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Studies on Physicochemical Properties and Elemental Analysis of Citron and Pomelo Fruits Peels Pectins

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Abstract

This research work is aimed to study the physicochemical properties and elemental analysis of citron and pomelo fruits peels pectins which have been extracted. In the present work, determination of some physicochemical parameters such as color, moisture content, ash content, setting time, equivalent weight, methoxyl content, anhydrouronic acid (AUA), degree of esterification (DE), molecular weight and elemental analysis of the prepared pectins have been performed. The color of citron peel pectin and pomelo peels pectins were determined by eye test. Moisture contents were determined by oven drying method, ash contents were determined by the method used in the food analysis, setting times were determined by a simple method of Owens, equivalent weights, methoxyl contents, anhydrouronic acids (AUA) and degree of esterifications (DE) were determined by titrimetric method, molecular weights were determined by viscometric method and elemental analysis were determined by Energy Disperse X-ray Fluoresce (ED XRF) analysis. Physicochemical properties of the citron and pomelo peels pectins were found to be white-yellow and brown-yellow colors, 7.96% and 2.15 % of moisture contents, 1.07 % and 5.72 % of ash contents, 5 minutes and 12 minutes of setting times, 2380 and 2500 of equivalent weights, 1.86 % and 2.29 % of methoxyl contents, 17.95 % and 20.06 % of anhydrouraonic acid contents and 58.83 % and 64.92 % of degree of estrifications and $6.61\times 10^4$and 5.62×10^4 Da of molecular weights respectively.

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In the citron pectin, the relative abundance of Ca, K, Fe, Cu, Sr 20.06 % of anhydrouraonic acid contents and 58.83 % and 64.92 % of degree of estrifications and $6.61\times 10^4$and 5.62×10^4 Da of molecular weights respectively. In the citron pectin, the relative abundance of Ca, K, Fe, Cu, Sr and Zn has been found to be 61.87 %, 22.67 %, 7.94 %, 3.11 %, 2.29 % and 2.13%, respectively (determined by ED XRF technique). Whereas in the pomelo pectin, the relative abundance of Ca, K, Fe, Cu, Sr and Zn has been found to be 66.32 % 11.82 %, 11.73 %, 2.75 %, 2.32 % and 5.06 %, respectively.

Keywords: citron and pomelo peel pectins; molecular weight; degree of esterification ; ED XRF elemental analysis.

1. Introduction

Pectin (derived from Greek meaning- ‟congealed, and curdled″) is natural, non-toxic, and amorphous carbohydrate present in cell wall of all plant tissues, which functions as an intercellular and intracellular cementing material. It was first isolated and described in 1825 by Heneri Bracannot. It is commercially in form of white to light brown powder, mainly extracted from apple and citrus fruits. As a secondary product of fruit juice, sunflower oil, and sugar manufacture industries, pectin is both inexpensive and abundantly available. Therefore, pectin is an excellent candidate for eco-friendly biodegradable applications. Pectin is commonly used in the food industry as a gelling and stabilizing agent. It is included in non-starch polysaccharide. It is used in food as a gelling agent particularly in jams and jellies. It is also used in filling, medicine, sweets, as a stabilizer in fruit juices and milk drinks, and as a source of dietary fiber. Pectin is an essentially linear polysaccharide. Like most other plant polysaccharides, it is both polydisperse and polymolecular and its composition varies with the source and the conditions applied during isolation. In any sample of pectin, parameters such as the molecular weight or the content of particular subunits will differ from molecule to molecule. Galacturonic acid residues can be partially esterified by methanol on the carboxyl group and by acetyl on the secondary hydroxyls. In nature, around 80 percent of carboxyl groups of galacturonic acid are esterified with methanol. The proportion is decreased to a varying degree during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. Pectin from gels under certain circumstances, the gelling mechanism is highly dependent on the degree of methoxylation (DM). Conventionally, pectin is divided into high methoxy (HM) pectin and low methoxy (LM) pectin. The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5 percent the salts are called pectates, the insoluble acid from, pectic acid. Low-ester pectin can do at lower soluble solids and higher pH-values than high-ester pectins. The main use for pectin is as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in gelling sugar (also known as ‟jam sugar″) where it is diluted to the right concentration with sugar and some citric acid to adjust pH. In some countries, pectin is also available as a solution or an extract, or as a blended powder, for home jam making. For conventional jams and marmalades that contain above 60 % sugar and soluble fruit solids, high-ester pectins are used. With low-ester pectins and amidated pectins less sugar is needed, so that diet products can be made. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, and as a fat substitute in baked goods. Typical levels of pectin used as a food additive are between 0.5 and 1.0 %, this is about the same amount of pectin as in fresh fruit. In

cosmetic products, pectin acts as stabilizer. Pectin is also used in jellybeans [1]. Trace minerals are inorganic substances that human body needs in small amounts to function properly. In general, human need less than 20 milligrams of each trace mineral daily, although specific amounts depend on human age and sex. Although human only need to consume a small amount of trace mineral, they are vital to human health because each mineral provides a unique benefic [2]. In the present study, some physicochemical properties and elemental analysis of citron and pomelo fruits (Figure 1) peels pectins in chemical and medicinal purpose.

(i)

(ii)

Figure 1: Photographs of (i) citron and (ii) pomelo fruits

1.1 Aim and objectives of the present work

The aim of the present research work was to study the physicochemical properties and elemental analysis of citron and pomelo peels pectins in chemical and medicinal purpose.

To achieve this aim, the research was carried out according to the following objectives;

- Determining some physicochemical properties of the extracted pectins such as color, moisture content, ash content, setting time, degree of esterification and molecular weight by appropriate analytical methods
- Analyzing the elements present in the extracted pectins by using ED XRF analysis

2. Materials and methods

2.1 Determination of some physiochemical properties and elemental analysis of the extracted pectins

The pectin samples used in this research work were citron peels pectin (CPPT) and pomelo peels pectin (PPPT) [3], which have been extracted. In this study some physicochemical properties such as color, moisture content, ash content, setting content, equivalent weight, methoxyl content, anhydrouronic acid, degree of esterification , molecular weights and elemental analysis of the extracted dry pectins (CPPT and PPPT) were determined.

2.2 Color of pectins

The color of citron peel pectin and pomelo peels pectin were determined by eye test.

2.2 Procedure for determination of moisture content

The pectin should be stored under dry and cooled conditions. In moist climate, however, weighing of dry pectin was difficult since it rapidly absorbed water vapour. Under these conditions samples should be exposed to air for 1 to 2 days until they reached equilibrium moisture level. An accurately weighed pectin samples (1 g) were taken in a weighed porcelain crucibles with lids. The samples were dried (60 °C) in an oven (20 hours). The crucibles were cooled to room temperature in a desiccator and weighed. (Desiccant should be P_2O_5). The experiment was repeated until the constant weights of dry pectin samples were obtained. The percentage of moisture content was then calculated (Appendix I) [4].

2.3 Procedure for determination of ash content

Total ash of pectin was estimated by the method used in food analysis. A pectin sample was weighed (1 g) into a porcelain crucible and heated slowly. It was then ignited at red heat by Bunsen burner. The crucible was cooled to room temperature in a desiccator and weighed. Ignition was repeated to a constant weight of ash. The percentage of total ash was then calculated (Appendix II) [5].

2.4 Procedure for determination of setting time

Rate of cooling, presence of metal ions, pH and solid content (sugar and pectin) influences the setting time of jelly. Thus for comparison sake, jellies were prepared from various pectin under the same conditions; that is jelly preparations were made with 65 % sugar solution, 0.5 g of pectin, pH adjusted to 3, and final volume adjusted to 50 mL. Several methods had been proposed to measure this factor. Here a simple method [5] was applied as follows.The accurately weighed pectin (0.5 g) was dissolved in distilled water (50 mL) by warming

and sugar (32.5 g) was then added with constant stirring. When all the pectin and sugar were dissolved, the solution was cooled to room temperature and a few drops of citric acid solution (1 %) were added to get a pH of 3. The solution was then heated to a constant volume (50 mL). The jelly was immediately poured into sample glass. The timer was started when sample glass was filled with jelly. The sample glass was then placed in water bath maintained at 30 °C so that the glass was surrounded by water almost to the top. When the jelly at the top just congealed, the timer was stopped to obtain the setting time. The time taken for complete setting of jelly was also noted. It was taken as setting time.

2.5 Procedure for determination of equivalent weight

Equivalent weights were done by weighing 0.5 g pectin (moisture free) in a 250 mL conical flask, moistened with 5 mL ethanol. 1 g of sodium chloride was added to sharpen the end point. A 100 mL of carbon dioxide free distilled water and 6 drops of phenol red indicator were added. The pectic substances were stirred rapidly to dissolve, then titrated slowly with 0.1 M NaOH until the colour of the indicator changed (pH 7.5) and persisted for at least 30 seconds. The values of equivalent weights were calculated from the following equations (Appendix III) [7].

$$\text{Equivalent weight} = \frac{\text{weight of sample(mg)}}{\text{mass equivalent of NaOH}}$$

(or)

$$\text{Equivalent weight} = \frac{1000 \times \text{weight of sample(g)}}{\text{M} \times \text{volume of NaOH}} \qquad (1)$$

The neutralized solution was kept for determination of methoxyl content.

2.6 Procedure for determination of methoxyl content

Methoxyl (MeO) contents were determined by adding 25 mL of 0.25 M NaOH to the neutral solution, mixing thoroughly (Section 2.5), and allowed to stand for 30 minutes at room temperature in a stoppered flask. 25 mL of 0.25 M HCl was then added and titrated with 0.1 M NaOH to the same end point as before. The value of methoxyl contents were calculated from the following equations.

$$\text{MeO \%} = \frac{\text{meq of sodium hydroxide} \times 31 \times 100}{\text{wt. of sample (mg)}}$$

(or)

$$\text{MeO \%} = \frac{\text{M} \times \textbf{volume of sodium hydroxide(mL)} \times \textbf{3.1}}{\textbf{wt. of sample (g)}} \qquad (2)$$

Where, 31 is the molecular weight of MeO.

The calculation of methoxyl content of citron peel pectin (CPPT) and pomelo peel pectin (PPPT) is shown in

(Appendix IV) [7].

2.7 Procedure for determination of anhydrouronic acid content (AUA)

If the equivalent weight and methoxyl content of pectin are known, its AUA can be calculated as follows.

$$\text{AUA\%} = \frac{\mathbf{176 \times 100}}{\mathbf{Z}} \quad (3)$$

Where, 176 is the molecular weight of AUA and

$$Z = \frac{\mathbf{wt.of\ sample\ (mg)}}{\mathbf{meq\ of\ alkali\ for\ free\ acid + meq\ of\ alkali\ for\ methoxyl}} \quad (4)$$

The calculation of anhydrouronic acid content (AUA %) of citron peel pectin (CPPT) and pomelo peel pectin (PPPT) is shown in Appendix V [7].

2.8 Procedure for determination of degree of esterification (DE)

The degree of esterification (DE) of pectin can be determined according to the formula given below.

$$\text{DE \%} = \frac{\mathbf{176 \times CH_3O\ \% \times 100}}{\mathbf{31 \times AUA\ \%}} \quad (5)$$

Where, CH_3O is % methoxyl content.

The calculation of degree of esterification (DE) of citron peel pectin (CPPT) and pomelo peel pectin (PPPT) is shown in Appendix VI [7].

2.9 Procedure for determination of the average molecular weight

The effect of molecular weight for polymer is one of the important factors for its applications. The extracted pectins were characterized by their molecular weights determined by means of viscosity measurements [7]. Specific viscosity, ηsp was measured by registering pectin solutions flow time in an Ubbelohde capillary viscometer at 25 ± 0.1 ^{o}C, immersed in a temperature controlled bath. Pectin solutions were prepared at different concentrations, dissolving dried pectin in an aqueous solution containing 0.1 M NaCl at pH 7 for 18 hours with stirring at room temperature. Pectin solutions and solvent were filtered using 0.45 µm membrane filters before viscosity measurement, η_r of pectin solutions by extrapolation of Kraemer and Mead and Fouss curves to "zero" concentration. NaCl was used in order to prevent pectin aggregation. The viscosities of the samples were calculated by the following equation: The viscosimetric molecular weight(M_v) was then calculated (Appendix VII) from the Mark-Houwink relationship $M_{v} = ([\eta]/K)^{1/\alpha}$, where the constants k and α are 0.0436 and 0.78, respectively.

2.10 Qualitative elemental analysis by Energy Dispersive X-Ray Fluorescence (ED XRF) method

The elemental contents in extracted pectins were qualitatively determined by ED XRF method. The spectra were taken using ED XRF spectrometer (ED XRF-700 Spectrometer, Shimadzu, Japan) at the Universities' Research Centre (URC) which situated in the University of Yangon campus, Myanmar.

3. Results and discussion

3.1 Some physicochemical properties of the extracted pectins and elemental analysis

Some physicochemical properties such as color, moisture content, ash content, setting content, equivalent weights, methoxyl content, anhydrouronic acid, degree of esterification and molecular weights of the extracted dry pectins (CPPT and PPPT) were determined as described in Section 2.1. The results are summarized in Table 3.

3.2 Color of pectins

The color of citron peel pectin is white-yellow and pomelo peel pectin is brown-yellow as shown in Figure 2. So consistent with literature range white to light brown color [1].

(i)

(ii)

Figure 2: Photograph of pectin (in powder form) obtained from (i) citron peel and (ii) pomelo peel

3.3 Moisture content

The moisture content of citron peel pectin was 7.96 % and that of pomelo peel pectin was 2.15 %, which were less than the literature value of moisture content of sun dried peel pectin range 9.6 % to 17.1 % [9]. 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.

3.4 Ash content

The ash contents were found to be 1.07 % in CPPT and 5.72 % in PPPT. It represents the amount of inorganic residues present in the materials. Therefore the ash contents of CPPT and PPPT were observed to be close to that of reported ash contents (1.9 ~ 5.2 %) of commercial high methoxyl and low methoxyl pectins [7]. Ash content also can affect the gelling of pectin. A low ash content is more favorable for gel formation.

3.5 Setting time

It was found that the pectin obtained from both of the citrus peels had rapid setting characteristics (5 minutes for citron peel pectin and 12 minutes for pomelo peel pectin). Because if jelly sets in 10-20 minutes, the pectin is considered as rapid setting while above 25 minutes is called slow setting as observed by [6,10].

3.6 Degree of esterification

The degree of esterification (% DE) of pectins was determined from the equivalent weights and percent anhydrouronic acid (% AUA) (Section 2.8). The equivalent weights obtained were used for the calculation of percentage of anhydrouronic acid (% AUA) and the percent anhydrouronic acid (% AUA) was then used for the calculation of degree of esterification (% DE). The methoxy content of CPPT was found to be 1.86 % and that of PPPT was 2.29 %. This indicates that pectin extracted from both of the citrus peels (CPPT and PPPT) are of the high methoxyl pectin (HMP) [6], since HMP is classified as having % DE higher than 50 % (58.83 % DE for citron pectin and 64.92 % DE pomelo pectin). The content of methoxyl plays a key role in the ability for gel formation. HMP can form gels in the presence of high sugar concentration. Hence, this will be more useful in making high sugar jams and marmalades because of their high methoxyl contents.

3.7 Average molecular weight of extracted pectins

The molecular weight is one of the important criteria to characterize the extracted pectins. Depending upon the sources, raw material quality, extraction methods and chemical treatment after extraction, pectin of variable average molecular weight can be obtained. Generally a high molecular mass is more favorable for gel formation. In this research work, the relative molecular weight was determined by viscosity method by applying the Mark-Houwink equation, relating the intrinsic viscosity, [η], with viscosity-average molecular weight, M_v (Section 2.9). The viscosities average molecular weights of extracted pectins were determined by dilute solution viscometry with Ubbelohde-type viscometer and estimated by using Mark-Houwink empirical equation:

$$[\eta] = KM^a \qquad (6)$$

Where, the intrinsic viscosity [η], also called the limiting viscosity number, is proportional to the polymer molecular weight, *M*, through the constant *K* and Mark-Houwink exponential "α" which relates to the stiffness of polymer chains, and valid for each polymer-solvent system at a given temperature. The value of *K* is 0.0436 mL g^{-1} for sodium chloride solution (0.1 M; pH = 7) and $\alpha = 0.78$ [8]. Equations relating relative viscosity (solution to solvent) η_r with [η] and pectin concentration 'C' are:

$$\frac{\eta_r - 1}{C} = [\eta] + k_H [\eta]^2 C \quad (8)$$

$$\frac{\ln \eta_r}{C} = [\eta] + k_K [\eta]^2 C \quad (9)$$

Where, k_H and k_K are the Huggin's and Kramer's coefficients respectively. Intrinsic viscosity [η] can be determined by the combined extrapolation at C = 0 of Huggin's and Kramer's equations. The expression $(\eta_r - 1)/C$ and $\ln(\eta_r)/C$ are known as viscosity index, respectively [11]. Determinations were made in the range of pectin concentration 2-10 mg/mL. Viscosity measurements were performed at room temperature. The resulting parameters were illustrated in Tables 1 and 2 and plots used to determine the intrinsic viscosities are shown in Figures 3 and 4 .The intrinsic viscosities [η] of the citron peel pectin and pomelo peel pectin from the plots were graphically found to be 251 and 227 mL/g, respectively. The viscosity average molecular weights M^a for citron peel pectin and pomelo peel pectin were 6.61×10^4 and 5.62×10^4 Dalton, respectively (Table 3), which were consistent with the literature range 1×10^4- 4×10^5 Dalton [12].

Table 1: Resulting parameters from determination of viscosity average molecular weights of citron peel pectin

Concentration of pectin in 0.1M NaCl solution (g/mL)	**Flow time (sec)**	$\eta_r = \frac{t}{t_0} = \frac{\eta}{\eta_0}$	$\eta_{sp} = \frac{\eta}{\eta_0} - 1$	$\frac{\eta_{sp}}{C}$ **(mL/g)**	$\frac{1}{C}\ln\frac{\eta}{\eta_0}$ **(mL/g)**
Pure solvent	49	0	0	0	0
0.002	77	1.57	0.57	285	238
0.004	118	2.40	1.40	350	218
0.006	160	3.26	2.26	376	196
0.008	200	4.08	3.08	385	175
0.010	280	5.71	4.71	471	174

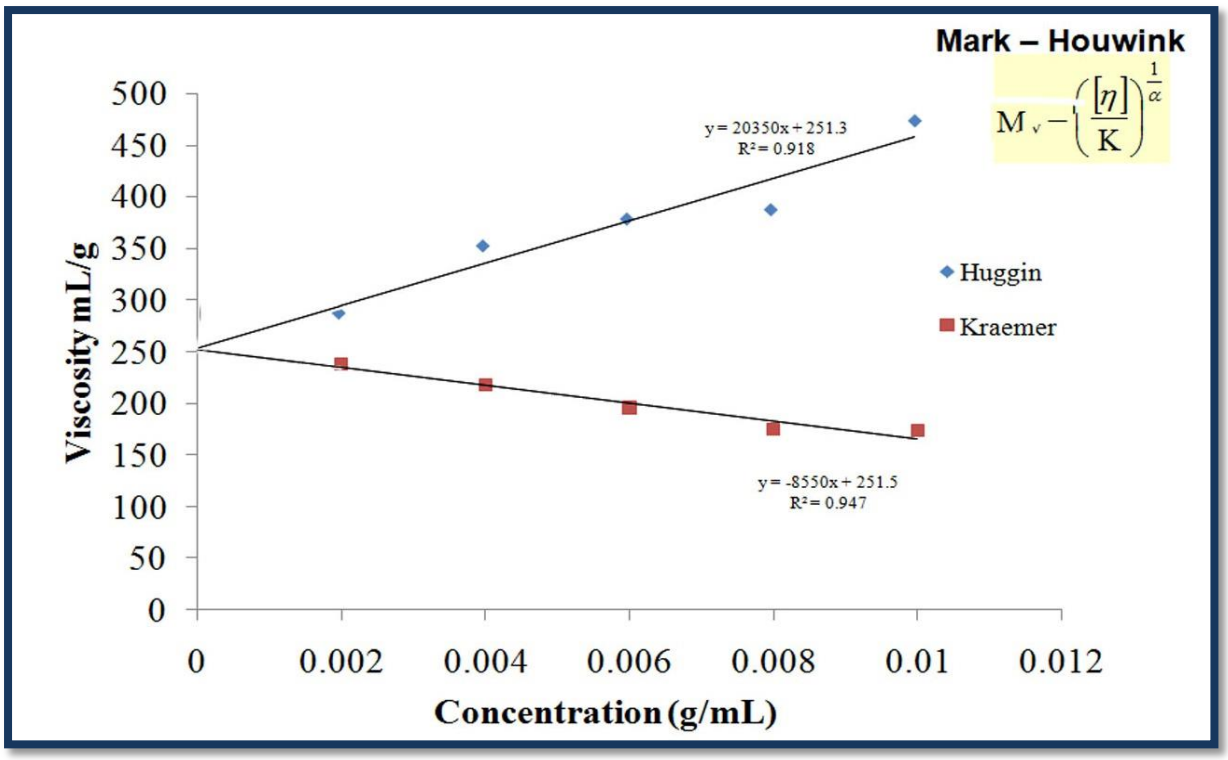

Figure 3: Huggin's and Kramer's plot of citron peel pectin for determination of intrinsic viscosity

Table 2: Resulting parameters from determination of viscosity average molecular weights of pomelo peel pectin

Concentration of pectin in 0.1M NaCl solution (g/mL)	**Flow time (sec)**	$\eta_r = \frac{t}{t_0} = \frac{\eta}{\eta_0}$	$\eta_{sp} = \frac{\eta}{\eta_0} - 1$	$\frac{\eta_{sp}}{C}$ **(mL/g)**	$\frac{1}{C}\ln\frac{\eta}{\eta_0}$ **(mL/g)**
Pure solvent	49	0	0	0	0
0.002	73	1.49	0.49	244	222
0.004	100	2.04	1.04	260	178
0.006	130	2.65	1.65	275	161
0.008	165	3.36	2..36	295	151
0.010	200	4.08	3.08	308	140

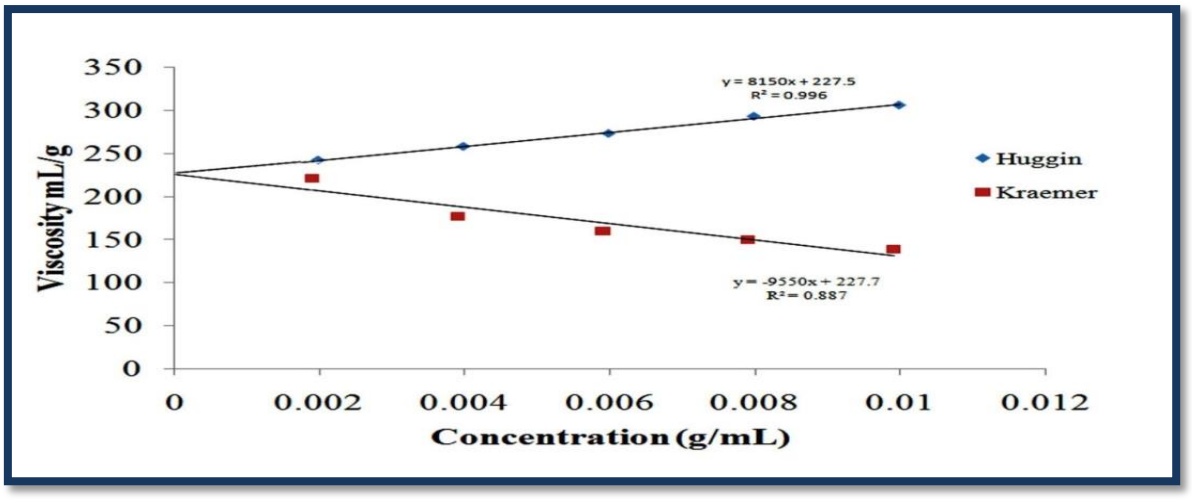

Figure 4: Huggin's and Kramer's plot of pomelo peel pectin for determination of intrinsic viscosity

Table 3: Results of some physicochemical properties of exteacted pectins

Sr. No	Parameters	Exteacted Pectins		Reported Data
		Citron peel	Pomelo peel	
1	color	white-yellow	brown-yellow	white to light brown*
2	Moisture content(%)	7.96	2.15	9.6 – 17.1**
3	Ash content(%)	1.07	5.72	1.9-5.2***
4	Setting time (min)	5	12	10-20 (rappid set) 20-25(slow set)****
5	Equivalent weight	2380	2500	
6	Methoxyl content (MeO %)	1.86	2.29	
7	Anhydrouronic acid content(AUA%)	17.95	20.06	
8	Degree of esterification (DE%)	58.83	64.92	<50 %DE (low methoxyl pectin); >50 % DE (high methoxyl pectin*****
9	Molecular weight (Daltons)	6.61×10^4	5.62×10^4	1×10^4- 4×10^5******

*	[1]	***	[7]	*****	[7]
**	[8]	****	[6]	******	[12]

3.8 ED XRF qualitative elemental analysis

In the citron pectin, the relative abundance of Ca, K, Fe, Cu, Sr and Zn has been respectively found to be 61.87 %, 22.67 %, 7.94 %, 3.11 %, 2.29 % and 2.13 %, determined by ED XRF technique. Whereas in the pomelo pectin, the relative abundance of Ca, K, Fe, Cu, Sr and Zn has been found to be 66.32 %, 11.82 %, 11.73 %, 2.75 %, 2.32 % and 5.06 %, respectively. ED XRF elemental analysis of citron pectin showed that the presence of Ca and K as major elements and Fe, Cu, Sr and Zn as minor elements and that of pomelo pectin showed the presence of Ca, K and Fe as major elements and Cu, Sr and Zn as minor elements. The ED XRF spectra of the two extracted pectins CPPT and PPPT are shown in Figures 5 and Figure 6. The relative abundances of the two extracted pectins are shown in Table 4. Calcium is an extremely important element in the human body. Calcium is necessary to build healthy bones and teeth. Calcium aids in maintaining bone health, dental care, prevention on colon cancer and reduces obesity. We need calcium right from birth till old age. Potassium is extremely important to cells, and without it, we could not survive. Potassium, the third most abundant mineral in the human body, is the synonym for health insurer. Iron has many functions in the body. Iron is used by the body to make tendons and ligaments. Iron is necessary for cell function and blood utilization. About two-thirds of the bodily iron is found in hemoglobin. Other symptoms of iron deficiency are chronic disease, cough, anemia in pregnancy, pre dialysis anemia, and many more. Copper is an element that is very important for good health. Copper is critically important for dozens of body functions. The health benefits of copper include proper growth, utilization of iron, enzymatic reactions, connective tissues, hair, eyes, ageing and energy production. Apart from these, heart rhythm, thyroid glands, arthritis, wound healing. Strontium has been found to be involved in the utilization of calcium in the body. It has promoting action on calcium uptake into bone at moderate dietary strontium levels. Although the average adult body only has about 2-3 grams of zinc, zinc is a very important trace element that is essential to many biological factors. Zinc is involved in over 100 different reactions in the body. Some of these reactions help our bodies construct and Maintain DNA [13].

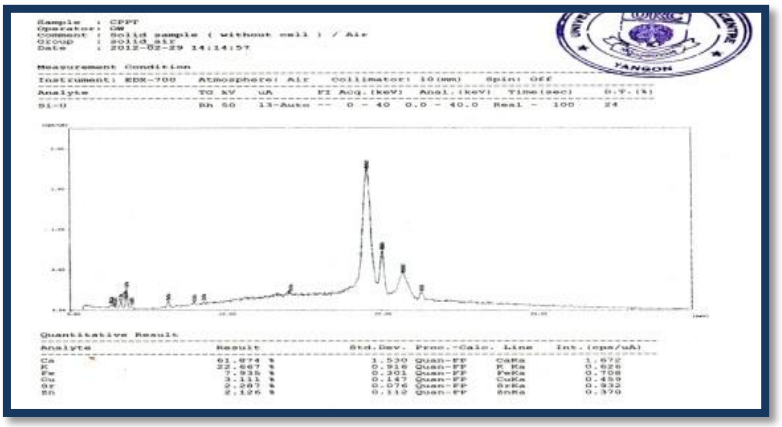

Figure 5: ED XRF spectrum of extracted citron peel pectin

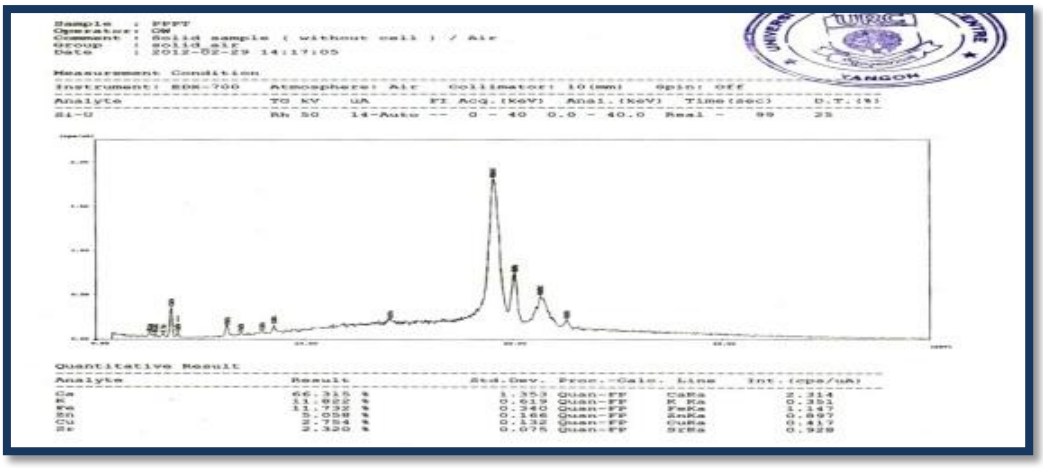

Figure 6: ED XRF spectrum of extracted pomelo peel pectin

Table 4: Relative abundance of some elements in the citron peel pectin and pomelo peel pectin (ED XRF analysis)

Sr. No	Elements	Relative Abundance%	
		Citron Peel Pectin	**Pomelo Peel Pectin**
1	*Ca*	61.87	66.32
2	K	22.67	11.82
3	Fe	7.94	11.73
4	Cu	3.11	2.75
5	Sr	2.29	2.32
6	Zn	2.13	5.06

4. Conclusion

This study reports some physicochemical properties of Citron Peel Pectin (CPPT) and Pomelo Peel Pectin (PPPT) as well as by comparing with the reported data and their elemental analysis. Total outcome of the research work were concluded as follow. The color of citron peel pectin is white-yellow and pomelo peel pectin is brown-yellow. The moisture content of dry citron peel pectin was 7.96 % and that of dry pomelo peel pectin was 2.15 %, which were less than the literature value of moisture content of sun dried peel pectin range. Only the ash content of citron pectin was observed to match with the commercial range, and pomelo pectin cannot be used for commercial purpose because of high ash content. It was found that the pectin obtained from both of the citrus peels had rapid setting characteristics. The pectins extracted from both of the citrus peels are of the high methoxyl pectin (HMP). The viscosity average molecular weights M^a of citron peel pectin and pomelo peel pectin were 6.61×10^4 and 5.62×10^4 Dalton respectively. So, both of the extracted pectins have high molecular

weights. ED XRF elemental analysis showed the presence of Ca and K as major elements in CPPT and Ca, K and Fe as major elements in PPPT. The contributions for the present research work were the citron peels pectins could be used as commercial purpose as making high sugar jam and marmalades and also as medicine and pomelo peels pectin could be used only for medicine due to high ash content. From the elemental analysis, both CPPT and PPPT can be used as supplement for human who deficiency in calcium, potassium, iron, copper, strontium and zinc elements.

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Appendix (I)

Determination of moisture content of the extracted pectins

$$\text{Moisture content (\%)} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

Appendix (II)

Determination of ash content of the extracted pectins

$$\text{Ash content (\%)} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Appendix (III)

Calculation of equivalent weight of extracted pectins by titration

$$\text{Equivalent Weight} = \frac{100 \times \text{weight of sample (g)}}{\text{M} \times \text{volume of NaOH}}$$

M = Molarity of the NaOH

For CPPT,

$$\text{Equivalent weight} = \frac{1000 \times 0.5}{0.1 \times 2.1} = 2380$$

For PPPT,

Equivalent weight = $\frac{1000\times 0.5}{0.1\times 2}$ = 2500

Appendix (IV)

Calculation of methoxyl content of extracted pectins by titration

MeO % = $\frac{meq\ of\ sodium\ hyroxide \times 31\times 100}{weight\ of\ sample\ (mg)}$

Where,

meq = mass equivalent weight

= (M× volume of sodium hydroxide)

For CPPT,

MeO % = $\frac{(0.1)(3)\times 31\times 100}{0.5\times 10^3} = \frac{0.3\times 31\times 100}{0.5\times 10^3}$ =1.86 %

For PPPT,

MeO % = $\frac{(0.1)(3.7)\times 31\times 100}{0.5\times 10^3} = \frac{0.3\times 31\times 100}{0.5\times 10^3}$ =2.29 %

Appendix (V)

Calculation of anhydrouronic acid content of extracted pectins

AUA % = $\frac{176\times 100}{Z}$

Where,

176 = Molecular weight of anhydrouronic acid

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

meq = mass equivalent weight

= (M× volume of sodium hydroxide)

For CPPT,

$$\text{AUA \%} = \frac{176 \times 100}{Z}$$

$$Z = \frac{0.5 \times 10^3}{0.1 \times 5.1} = \frac{0.5 \times 10^3}{0.51} = 0.980 \times 10^3$$

$$\text{AUA \%} = \frac{176 \times 100}{0.980 \times 10^3} = 17.95\%$$

For PPPT,

$$\text{AUA \%} = \frac{176 \times 100}{Z}$$

$$Z = \frac{0.5 \times 10^3}{0.1 \times 5.7} = 0.877 \times 10^3$$

$$\text{AUA \%} = \frac{176 \times 100}{0.877 \times 10^3} = 20.06\%$$

Appendix (VI)

Calculation of degree of esterification of extracted pectins

$$\text{\% DE} = \frac{176 \times CH_3O\,\% \times 100}{31 \times \text{AUA \%}}$$

DE = Degree of esterification

176 = Molecular weight of anhydrouronic acid

CH_3O % = methoxyl content (%)

31 = Molecular weight of methoxyl group

AUA = Anhydrouronic acid content (%)

For CPPT,

$$\text{DE \%} = \frac{176 \times CH_3O\,\% \times 100}{31 \times \text{AUA \%}}$$

$$= \frac{176 \times 1.86 \times 100}{31 \times 17.95} = \frac{327.36 \times 10^2}{556.45} = 58.83\%$$

For PPPT,

$$\text{DE \%} = \frac{176 \times CH_3O\,\% \times 100}{31 \times \text{AUA \%}}$$

$$= \frac{176 \times 2.294 \times 100}{31 \times 20.06} = \frac{403.744 \times 100}{621.86} = 64.92\%$$

Appendix (VII)

Calculation of molecular weight of extracted pectins

Mark – Hauwink's(MH) empirical equation

$$M_v = \left(\frac{[\eta]}{K}\right)^{\frac{1}{\alpha}}$$

Where,

$[\eta]$ = ntrinisic viscosity

M_v = molecular weight of the pectin

K ,$\propto$ = Empirical parameters characteristics of a particular solute –solvent pair

For extracted pectin from citron peel in sodium chloride solution

$$M_v = \left(\frac{[\eta]}{K}\right)^{\frac{1}{\alpha}}$$

$\text{Log } M_v = \frac{1}{\alpha}\log\frac{[\eta]}{K}$

$= \frac{1}{0.78}\log\frac{[251]}{0.0436} \quad \frac{mL/g}{mL/g}$

= (1.282) log (5756)

= (1.282) (3.76)

$\text{Log } M_v$ = 4.82

M_v = anti 4.82

= 66069

= 6.61×10^4 Da

For extracted pectin from Pomelo peel in sodium chloride solution

$M_v = \left(\frac{[\eta]}{K}\right)^{\frac{1}{\alpha}}$

$\text{Log } M_v = \frac{1}{\alpha}\log\frac{[\eta]}{K}$

$= \frac{1}{0.78}\log\frac{227}{0.0436}\frac{mL/g}{mL/g}$

= (1.282) log (5206)

= (1.282) (3.71)

= 4.75

M_v = anti 4.75

= 56234 Da

= 5.62×10^4 Da

20, May, 2015

External Examiner's Assessment Report

1. Candidate - Thida Hlaing (ဌ-ဝါ-ဓ-ဘဌ)
2. Title - PREPARATION, CHARACTERIZATION AND SOME BIOACTIVITIES OF EXTRACTED CITRUS PECTIN-ZnO NANOCOMPOSITES FROM CITRON (*CITRUS MEDICA* L.) AND POMELO (*CITRUS MAXIMA MERR.*) FRUITS PEELS
3. Research Area - nanocomposites
4. Assessment
   (a) The candidate has collected data and also experiment work done to extract pectin from Citron (*CITRUS MEDICA* L.) and Pomelo (*CITRUS MAXIMA* MERR.) fruits' peels.
   (b) She has also determined some physicochemal properties of extracted pectin and prepared pectin-ZnO nanocomposites from different sources of citron and pomelo fruits' peels.
   (c) She has studied the antimicrobial and antitumor bioactivities of the prepered pectin-ZnO nanocomposite.
   (d) I enjoy reading her thesis and it is well written and well presented. I am quite satisfied with her work and believe that her work can be a significant contribution for the aspect of the application of the pectin-ZnO nanocomposites.
5. Viva Voce Examination- Satisfactory
6. Recommendation- I am pleased to recommend Thida Hlaing should be awarded the Ph.D Degree in Chemistry for having done this important research.

Dr. Aung Min
Rector
Yangon Institute of Education

Prof. Dr. Daw Hla Ngwe
Professor/ Head of Department
Department of Chemistry
University of Yangon

Figure 7