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Hydrogel formation from Kokiyan Biri (*Limonia acidissima*) polysaccharide hydrocolloids

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Abstract

This study investigated the effect of temperature, time and pH on the yield, physicochemical characteristics and hydrogel formation of water soluble pectin extracted from *Limonia acidissima* fruits. The pectin was extracted using the acid extraction method followed by 96% alcohol precipitation. The optimum temperature, time and pH for the extraction of pectin for dried and wet fruit were determined to be 85°C, 90 minutes and 2 respectively. The yields of pectin under these optimum conditions were found to be 16.55 and 18.92% for dried and wet fruit extracts respectively. Temperature, extraction time and pH showed a significant effect on the pectin yield with the wet sample extracts been highest. The physicochemical properties determined under this optimum condition were found to be; equivalent weight; 765.40 and 784.25 mg/mol, methoxyl content; 4.22, and 6.45%, anhydrouronic acid content; 58.60, and 65.25%, degree of esterification; 67.80 and 78.45%, moisture content; 1.30 and 1.70%, and ash content; 0.72 and 0.48% for dried and wet fruit extracted pectin respectively. Hydrogels were formulated through physical method and characterized. The swelling ratio was observed to increase with increasing temperature, and swelling medium also has significant influence on the swelling ratio, as it increased with high pH due to complete hydrolysis of carboxylate. FTIR was also utilized for characterizing the pectin and hydrogel which was favourably compared with the available standards. Generally, the findings of the study showed that the pectin extracted and hydrogels formed from *Limonia acidissima* fruits can find industrial applications, especially in food processing and pharmaceutical industries.

Keywords: Polysaccharide, Hydrocolloids, Tsamiyan Biri, *Limonia acidissima*, Hydrogel

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1. Introduction

Pectin is structural hetero-polysaccharide hydrocolloids contained mainly in the primary cell walls of many plants. It's also a multifunctional food ingredient that is widely used as gelling agent and mostly as stabilizers in food processing industries [1]. Pectin is produced commercially in form of white to light brown powder, mainly extracted from citrus fruits as it is contained in the literature. Despite the fact that pectin occurs commonly in most of the plant tissues, the commercial source of pectin is limited; this is because the ability of pectin to form gel depends on the molecular size and the degree of esterification. The pectin obtained from various sources differ in their gelling ability due to variation in certain parameters such as temperature and pressure during extraction. In medicine, pectin increases viscosity and volume of stool so that it is used against constipation and diarrhea. It has also been used in gentle heavy metal removal from biological systems. In ruminant nutrition, it depends on the extent of lignification of the cell wall; pectin is up to 90% digestible by bacterial enzymes. The ruminant nutritionists recommend that the digestibility of energy concentration in forage can be improved by increasing pectin concentration in the forage [1].

The fruits of *Limonia acidissima* are good sources of carbohydrates, proteins, fats, and minerals and other medicinal importance [2]. However, little has been done on the characterization of the pectin contents of these fruits and the evaluation of its potential industrial applications. These fruits are of economic importance and it is readily available and affordable in some local areas of Gombe and Adamawa state in the north-eastern part of Nigeria which could serve as a source of raw material for pharmaceutical and food processing industries in those areas and Nigeria at large. Therefore, it has become necessary to intensively characterized and credibly evaluate the potential industrial applications of the pectin hydrocolloids formulated

hydrogels from these fruits as a step forward towards the growth of indigenous industries in the country. Considering the importance and many values of fruits plant, the characterization and the application of pectin for hydrogel formation is very crucial since hydrogel has found wider areas of applications. Therefore, this research work focused particularly on the fruits of indigenous plant, *Limonia acidissima*.

2. Materials and methods

2.1. Sample collection and preparation

The ripped fruits of *Limonia acidissma* were collected mechanically at Yiri-Tula, Kaltungo, Gombe State, Nigeria. The fresh sample was washed with distilled water and the pulp was separated from the seed. The sample was divided into two portions, first portion was dried under shade at room temperature for about seven days, which was then powdered using a mortar and pestle and was kept in a desiccator pending extraction while the second portion was utilize immediately for wet sample extraction of pectin following standard method described below.

2.1.1. Extraction of pectin

The extraction of the pectin hydrocolloids from both dried and wet samples were carried out following standard methods described by Joel *et al*., [1].

2.1.2. Percentage yield of pectin

The percentage yield of the extracted pectin hydrocolloids was calculated using the following equation.

$$Y_{pec}(\%) = P/Q \times 100$$

Where; Ypec (%) is the extracted pectin yield in percent (%), P is the amount of extracted pectin in grams (g) and Q is the initial amount of powder fruit samples (50g), and the results are given on Table 1.

2.2. Physicochemical characterization of pectin

The dried pectin samples obtained from the two fruits were subjected to quantitative and qualitative test in order to determine its physicochemical characteristics.

2.2.1. Qualitative test

2.2.1.1. Colour

This was done by visual observation

2.2.1.2. Solubility of dry pectin in cold and hot water

About 0.25% of the pectin samples were differently placed in a conical flask with 10 mL of 95% ethanol added followed by 50 mL distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 85°C for 15 min. [1].

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

About 1 mL of 0.1 M NaOH was added 5ml pectin solution and then heated at 85°C for 15 minutes [1].

2.2.1.4. pH determination

The choice of the pH was made by preparing a buffer at pH 7.0 and the temperature adjusted to 28°C, the glass electrode standardized with standard buffer solution with the electrode rinsed with distilled water before inserting into the pectin solution and pH determined taken from the pH meter and the results are given in Table 2.

2.3. Quantitative analysis of extracted pectin

2.3.1. Determination of ash and moisture contents

The moisture and ash content were determined by adopting AOAC [3] method. As explained below:

2.3.2. Ash content

The ash content of the extracted pectin samples was determined by weighing 1.0 g of pectin in a tared crucible and then heated in a muffle furnace at 550°C for four hours. The residue was then cooled in a desiccator and weighed to a constant weight for three times. The percentage ash content of the sample was then calculated according to the expression below and results were reported in Table 2:

$$\text{Ash Content } (\%) = \frac{\text{Weight of the Ash (g)}}{\text{Weight of Pectin (g)}} \times 100$$

2.3.3. Moisture content

The moisture content of the pectin samples was determined by weighing 2.0g of the sample into a well dried crucible that was heated in an oven and cooled in a desiccator thereafter, the crucible and sample were heated in an oven set at 105±5°C, which was then removed and cooled in a desiccator and weighed. The process was repeated until a constant weight was obtained for all the samples. The moisture content was calculated from the expression given below and the results were reported in Table 2:

$$\text{Moisture Content } (\%) = \frac{\text{Weight of the Residue (g)}}{\text{Weight of Pectin (g)}} \times 100$$

2.3.4. Determination of equivalent weight

The determination of equivalent weight was carried out according to the method described by Joel *et al*., [1]. The following equation was used to calculate the equivalent weight of the pectin after titration and the results for all samples were reported in Table 2:

$$\text{Equivalent Weight (mg/mol)} = \frac{\text{Weight of the pectin sample (g)}}{\text{Volume of Alkali } (cm^3) \times \text{Molarity of Alkali (M)}} \times 100$$

2.3.5. Methoxyl content analysis of extracted pectin

The MeO content in a pectin sample is an important factor in controlling the setting time of pectins, the sensitivity to polyvalent cations and their functions in the preparation of low solid gels and fibers. Therefore, it is very necessary for it to be determined. The determination of the methoxyl (MeO) content of the pectin sample was carried out by adopting the methode of Joel *et al*., [1]. Methoxyl (MeO) content was calculated from the expression given below and the results were reported in Table 2:

$$\text{Methoxyl Content (\%)} = \frac{\text{Volume of Alkali}(cm^3) \times \text{Molarity of NaOH (g)} \times 31 \times 100}{\text{Weight of Pectin Sample (mg)} \times 1000}$$

Where: 31 is the molecular weight of methoxyl (CH_3O)

2.3.6. Anhydrouronic acid (AUA) analysis of extracted pectin

The Anhydrouronic acid (AUA) analysis was done by using the values of the equivalent weight and the methoxyl content obtained following the method specified by Ania *et al*., [4]. Thus, the anhydrouronic acid (AUA) content was calculated from the expression given below [4]. While the results obtained were reported in Table 2 as well.

$$\frac{176\ X\ 0.1z\ x100}{W\ x\ 1000} \text{ and } \frac{176\ X\ 0.1y\ x100}{W\ x\ 1000}$$

Where: Molecular unit of AUA (1 unit) = 176g, z = volume cm^3 (titre) of NaOH from equivalent weight determination, y = volume cm^3 (titre) of NaOH from methoxyl content determination, and W = weight of pectin sample

2.3.7. Determination of degree of esterification

The pectin degree of esterification (DE) was determined by titrimetric method described by Bello *et al*., [5]. The degree of esterification (DE) was calculated using the following equation and the results were reported in Table 2.

$$\frac{V_2}{V_1 + V_2}\ x\ 100$$

Where V_1 = Volume (cm^3) of sodium hydroxide (0.1M) used in first titration, and V_2 = Volume (cm^3) of sodium hydroxide (0.1M) used in second titration.

2.4. Swelling studies of pectin samples

The swelling studies of the extracted pectin samples were carried out in deionized water by gravimetric method. About 2.0g of the extracted pectin sample was weighed and immersed in excess water in a beaker for 30 minutes for different time intervals at 37°C and then the samples were removed, wiped with tissue paper to remove excess of solvent, and then it was weighed immediately. The difference in weight was calculated to obtain the weight gain at different time intervals [6]. The percentage swelling was calculated from the expression below and the results were given in table 6 and figure 12.

$$Swelling\ Ratio\ (\%) = \frac{W_t - W_d}{W_d} \times 100$$

Where W_t, is the weight of the swollen pectin at time, t and W_d is the initial weight of the dried pectin before immersion.

2.5. Hydrogel formation

Physical or reversible method of hydrogel formation was used due to its relative ease of production and the advantage of not using cross-linking agents. These agents affect the integrity of substances to be entrapped (e.g. cell, proteins, etc.) as well as the need for their removal before application. Careful selection of hydrocolloid type, concentration and pH was applied for the formulation of the hydrogel with a broad range of gel textures; the method that was used to obtain physically cross-linked hydrogels is described below.

2.5.1. Heating and cooling a polymer solution

The formation of hydrogels from the extracted pectin polysaccharide was achieved by heating and cooling hot solutions of the pectin made by dissolving 2.0g in a 10ml of acidified solution of pH 2 at a temperature of 60°C. This was done following the procedure outlined by Funami *et al*., [7]. The gel formation is due to helix-formation, association of the helices, and forming junction zones carrageenan in hot solution above the melting transition temperature. Upon cooling, it transforms into rigid helical rod structures in the presence of potassium salt.

2.6. Characterization of hydrogels

2.6.1. Solubility

The hydrogel content of a given material is estimated by measuring its insoluble part in dried sample after immersion in deionized water for 16 hrs [8] or 48 hrs at room temperature [9]. The samples for solubility test were prepared at a dilute concentration (0.05M) to ensure that hydrogel material is fully dispersed in water. The gel fraction was then measured as follows:

$$\text{Gel Fraction (Hydrogel \%)} = \frac{W_d}{W_i} \times 100$$

Where: W_i is the initial weight of dried hydrogel sample and W_d is the weight of the dried insoluble part of sample after extraction with water.

2.6.2. Swelling measurement and equilibrium studies

The swelling measurement and dynamic equilibrium experiment for the hydrogel was conducted at 30°C in buffer solution of pH 2, 7.2 and 9.6 in other to determine the effect of swelling medium on the polymer sample while the ionic strength was maintained at I = 0.15M. The weights gain of the samples were obtained by removing it at time interval of 30 minutes from the swelling media, then the excess water is blotted with a tissue paper before weighing, and the swelling ration was then

determined. When the samples reached equilibrium swollen state, the weight of the polymer at this state was then taken again in other to determine the equilibrium swelling ration or water uptake were also calculated from the expressions stated below; and the results were given in table 7 and figure 14. Similarly; the swelling ratios under different temperatures of 30, 45 and 60°C of polymer sample was carried out in deionized water maintained at these temperatures in a water bath for time interval of 30 mins. The same equation was applied for calculating the swelling ratio and the results obtained were given on table 3 and figure 3 [9].

$$\text{Swelling Ratio} = \frac{W_t - W_d}{W_d}$$

Where W_t, is the weight of hydrogel in swollen state at time, t and W_d is the weight of hydrogel in dry state before water sorption. The terms 'swelling ratio' [10], or 'degree of swelling' has been used for more or less similar measurements [10].

2.6.3. Spectroscopic characterization of pectin and the hydrogel

The FTIR studies were performed on both the pectin and hydrogel at wave number ranging from 4000-400 cm^{-1} in other to determine the characteristic functional groups of the formulated hydrogel.

2.7. Statistical analysis of data

For each experimental determination the results were obtained in replicate of at least two and the statistical method was applied in calculating mean and standard deviation. And all the results were expressed as mean values.

3. Results and discussion

3.1. Yield of extracted pectin

The percentage yield of the extracted pectin from *Limonia acidissima* fruit for both dried and wet samples under the varying experimental conditions of temperature, extraction time and pH are given in Table 1.

The yield of pectin from both dried and wet *Limonia acidissima* fruit were in the ranges from 4.76 to 16.55 % and 5.86 to 18.92 %, respectively.

3.2. Effect of extraction process parameters on pectin yield

3.2.1. Effect of extraction time on the pectin yield

From the results shown on Figure 1, the pectin yield was observed increased significantly with the increase in the extraction time, it is clear that the yield of pectin increases upto 90 minutes and thereafter its start declining. This may due to the fact that a relatively long period of extraction would cause a thermal degradation on the extracted pectin, thus causing a decrease in the amount extractable by the alcohol during precipitation process [1]. Apart from that, the color of the pectin extract became dark brown for longer periods of extraction which invariably required a higher number of alcoholic washing of the precipitate. Also, as the extraction proceeds, the concentration of the pectin in the solution will increase and the rate of extraction will also progressively decrease; due to fact that the concentration gradient will be reduced and, consequently, the solution becomes more viscous. Generally, the result shows that the yield increases with increase in extraction time as the proto-pectin naturally present in cells takes time to solubilize and go into the solution. However, at longer extraction time and higher temperatures, the pectin yield begins to decline; this probably could due to hydrogen bond dissociation as a result of thermal degradation of pectin backbone that may likely occur at high temperatures [1].

In this current work, the optimum pectin yield was obtained at a temperature of 85°C and extraction time of 90 minutes as 16.55% and 18.92% for DLA, and WLA fruits extracts respectively as given on Table 1.

3.2.2. Effects of temperature on pectin extraction yield

As shown in Table 1 the pectin yield was greatly influenced by temperature. The yield increases with the increasing temperature for both *Limonia acidissima* and *Azanza garckeana* fruits samples until the temperature above 85°C. Because increasing the extraction temperature would increase the solubility of the extracted pectin, giving a higher rate of extraction. However, further increase in temperature from 85 to 105°C shows a declining tendency of pectin yield, since too high temperature would lead to break down of pectin molecules as pectin is composed of α-(1-4) linked units of galacturonic acid or methyl ester resulting in pectin of lower molecular size which is not stable and extractable with alcohol and consequently, the pectin yield declined. Also, high temperature encourages energy loss through vaporization and increases the cost of extraction process from the industrial point of view. At lower temperature, the lower viscosity of pectin might cause poor diffusion between the phases that could cause slower rate of extraction leading to low yield of pectin.

From the previous research works, it was shown that temperature; extraction time and pH had notable influence on the pectin yields. This is why these conditions were chosen and monitored. Thus, the results obtained were similar and agreed with those obtained by other researchers [11]. Based on the current literatures Joel *et al*., [1], Udonne *et al*., [11], and Ania *et al*., [5], it could be seen that low pH gives high pectin yield, although it also depends on the plant materials this is why a low pH of 2.0 was chosen for this research work while other parameters were varied.

3.3. Physicochemical characterization of pectin

3.3.1. Qualitative test of extracted pectin

The qualitative test for all the samples were given under Table 2. It was observed that in cold water, the pectic

sample were insoluble but dissolved slightly and forms suspension after vigorous shaking for about 15 mins for both dried and wet samples. However, when the temperature was raised to 85°C, the solubility was gradually increased.

The solubility of the pectic substance in an alkaline medium was investigated, the test shows that the pectin suspension forms a yellow precipitate in cold alkali medium whereas, in an elevated temperature, the suspension dissolved and turned milky, this could indicate the breaking of backbone chain of the functionality especially the esters, $RCOOCH_3$ with evolution of CO_2. Since this is a general qualitative test, it shows similar properties for the pectin extracted from plant fruits of *Limonia acidissima* both on the dried and wet basis. The results obtained in this research work shows similar characteristics with that of earlier researchers [11, 12] which stated that pectin is unstable under alkaline solution, this agrees with what was obtained from this work.

3.4. Quantitative analysis of the extracted pectin

3.4.1. Physicochemical characterizations

Physicochemical characterizations of the extracted pectin were carried out for various parameters in other to evaluate its suitability in industrial applications. The extraction time and pH had significance effect on the degree of esterification and moisture content of all the samples. The physicochemical characterizations of pectin depend mainly on the raw material source and conditions selected for isolation and purification. Table 3 gives a brief summary of the parameters that were determined in order to evaluate the physicochemical quality characteristics of *Limonia acidissima* fruit pectin. The values of equivalent weight of *Limonia acidissima* fruits pectin were slightly lower than those obtained from *Azanza garckean* fruits extracted pectin [1]. Methoxyl content is an important factor in controlling the setting time of pectin and the ability of the pectin to form gels [4]. The values of methoxyl contents and the pH obtained were 4.22 and 3.80% and 4.10 and 6.45% for DLA and WLA respectively. Which is within the range, from previous study literature had it that methoxyl content of extracted pectin vary from 0.2-12% depending on the source and mode of extraction. Since all the values obtained experimentally were below 7.5%, hence the pectin was of low ester content, which is a characteristic indicating that the pectin obtained is good in terms of quality [1]. Degree of methylation and pectin yield are important factors in determining the firmness of the gel and, subsequently, the value and possible use of raw material in the food industry and other applications.

The moisture and ash content of 1.30, 1.70 and 0.72, 0.48 (% w/w) for DLA, and WLA respectively were obtained in these extracting conditions. The results showed that pectin samples have a minimal moisture content and low ash content which are very important factors required for checking quality of pectin for both utilization and storage. Anhydrouronic acid content and degree of esterification in the samples were found to be 58.60 and 60.25 % and 67.80 and 78.45 % respectively for both dry and wet sample of *Limonia acidissima* fruits pectin. Therefore, with respect to this parameter, the pectin extracted in this study may be considered to be of satisfactorily good quality. The inorganic impurities in pectin were indicated by the ash content. Lower ash content indicates good quality of pectin. The ash content of extracted pectin was close to the reported values by other researchers.

3.5. FT-IR spectroscopy

Figure 2 shows the over-laid as DLA and WLA which represents the characteristic absorption peaks for dried and wet *Limonia acidissima* fruit extracted pectin respectively. The spectra presented as DLA gives a characteristic absorption peak at 3419.48 cm^{-1} which corresponds to O-H stretching vibration, 2938.95 cm^{-1} is due to sp^3 C-H, 2364.07cm^{-1} is due to C=C stretching vibration, 1647.27 through 1743.80 cm^{-1} is due to C=O stretching vibration, 1541.02 cm^{-1} is due to N-H bending motion of the amine, 1381.61 through 1440.78 cm^{-1} is due to sp^3 CH_2 of methylene bridge, 1060.61 through 1246.39 cm^{-1} due to C-O stretching vibration and 752.91 cm^{-1} is due to sp^2 C-H bending vibrations [1]. However, the characteristic absorption peaks for wet *Limonia acidissima* from the spectra presented as WLA it could be seen that the broad band at 3417.79 cm^{-1} could probably be due to the sp O-H stretching vibration, 2941.37 cm^{-1} is likely to be sp^3 C-H, 2366.36 cm^{-1} is due to C=C stretching vibration, 1647.53 through 1743.15 cm^{-1} is due to C=O stretching vibration, 1540.15 cm^{-1} is due to N-H bending motion of the amine, 1381.71 through 1437.33cm^{-1} is due to sp^3 CH_2 bending of methylene bridge, 1058.39 through 1243.47 cm^{-1} due to C-O stretching vibration and 753.56 is due to sp^2 C-H bending vibrations [1].

3.6. Swelling studies of pectin samples

Pectin is soluble in water but not in organic solvents. Therefore, the swelling study was conducted in water. The presence of carboxylic acid groups makes pectin a polyelectorolyte and a weak organic acid. When pectin is added to water, carboxylic acid groups dissociate and the pectin molecules become negatively charged. Solubility is increased by all factors diminishing possibilities of intermolecular association.

From the results obtained, it showed that the pectin swelling increases with increasing temperatures as seen from Figure 3. The swelling value at 60°C is twice as big as the value at 30°C. It is possible to suggest that the H-bonds inside the pectin sample were broken due to temperature rise and thus increase the amount of swelling. Also, the increase in swelling value with increasing temperature of the pectin

might be caused by the increase in thermal mobility of polymer molecules inside the pectic substance [1].

3.7. Characterization of the hydrogel

3.7.1. Swelling studies of hydrogel

The swelling characteristics of hydrogels have a significant influence on the diffusion behaviors of small molecules through the gels network and determines its applicability in any particular area. Therefore, in order to have background understanding on the water transport process through the hydrogel. The effect of temperature and swelling medium pH on the swelling behavior were studied for the hydrogel formulated from the pectin extracted at 85°C for 90 mins at a pH 2.0.

3.7.2. Effect of swelling medium on the swelling ratios of hydrogel

The effects of swelling medium buffered solution pH 2, 7.2 and 9.6 The swelling of hydrogel of polymer that shows the presence of ionic groups and the swelling behavior is greatly influenced due to the fact that, the charges on the polymer chains are delocalized. Thus, the ionization of carbonyl group differs with the pH of the reacting or immersion medium.

The effect of swelling medium pH can be seen in Figure 4. The ionization of carboxylic groups at pH above its pKa, while at lower pH, protonation of the carbornyl groups are observed at different pH's. The swelling ratio at pH 7.2 was higher compare to other pHs of 2 and 9.6 the results shows that at pH 2.0, the ionized groups was less in the cross-linked network of the polymer; at pH 9.6, the result obtained suggested that the carboxylic groups on the polymer network were ionized more completely which resulted to higher counter ions concentration inside the gel, this could lead to electrostatic repulsion been reduced in the cross-linked hydrogel network and consequently the reduction in and equilibrium swelling which was observed from the results given. Thus, the swelling ratio at pH = 9.6 was observed to be lower than that obtained at pH = 7.2. The influence of swelling medium pH on the equilibrium swelling ratio is given on table 7 from the results, it could be observed that swelling ratios increased with increasing pH of buffer at pH less than 7.2, because the pKa of carboxylic acid contained in the polymer is about 4.6, at this point the carboxylic groups present in hydrogel polymer tend to dissociate at a pH greater than the pKa values of 4.6. This also shows that the osmotic pressure inside the hydrogel is been increased [1].

3.7.3. FTIR spectroscopy of hydrogels

From Figure 5, the characteristics absorption peaks for WLA-Hydrogel denoted, the peaks at 3500.01 and 3302.21 cm^{-1} are most likely to be stretching vibration absorption by OH and NH, the small peak at 2942.70 cm^{-1} can be associated with the sp^3 C-H at the methylene linkage, the peak between 2578.25 to 2660.73 cm^{-1} are a characteristic of S-H stretching vibration, 2348.06 cm^{-1} is due to C≡N stretching vibration , the doublet peak of 1711.43 and 1744.86 cm^{-1} are due to stretching vibrations of (C=O), the peak at 1412.71 cm^{-1} is due to sp^3 CH_2 bending vibration, also the peaks between 1228.52–1000 cm^{-1} are due to C-O at stretching vibration, while the peaks at 772.28–946.36 $cm^{-1}$are characteristic of sp^2 C-H bending vibration [1].

3.8. Possible applications of pectin and hydrogel

Hydrogels have a unique characteristics combination that makes them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can absorb large amounts of water (usually above 90%, w/v). Therefore, the molecular release mechanisms from hydrogels are very different from hydrophobic polymers. Both simple and sophisticated models have been previously developed to predict the release of a drug from a hydrogel device as a function of time. The water absorption capacity and response to change in temperature and ionic environment of these polymers makes it possible to be applied in various areas.

Elevated cholesterol levels considerably increase the risk of developing heart disease. High cholesterol levels in the blood cause plaque buildup in the arteries and when there is a blockage in the artery leading to the heart, a heart attack can occur. One of the best ways to reduce the risk of heart disease is to control the cholesterol levels through dietary changes, exercise and intake of medication. Grapefruit pectin is known to be a beneficial remedy for lowering cholesterol levels. In one study, individuals with high cholesterol consumed grapefruit pectin for a period of 16 weeks. The results showed that the pectin resulted in a reduction of bad cholesterol in the blood. Pectin increases the viscosity and bulk of the stools and thus helps to relieve diarrhea. It is used in many commercial diarrhea medications. Pectin obtained directly from fruits supplies nutrients to the 'good' bacteria in the colon and helps to repair damages tissue in the large intestine.

Individuals with arthritis commonly experience symptoms such as joint pain, stiffness, inflammation and degeneration of the joints are constantly on the lookout for beneficial remedies which are non-habit-forming. Pectin which is naturally present in citrus fruits, bananas, apples and some vegetables adheres to heavy metals and removes them from the joints. This is known as chelation. The joints get damaged when heavy metals accumulate in them and cause them to become painful and stiff. Getting rid of these heavy metals through chelation enables the body to repair itself. Pectin also stimulates the production of synovial fluid which protects the joints and enables them to function properly. Pectin helps to cleanse the intestinal tract but if it is consumed in large quantities, it may cause diarrhea. When there is a high intake of fiber through the diet, the absorption

of other nutrients may be reduced in the intestinal tract and this can trigger diarrhea. Therefore, it is advisable to drink plenty of water when taking fiber supplements such as pectin.

The fiber in pectin may expand inside the stomach and exert pressure on the stomach walls and the receptors in the digestive tract send a signal to the brain indicating that you are full. Hence there may be a decrease in appetite and weight loss. The passage of fiber along the digestive tract may disrupt the absorption of certain important nutrients such as calcium, zinc, iron and magnesium. These minerals may get trapped in the fiber particles and may not be able to enter the blood stream. Therefore, pectin and other nutritional supplements must be taken separately. These and many more could be the possible applications of pectin and hydrogels. Thus, from the above explanations it can be inferred that the pectin extracted from the fruits of *Limonia acidissima* could serve similar functions in the human body. However, as stated earlier dietary fibers should be consumed with cautions, in other to avoid its side effects.

Table 1. Pectin Yield of *Limonia acidissima* fruit obtained at extraction pH 2.0 under Varying extraction temperature and time, followed by precipitation with 96% ethanol

Time (mins.)	Temperature (^{0}C)	Pectin yield (% w/w)	
		DLA	WLA
45	70	4.70	5.86
60		6.20	7.46
90		8.72	10.68
45	85	10.40	12.32
60		12.35	15.63
90		16.55	18.92
45	105	14.28	17.12
60		13.26	15.52
90		12.20	13.15

DLA = Dried *Limonia acidissima* fruits, and WLA Wet *Limonaia acidissima* fruits

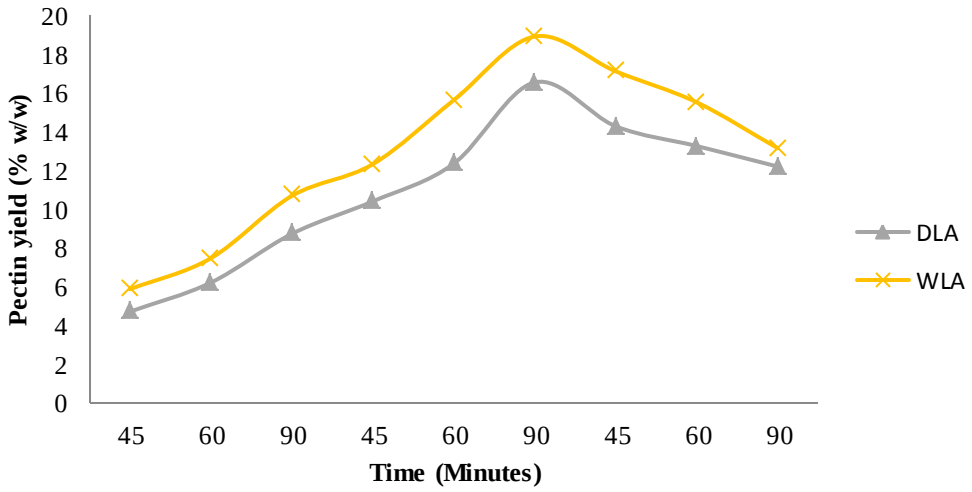

Figure 1. Effect of extraction time on the pectin yield

Table 2. Quantitative Test for Pectin Extracted at 85^{0}C, pH 2.0 and 90 minutes Extraction time

Parameter	Pectin Source	
	DLA	WLA
Solubility in cold water	Insoluble, forms suspension after vigorous shaking	Insoluble, forms suspension after vigorous shaking
Solubility in water at 85^{0}C for 15 minutes	The mixture dissolved gradually	The mixture dissolved gradually

Solubility of pectin solution in cold alkali (NaOH) 0.1M	A yellow precipitate was formed	A yellow precipitate was formed
Solubility of pectin solution in hot alkali (NaOH, 0.1M) at 40^{0}C for 10mins	The yellow precipitate dissolved and turned milky	The yellow precipitate dissolved and turned milky
Colour	Yellowish-brown	Yellowish-brown

DLA = Dried *Limonia acidissima* fruits, and WLA Wet *Limonaia acidissima* fruits

Table 3. Physicochemical composition of the extracted pectin under optimum conditions of 85°C, 90 minutes and pH 2.0

Composition	Pectin source	
	DLA	WLA
Yield of Pectin (% w/w)	16.55	18.92
Moisture content (% w/w)	1.30	1.70
Ash content (% w/w)	0.72	0.48
Equivalent weight (g/ml)	765.40	784.25
Methoxyl content (%)	4.22	6.45
Anhydrouronic Acid (% AUA)	58.60	65.25
Degree of Esterification (% DE)	67.80	78.45
pH	3.80	4.10

DLA = Dried *Limonia acidissima* fruits, and WLA Wet *Limonaia acidissima* fruits

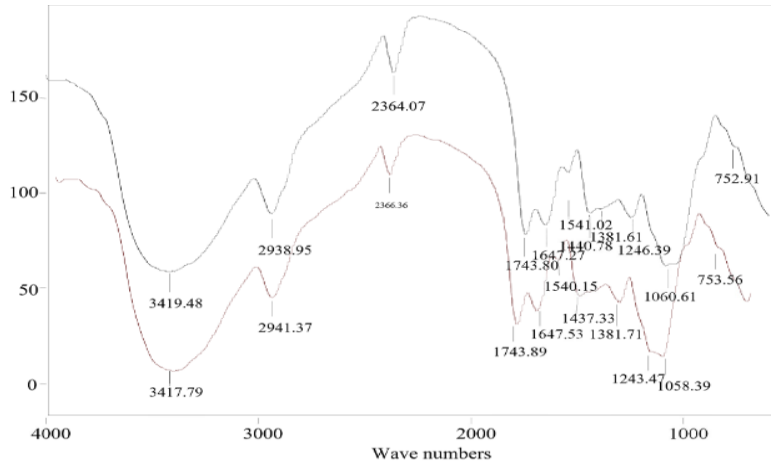

Figure 2: FTIR spectra of pectin extracted from dried and wet *Limonia acidissima* fruit

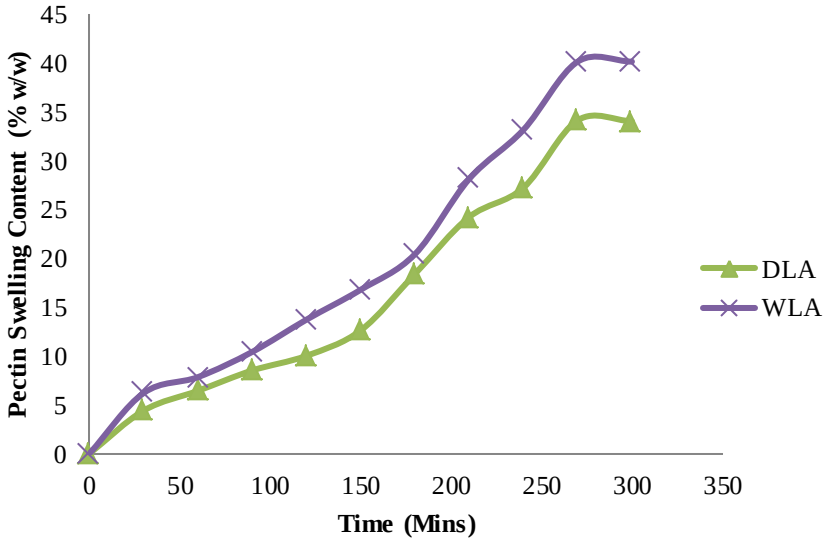

Figure 3. Swelling ratio, %, of pectin extracted at 85°C as a function of time

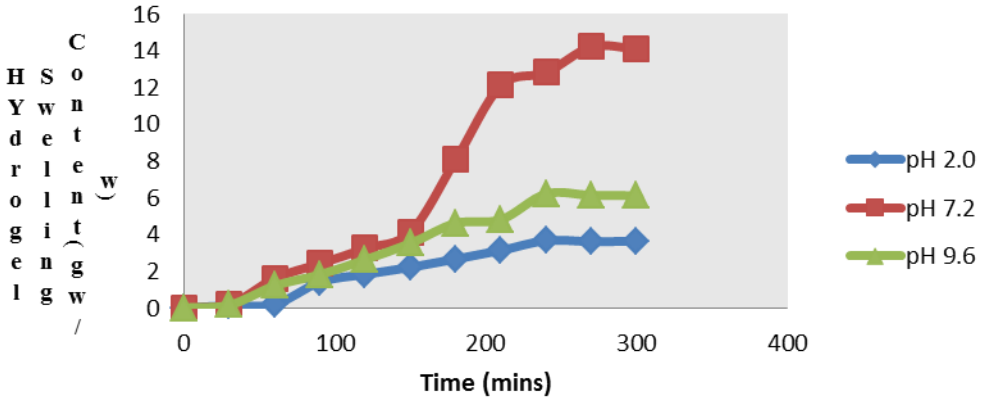

Figure 4: Effect of swelling medium pH on the swelling ratios of polymer hydrogel

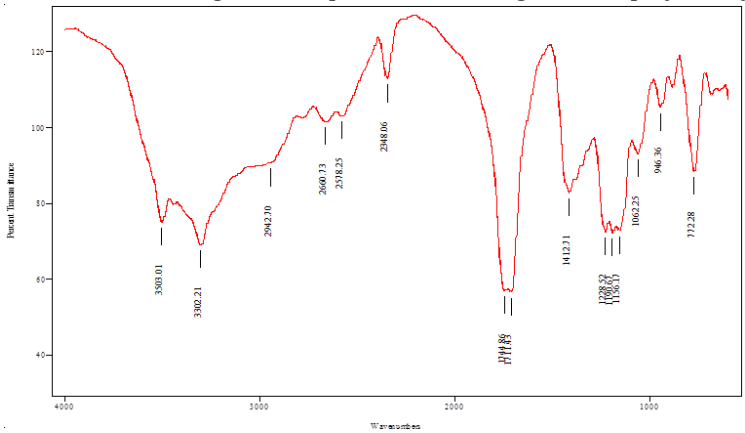

Figure 5: FTIR spectrum of WLA hydrogel formulated from pectin extracted from *Limonia acidissima* fruits

4. Conclusions

This research work showed that *Limonia acidissima* fruits can serve as a good alternative source of pectin for commercial purpose which is a potential raw material for food and pharmaceutical industries. Considering the fact that a reasonable percentage yield was obtained under optimal conditions of temperature, extraction time and pH and these conditions are technologically attainable. The physico-chemical characteristic properties were found to be within the commercially available products and this can be utilized for the formulation of natural hydrogel polymer instead of synthetic ones which the cross-linking agents needed to be removed before it application. Pectin and hydrogels have wide fields of application such as food industries, pharmaceutical industries and water treatment since it has the potential of removing metals like lead and mercury.

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