thermal degradation of the

extracted pectin. The degradation is mainly caused by the depolymerisation mechanism of galacturonan chain of pectin, which is known as beta-elimination<sup>32</sup>. Thus, the pectin cannot be recovered by precipitation with alcohol<sup>33</sup>.

The pH during extraction was maintained at 1.5. Kertes<sup>33</sup> reported that high concentration of hydrogen ions present in the solvent (at low pH) stimulates the hydrolysis of protopectin. Protopectin is a compound formed by the combination of cellulose with pectin molecules. During acid hydrolysis, the combination is split up to produce soluble pectin and cellulose by eliminating water molecules. Besides, the removals of calcium and magnesium ions do occur. As a result, protopectin becomes soluble pectin. The research of Joye D. D. et al<sup>34</sup> demonstrated that extraction under strong acidic conditions (below pH 2.0) was sufficient to extract the non-calcium sensitive pectin (NSCP) and the remaining pectin present in citrus peel, which is primarily calcium sensitive- pectin (CSP). Extraction under intermediate acidic conditions (approximately pH 3.0) was reported to extract only non-calcium-sensitive pectin.

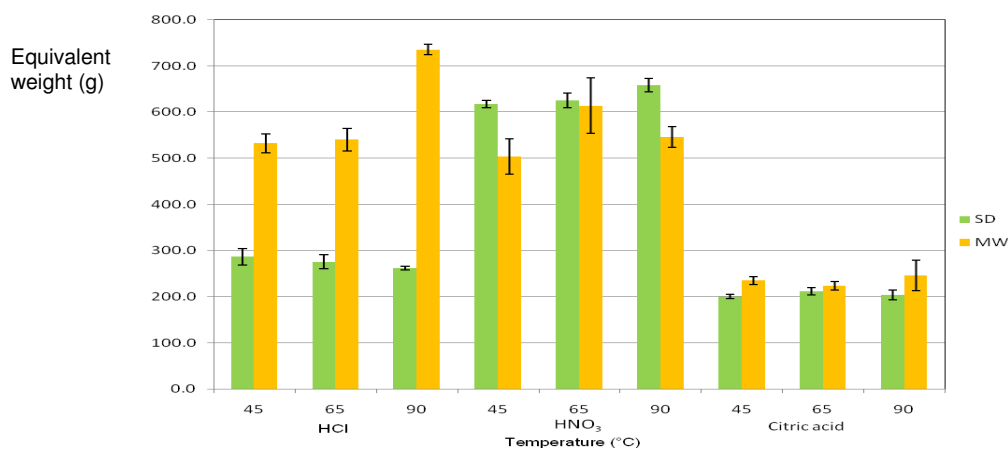
At lower pHs, the highly hydrated carboxylate groups are repressed in the larger hydrogen ion concentrations and therefore, converted into slightly hydrated carboxylic acid groups<sup>35</sup>. The lost of charge is able to reduce the repulsion of the polysaccharide molecules which promote the gelation properties of pectin giving more precipitated pectin at lower pH. Thus, the decreased in pH is able to promote the liberation of pectin molecules from the peel during acid-washing stage because of the interaction of pectins to the hemicelluloses fractions are cleaved<sup>36</sup>. Pectin yield is lesser in higher pH might be due to some pectin is still attached to the cell wall components, although pectin molecules can be partially solubilised from plant tissues without degradation by weakly acidic aqueous solvents<sup>37</sup>.

In order to improve the yield, this type of pectin constituent (protopectin) is suggested to be hydrolysed by acid. Alkaline

conditions were found by Knee<sup>38</sup> and Jarvis *et al*<sup>39</sup> to break the bonds between the pectin molecule and the cell wall in a similar manner to acidic solvents. Knee<sup>38</sup> and Jarvis *et al.*<sup>39</sup> found that substantial amounts of pectin were extracted under alkaline conditions as compared with neutral conditions. Nevertheless, alkaline conditions cause instability in the backbone of pectin molecule (galacturonic acid) and consequently, the pectin molecule tends to decompose<sup>32</sup>. Due to the decomposition of pectin molecules, the extracted pectin cannot be precipitated with alcohol. Therefore, the recovery of the extracted pectin tends to be reduced under alkaline conditions. Thus low pH is essential for higher yield that is not achievable at higher pH condition.

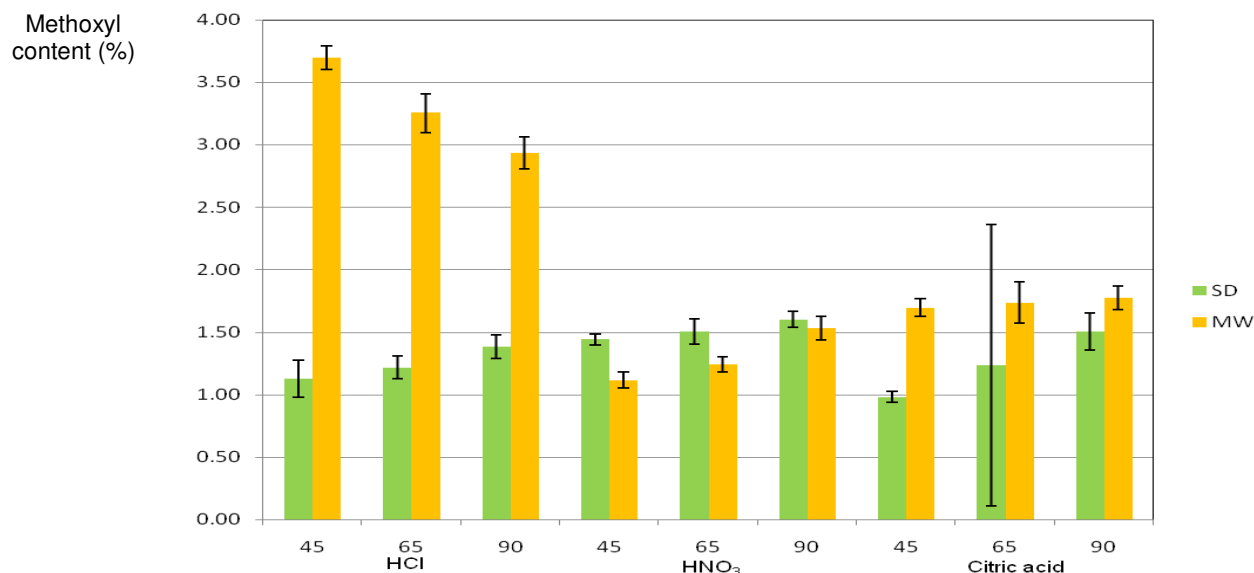
According to Adamson<sup>40</sup> the capillary pressure of the plant tissues affects penetration of solvents significantly. The capillary pressure is influenced by factors such as the surface tension between the solvent and the gas phase, the solvent contact wetting angle and the capillary radius. Because of the presence of waxes and resins on the surfaces of plant tissues, the surfaces become resistant to the solvent giving a high wetting angle. Hence the amount of extractable pectin is reduced considerably. The ethanol, as a surfactant solvent, significantly reduces the wetting angle of the plant tissues by modifying the drainage properties of the plant tissues<sup>41</sup>. Consequently, the capillary pressure of the plant tissues is increased, and this condition causes an improvement in the penetration rate of the solvent.

**Equivalent weight:** The value for equivalent weight was highest (735.4g) using microwave dried peel using hydrochloric acid at 90°C. However, the value was highest (658.1g) using sun dried peel at 90°C using nitric acid. This could not be used for comparison as the acids used are monobasic. Thus the weight of the pectin contained one equivalent of a proton (of the acid) which applies for the samples treated under different acids. The equivalent weights obtained were used in the calculations of % AUA and % DE (figure-3).

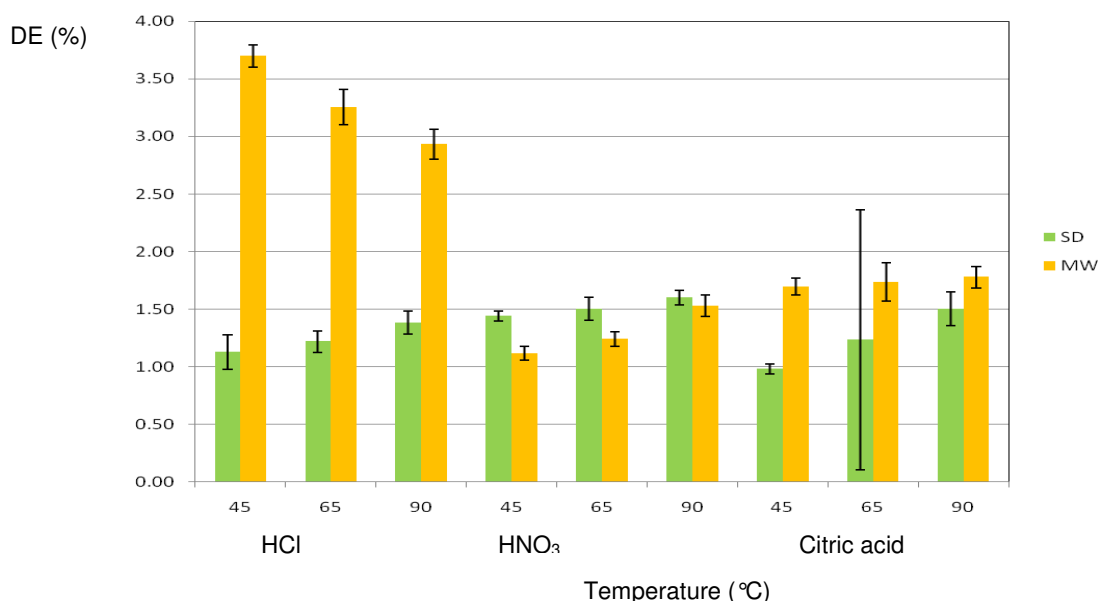


**Figure-3**  
 Comparison between peel drying, different temperature and extracting acid to equivalent weight

**Methoxyl Content**



**Figure-4**  
 Comparison between peel drying, different temperature and extracting acid to methoxyl content



**Figure-5**  
 Comparison between peel drying, different temperature and extracting acid to degree of esterification

Methoxyl content is an important factor in controlling the setting time of pectins, their combining power with metallic ions and the ability of the pectin to form gels. The methoxyl content was highest ( $3.70 \pm 0.09\%$ ) using microwave dried peel at  $45^\circ\text{C}$  using hydrochloric acid. For sun dried peel, the value was highest ( $1.60 \pm 0.07$ ) using nitric acid at  $90^\circ\text{C}$  but the value is not as higher as in former (figure-4).

**Degree of esterification (DE):** The Kaffir lime pectin produced in this study can be categorized as low methoxyl pectin (LMP) because it has a %DE that is lower than 50% (figure-5) and methoxyl content between 0.17 and 2.42% for sun dried peel while for microwave dried peel between 1.05 to 3.78%. The types of pectin determine the mechanism for gel formation. LMP can form gels with the addition of low amount of sugar or without sugar in divalent cations. There can be an extensive

range of DEs dependent on species, tissue, and maturity. In general, tissue pectins range from 60 to 90% DE.

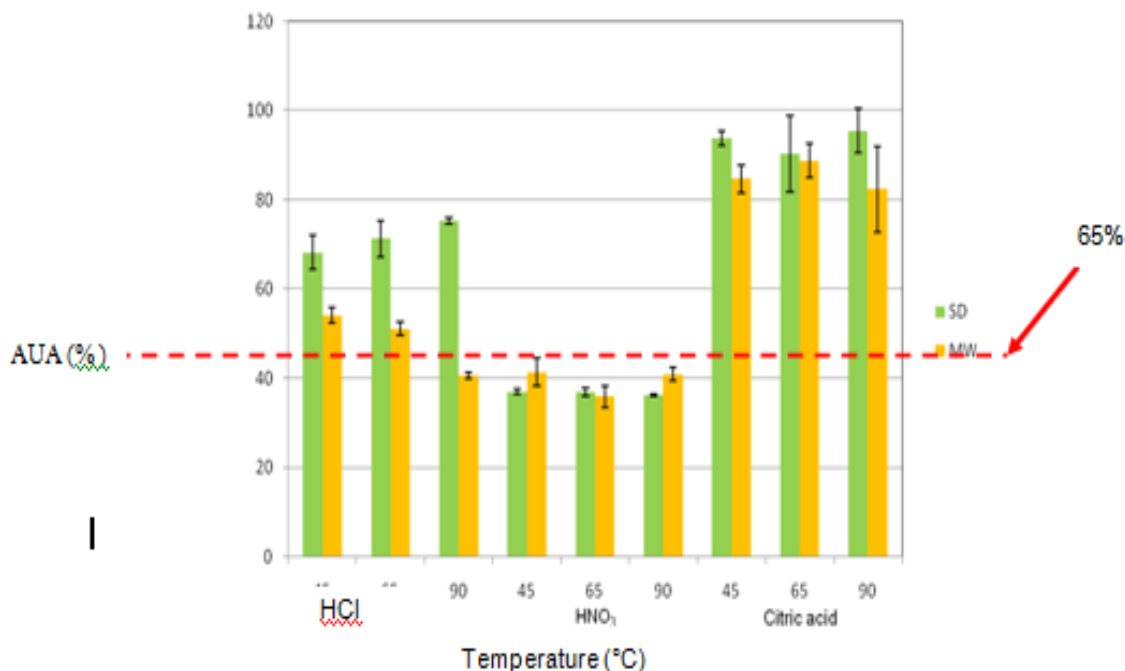
LMP produce gels independent of sugar content. They also are not as sensitive to pH as the HM-pectins are. LMPs require the presence of a controlled amount of calcium or other divalent cations for gelation. LMPs contain sufficient acid groups to gel or precipitate with calcium ions, although other ions such as aluminium or copper cause precipitation under certain conditions. Gel formation is caused by hydrogen bonding between free carboxyl groups on the pectin molecules and also between the hydroxyl groups of neighbouring molecules. In a neutral or only slightly acid dispersion of pectin molecules, most of the un-esterified carboxyl groups are present as partially ionised salts.

Those that are ionised produce a negative charge on the molecule, which together with the hydroxyl groups causes it to draw layers of water. The repulsive forces between these groups, due to their negative charge, can be adequately strong to prevent the formation of a pectin network. When acid is added, the carboxyl ions are converted to mostly unionised carboxylic acid groups. The decrease in the number of negative charges not only lowers the attraction between pectin and water molecules, but also lowers the repulsive forces between pectin molecules. Sugar further decreases hydration of the pectin by competing for water.

These conditions reduce the ability of pectin to stay in a dispersed state. Upon cooling, the unstable dispersing of less hydrated pectin forms a gel, a continuous network of pectin holding the aqueous solution. The rate at which gel formation takes place is also affected by the degree of esterification. A higher DE causes more rapid setting. Rapid-set pectins which are pectin with a DE of above 72% also gel at lower soluble solids and higher levels than slow-set pectins which is pectin with a DE of 58-65%.

**Anhydrouronic acid (AUA):** The content of AUA indicates the purity of the extracted pectin and is suggested to be not less than 65%<sup>42</sup>. From figure-6, only in extraction using citric acid did it give pure pectin for both type of peel. Extraction using nitric acid did not give pure pectin. Result indicates that the extract may not be sufficiently pure due to presence of sugars in the precipitated pectins.

The purification method used is alcohol precipitation procedure (APP). Yapo<sup>18</sup> had reported that metal-ion precipitation procedure (MPP) proves to be most effective method in comparison to alcohol precipitation method and dialysis method. MPP is the most efficacious for removing ash and proteins whereas APP is the least effective, which could be attributable to the poor solubility of these components, including longer neutral oligosaccharides and polysaccharides, in alcohols.



**Figure-6**  
 Comparison between peel drying, different temperature and extracting acid to AUA value



However, MPP is notably lower in terms of yields of other pectin isolates compared to APP. It shows that the total recovery of pectins from same crude aqueous acid extracts can be dependent on the employed purification process. A probable loss of neutral sugar-containing highly methyl-esterified pectin chains, following to form insoluble complexes with  $\text{Cu}^{2+}$  ions, as had been reported with  $\text{Al}^{3+}$  ions<sup>43</sup>, might also participate in the obtained lower yield of MPP. Even though MPP is the most effective purification method, it affects the pectin yield significantly. Moreover, MPP is likely to generate a large amount of effluents on an industrial scale demanding mitigating measures to avoid causing environmental damage.

In the present study, APP serves to be efficient for treatment using citric acid and hydrochloric acid but inefficient for treatment using nitric acid. It could be due to  $\text{NO}_3^{2-}$  ions might have resulted in the obtained lower yield. Also, the presence of sugars in the precipitated pectin's could possibly affect the performance of APP. APP is much more preferred than MPP because alcohol waste can be more easily managed with by recycling it.

## Conclusion

This research emphasized on pectin extraction and characterizations from Kaffir lime peel. The peels were subjected to pre-treatment with ethanol for 30 minutes before subjected to two different drying methods namely sun drying and microwave drying. In general, the research had been divided into three parts namely extraction of pectin using changing extraction condition, purification via alcohol-precipitation procedure (APP) and characterization of pectin. Based on the extraction of pectin using changing conditions, pH and extraction period was kept constant at 1.5 and 1 hour respectively. The changing conditions were different drying method for peels, different extracting temperature and different extracting solvent. The study was conducted to identify if Kaffir lime peel had the potential to yield pectin. The results indicated that different: pretreatment of drying, extracting temperature and extractants affect the extraction yield. The best condition were using microwave dried peels, extracting temperature at 90°C and using citric acid as the extracting solvent. This gave a yield of 61.80%. Purification using APP was sufficient to yield pectin of high purity. This was confirmed when the pectin's physicochemical properties (moisture, % AUA, degree of esterification, colour methoxyl content and equivalent weight) were analysed. The AUA % was mostly above 65% which indicates that the pectin is pure. Also, the pectin isolated from Kaffir lime peels can be classified as low methoxyl pectin as it demonstrated low degree of esterification and methoxyl content.

This study was intended to identify if Kaffir lime peel could be a potential source for pectin source and if there is any, the optimum conditions could be determined. From the results obtained, Kaffir lime peel gives a significant amount of pectin whereby it can be considered in commercial production of pectin alongside with other citrus sources.

**Suggestions:** The study in future should be commenced using a greater amount of dried peel so that more physicochemical properties like ash content, jelly grade, setting time and acetyl value could be studied. As there are no much studies been reported for pectin extraction using Kaffir lime, this study in future could serve as a preliminary study to evaluate Kaffir lime peels' capacity for pectin source. Faravesh et al.,<sup>4</sup> and Pagan *et al.*,<sup>44</sup> had reported that prolonged extraction time will lead to higher pectin quality under a constant pH and temperature. Therefore, it could be assumed that extraction time of 120 minutes favoured the extraction of high-quality pectin and would be the most suitable extraction time for obtaining pectin with high quality.

Besides, other factors such as the addition of alcohol during extraction are believed to affect DE. Alcohol is believed to change the thermodynamic condition of monophasic system into two-phase gel-liquid system such as the interaction between water molecules, carboxylic groups of pectin and alcohol functional groups<sup>45</sup>. Yeoh et al.,<sup>8</sup> had reported that solvent systems containing ethanol and EDTA both extracted approximately twice the amount extracted using distilled water. Besides, it would be more advantageous to pave the way for alcohol-precipitation with an industrially-practical membrane procedure such as ultrafiltration-diafiltration using membranes of sufficiently great nominal molecular weight cut-offs for an effective removal of pectin contaminants (ash, free neutral and protein components, etc.), thereby improving the compositional quality and possibly gelling properties of the final pectin products<sup>18</sup>.

## References

1. Chakraborty A. and Ray S., Development of a Process for the Extraction of Pectin from Citrus Fruit Wastes viz. Lime peel, Spent Guava Extract, Apple Pomace etc. *Internet Journal of Food Safety*, **13**, 391-397 (2011)
2. Yujaroen P., Supjaroenkul U. and Rungrodnimitchai S., Extraction of Pectin from Sugar Palm Meat, *hammasat International Journal of Science and Technology*, **13** Special Edition, 44-47 (2008)
3. Woo K.K., Chong Y.Y., Li Hiong S.K. and Tang P.Y., Pectin Extraction and Characterization from Red Dragon Fruit (*Hylocereus polyrhizus*): A Preliminary Study, *Journal of Biological Sciences*, **10(7)**, 631-636 (2010)
4. Faravash R.S. and Ashtiani F.Z., The influence of acid volume, ethanol-to-extract ratio and acid-washing time on the yield of pectic substances extraction from peach pomace, *Food Hydrocolloids*, **22**, 196-202 (2008)
5. Kashyap D.R., Vohra P.K., Chopra S. and Tewari R., Applications of pectinases in the commercial sector: A review, *Bioresour. Technology*, **77**, 215-227 (2001)
6. Novosel'skaya I.L., Voropaeva N.L., Semenova L.N., and Rashidova S.S., Trends in the Science and Applications of Pectins, *Chemistry of Natural Compounds*, **36**, 1 (2000)

7. Levigne S., Ralet M.C. and Thibault J.F., Characterization of pectins extracted from fresh sugar beet under different conditions using an experimental design, *Carbohydrate Polymers*, **49**, 145-153 (2002)
8. Yeoh S., Shi, J., T.A.G. Langrish, T.A.G., Comparisons between different techniques for water-based mextraction of pectin from orange peels, *Desalination*, **218**, 229-237 (2008)
9. Fellows P., *Food Processing Technology: Principles and practise*, Cambridge: Woodhead Publishing Limited (2009)
10. Walding A., Pectin-Rich Fruits, *Livestrong.Com*. (2011)
11. Institute N.B., Pectin Power: Why Fruits And Vegetables May Protect Against Cancer's Spread, *Science Daily* (2011)
12. Liu Y., Shi, J. and Langrish T.A.G., Water-based extraction of pectin from flavedo and albedo of orange peels, *Chemical Engineering Journal*, **120**, 203-209 (2006)
13. Berardini N., Schieber A. and Carle R., Characterization of gallotannins and benzophenone derivatives from mango (*Mangifera indica L. Cv. 'Tommy Atkins'*) peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry, *Rapid Communications in Mass Spectrometry*, **18**, 2208-2216 (2004)
14. Rehmann Z.U., Salatiya A.M. and Shah W.H., Utilization of mango peels as a source of pectin, *Journal of the Chemical Society of Pakistan*, **26**, 73-76 (2004)
15. Virk, B.S. and Sogi, D.S., Extraction and characterization of pectin from apple pomace (*Malus Pumila Cv Amri*) peel waste, *International Journal of Food Properties*, **7**, 1-11 (2004)
16. Schemin M.H.C., Fertoni H.C.R., Waszczyński N. and Wosiacki G., Extraction of pectin from apple pomace, *Brazilian Archives of Biology and Technology*, **48**, 259-266 (2005)
17. Kratchanova M., Pavlova E. and Panchev I., The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin, *Carbohydrate Polymers*, **56**, 181-185 (2004)
18. Yapo B.M., Pectin quantity, composition and physicochemical behaviour as influenced by the purification process, *Food Research International*, **42**, 1197-1202 (2009)
19. Johnson C.M., *Ind. Eng. Chem., Anal. Edn.*, **17**, 312 (1945)
20. Owens H.S., McCready R.M., Shepherd A.D., Schultz S.H., Phippen E.L., Swenson H.A., Miers J. C., Erlandsen R.F. and Maclay W.D., *Methods used at Western Regional Research Laboratory for Extraction and Analysis of Pectic Materials*, AIC-340, Western Regional Research Laboratory, Albany, California (1952)
21. Norziah M.H., Fang E.O. and Karim A.A., Extraction and characterization of pectin from pomelo fruit peels. In G. P. Williams (Ed.), *Gums and Stabilisers for the food industry*, Cambridge, UK: The Royal Society of Chemistry, **10**, 26-36 (2000)
22. Schultz T., *Methods in Carbohydrate Chemistry*. In T. Schultz, *Methods in Carbohydrate Chemistry*, New York: Academic Press, 189 (1976)
23. Canteri-Schemin M.H., Fertoni H.C.R., Waszczyński N. and Wosiacki G., Extraction of pectin from apple pomace, *Brazilian Archives of Biology and Technology*, **48**, 259-266 (2005)
24. Mohamed S., and Hasan Z., Extraction and Characterization of Pectin from Various Tropical Agrowastes, *ASEAN Food Journal*, **10**, 43-50 (1995)
25. Fishman M. L., Chau H. K., Hoagland P. and Aygad K., Characterization of pectin, flash-extracted from orange albedo by microwave heating under pressure, *Carbohydrate Research*, **323**, 126-138 (2000)
26. Liu Y., Shi J. and Langrish T.A.G., Water-based extraction of pectin from flavedo and albedo of orange peels, *Chemical Engineering Journal*, **120**, 203-209 (2006)
27. Kratchanova M., Panchev I., Pavlova E., and Shtereva L., Extraction of pectin from fruit materials pretreated in an electromagnetic field of super-high frequency, *Carbohydrate Polymers*, **25**, 141-144 (1994)
28. Iliina I.A., Zemskova Z.G., Donchenko L.V., Uvracheva T.V., *Izvlechenie pektina s predvaritel'noi SV-suchkoi siiria, Hranenie i pererabotka selhozirija, Kubanskii Gosudarstvennii Universitet, Krasnodar, Russia*, **12**, 23-25 (2000)
29. Kohg Z., Lin Z.D. and Lin Chen S.T., Study on the extraction of pectin from apple pomace with MW, *J. Zhengzhon Grain Colloids*, **21(2)**, 11-15 (2000)
30. Fishman M.L., Chau H.K., Coffin D.R. and Hotchkiss A. T., Jr., A comparison of lime and orange pectin which were rapidly extracted from albedo, In F. Voragen, et al. (Eds.), *Advances in Pectin and Pectinase Research*, 107-122 (2003)
31. Kalapathy U. and Proctor A., Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin, *Food Chemistry*, **73**, 393-396 (2001)
32. Albersheim P., Neukom H., and Deuel H., Splitting of pectin chain molecules in neutral solutions, *Archives of Biochemistry and Biophysics*, **90**, 46-51 (1960)
33. Kertesz Z.J., *The Pectic Substances*. New York: Interscience Publishers Inc., 432-441, 457-462 (1951)

34. Joye D.D. and Luzio G.A., Process for selective extraction of pectins from plant material by differential pH, *Carbohydrate Polymers*, **43**, 337-342 (2000)
35. BeMiller J.N., An Introduction to pectins: Structure and Properties, Chemistry and Function of Pectin. American Chemical Society, Washington, DC, **2-12** (1986)
36. Rombouts F.M. and Thibault J.F., Feruloyated pectic substances from sugar beet pulp, *Carbohydrate Resources*, **154**, 177-188 (1996)
37. Voragen A.C.J., Rolin C., and Marr B.U., Pectins. In: Polysaccharides, Voragen, A.C.J., Rolin, C., Marr, B.U., Challen, I., Riad, A., and Lebabar, R. (Eds). Wiley-VCH, New York, 189-195 (2003)
38. Knee M., Polysaccharide and glycoproteins of apple fruit cell walls, *Phytochemistry*, **12**, 637-653 (1973)
39. Jarvis M.C., Hall M.A., Threlfall D.R. and Friend J., The polysaccharide structure of potato cell walls: chemical fractionation, *Planta*, **152**(1), 93-100 (1981)
40. Adamson A.W., Physical Chemistry of Surfaces, Wiley Interscience, New York, **368** (1979)
41. Kirtchev N., Panchev I., and Kratchanov C., Pectin extraction in the presence of alcohols, *Carbohydrate Polymers*, **11**(4), 257-263 (1989)
42. Food Chemical Codex. IV monographs. pp. 283. Washington DC: National Academy Press (1996)
43. Joslyn, M.A., and De Luca, G., The formation and properties of aluminium pectinates, *Journal of Colloid Science*, **12**, 108-130 (1957)
44. Pagán J., Ibarz A., Llorca M., Pagán A. and Barbosa-Cánovas G.V., Extraction and characterization of pectin from stored peach pomace, *Food Research International*, **34**, 605-612 (2001)
45. Faravash R.S. and Ashtiani F.Z., The effect of pH, ethanol volume and acid washing time on the yield of pectin extraction from peach pomace, *International Journal of Food Science and Technology*, **42**, 1177-1187 (2007)