Original Article

Evaluation of Crude Pectin Obtained From Green Citrus Peels Of Sweet Orange (*Citrus Sinesis*) Grape (*Citrus Paradisi*) and Lime (*Citrus Acida*)

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Abstract
Crude pectin was extracted from the peels of sweet orange (Citrus sinesis), grape (Citrus paradisi) and lime (Citrus acida), using the hot water bath procedure. Varying extraction conditions one at a time, a maximum yield of 26 % was obtained from Citrus sinesis. Optimum conditions for the process were established to be pH of 1.5, temperature of 80 °C and time 60 minutes. Qualitative and quantitative analyses showed that the pectin from these three sources were the same in color and texture. They were all soluble in hot alkali and water. The moisture content, ash content and methoxyl content, were all higher in the pectin extracted from C. sinesis and had the values 7.6, 9.80, 4.717 respectively. However, the equivalent weight of the pectin extracted from the peels of lime was higher with 684.9 mg/mol. The total anhydrouronic acid content and degree of esterification were also calculated using the equivalent and methoxyl content gotten. The overall results point towards the amenability of the extracted pectin for industrial applications.

Keywords: Pectin, Citrus peels, Agricultural wastes

Introduction
Sweet orange, grape and lime are citrus fruits commonly grown in Benue State, Nigeria. These fruits are made up of two parts; the outer peels and the pulp. The pulp usually serves as the edible part of the fruit while the outer peels is thrown away thus generating waste to the environment (Maric *et al*, 2018). These peels have been reported to be a good source of pectin (McGready, 1996). Thus the once wastes could be processed into a valuable by-product such as pectin, and hence, protecting the environment from plant wastes (Picot-Allain *et al*, 2020)

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1925 by Heneri Bracannot (Gama *et al*., 2015). In the presence of saccharine and small quantities of organic acids (usually citrus acids), pectin is gelatinized and this property is exploited by the agro chemistry and pharmaceutical industries (Naqash *et al*, 2017).

Pectin substances are found in higher ûowering plants. They are the constituents of cell walls and, together with other components, determine the rigidity and elasticity of cell walls, turgor and the resistance of plants to drought and low temperatures (Thakur *et al.,* 1997). Pectin substances provide water-salt exchange, exhibit a high gelation capacity, play an important role in the nutrition of humans as components of food ûbers and have a wide spectrum of physiological activity (Pi *et al*, 2019; Nasseri *et al.,* 2008). The structure of pectin substances depends on many factors and can substantially vary during the growth and development of the plant. It is not surprising that pectin polysaccharides are considered as one of the most complicated and structurally dynamical classes of biopolymers.

Pectin is well suited for applications in acidic food products because of its good stability at low pH values (Dranca *et al*., 2018). It is widely used to impart gel formation, thickening and physical stability to a wide range of foods mainly fruit based products (Belkheiri *et al*., 2021). In dairy applications, especially low pH dairy drinks the consumption of pectin has been growing rapidly in recent years. Pharmaceutical and cosmetic applications are also areas where the much different pectin is being utilized (Devi *et al.,* 2014).

The present investigation aims to extract pectin from the peels of citrus fruits namely; *Citrus sinensis* (Orange), *Citrus paradisi* (Grape fruit) and *Citrus acida* (Lime) and to characterize the extracted pectin by both qualitative and quantitative methods.

Materials and Methods

Sample Preparation

Green lime (*citrus acida*), sweet orange (*citrus sinesis*) and grape fruit (*citrus paradisi)* were gotten from the local markets within Makurdi, Benue State. The fruits were physically examined to ascertain their wholesomeness. The three samples were washed with large quantity of water to remove the glycosides and the bitter taste of the peels. The three samples were finely peeled and oven dried for four days at 40 ^{o}C. The samples were then grinded into fine power which was sieved and kept in an air tight container and stored for further analysis.

Extraction of Pectin

The extraction procedure was based on method given by Kratchanova. 5 g of the peeled power was weighed into a 250 mL conical flask and 150 mL of distilled water added and stirred. To maintain a pH medium of 1.5 which was found to be the optimum pH, 45 g of citric acid (99.9%) was added. The mixture was heated in a hot water bath and stirred thoroughly after every 10 min for 1h. The solution was filtered using a muslin cloth and the residue discarded while the filtrate was kept in a beaker to cool. Ethanol (95 %) was added into the beaker and a gelatinous substance was formed. The solution was filtered using whatman filter paper (0.4mm). The gel was collected into a petri-dish, dried in an oven at 40 ^{o}C for 24 h and kept for further analysis. The percentage yield of pectin produced was calculated using equation 1 below:

$$\text{Percentage yield} = \frac{W1}{W2} x\ 100 \qquad - \qquad (1)$$

Where;
W_1 = dry weight of pectin in grams
W_2= weight of sample peel powder in grams

Pectin Characterizations

The dried pectin obtained from the various peels of the three samples was subjected to the following qualitative and quantitative tests to characterize them.

Color and Texture:

This was done by human observation.

Solubility of Dry Pectin in Cold and Hot Water:

0.25 g of the pectin samples were separately placed in two conical flasks with 10 mL of 95% ethanol added followed by 50 mL distilled water. The mixture in the second flask was shaken vigorously to form a suspension which was then heated at 85-95 ^{o}C for 15 min (Georgiev *et al*., 2012).

Solubility of Pectin Solution in Cold and Hot Alkali

To 1 mL 0.1 N NaOH, 5 mL pectin solution was added and then heated at 85-90 ^{o}C for 15 min (Muhamadzadeh *et al*., 2010).

Moisture Content

Dried empty petri-dishes were dried in an oven, cooled in a desiccator and weighed. 5 g of the pectin samples each were transferred into the petri-dishes and placed in the oven at 130 ^{o} C for 1h. Thereafter the petri-dishes were removed, cooled in a desiccator and weighed. This was repeated until a constant sample weight was obtained. The moisture content for each of the samples was calculated using equation 2 below:

$$\text{Moisture content (\%)} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100 - \quad (2)$$

Ash Content

5 g of each of the samples were transferred into a weighed empty crucible separately. The crucible was transferred to a furnace and set at 600 ^{o}C to burn off all the organic matter. The carbon charred and then burnt off as carbondioxide, leaving a dark ash. This process lasted for 24 h .The crucible was kept in a desiccators to cool. After cooling, the crucible was weighed again. This was calculated using equation 3 below:

$$\text{Ash Content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 - \quad (3)$$

Equivalent Weight Determination

Equivalent weight was used for calculating the anhydrouronic acid content and degree of esterification. It was determined by titration with sodium hydroxide to pH 7.5 using phenol red indicator (Ranganna, 1995). 0.5 g of pectin sample was weighed into a 250 mL conical flask and moistened with 5 mL ethanol, 1 g sodium chloride was added to the mixture to sharpen the end point followed by 100 mL distilled water and 6 drops of phenol red indicator. Care was taken at this point to ensure that all the pectin had dissolved and that no clumping occurred at the sides of the flask before the solution was then slowly titrated (to avoid possible de-esterification) with 0.1 M NaOH. Titration point was indicated by a purple color at the end point. This neutralized solution was stored for determination of methoxyl content. Equivalent weight was calculated using equation 4 below:

$$\text{Equivalent Weight} = \frac{\text{weight of sample} \times 1000}{\text{Volume of alkali} \times \text{Molarity}} \quad (4)$$

Methoxyl Content (MeO)

The methoxyl content is an important factor in controlling the setting time of pectin, the sensitivity to polyvalent cations and their usefulness in the preparation of low solid gels, fibres and film. It was determined by saponification of the pectin and titration of the liberated carboxyl groups. Determination of MeO was done following the Ranganna's method. The neutral solution was collected from determination of equivalent weight and 25 mL of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min, 25 mL of 0.25 N hydrochloric acid was added and titrated against 0.1 N NaOH to the same end point as in equivalent weight titration. The Methoxyl Content was calculated using equation 5 below:

$$\text{Methoxyl Content} = \frac{\text{mL of alkali} \times \text{Molarity of alkali} \times \text{molecular unit of MeO}}{\text{Weight of sample}} - (5)$$

Where; molecular unit of MeO = 31

Total Anhydrouronic Acid Content (AUA)

Estimation of anhydrouronic acid content is essential to determine the purity and degree of esterification and to evaluate the physical properties. Pectin which is a partly esterified polygalacturonide contains 10 % or more of organic materials composed of arabinose, galactose and perhaps sugars. Making use of the equivalent weight and methoxyl content value of titre used, the total AUA of pectin was obtained bythe formula below (Mohamed and Hassan, 1995).% AUA =

$$\frac{176 \ x \ 0.1z \ x \ 100}{W \ x \ 1000} + \frac{176 \ x \ 0.1Y \ x \ 100}{W \ x \ 1000}$$

Where;

molecular unit of AUA (1 unit) =176

z = titre value of NaOH from equivalent weight determination.

Y = titre value of NaOH from methoxyl content determination.

w = weight of sample

Determination of Degree of Esterification (DE)

The DE of pectin was measured on the basis of methoxyl and AUA content (Owens *et al.*, 1952) and calculated using equation 6 below:

$$\% \ DE = \frac{176 \ x \ \%MeO}{31 \ x \ \%AUA} \ x \ 100 \qquad (6)$$

Where; % MeO = Methoxyl content,
% AUA = Anhydrouronic Acid Content

Results

Percentage Yield

The percentage yield of pectin obtained for the three citrus peels samples; grape (*Citrus paradisi*), sweet orange (*Citrus sinesis*) and lime (*Citrus acida*) is as presented in figure 1 below. Citrus *Sinesis* recorded the highest yield of 26% while that for *Citrus paradisi*and *Citrus acida* were 17.6% and 9.1% respectively.

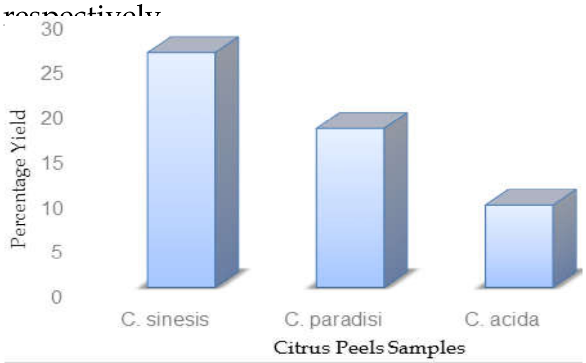

Fig 1: Comparative chart showing percentage yield of crude pectin.

Physicochemical parameters

The result on the quantitative and qualitative tests for the three citrus peels is as shown in tables 1 and 2 below.

Table 1 shows the quantitative results on some of the physicochemical parameters considered, some of which include; the moisture content, ash content, equivalent weight, methoxyl content, anhyrouronic acid content and the degree of esterification. Amongst the three citrus peels samples used, *C. Sinesis* recorded the highest percentage of each of the parameters considered except for the anhydrouronic acid content and degree of esterification where *C. paradisi* and *C. acida* recorded highest respectively.

Table 2 reflects results on parameters such as color, texture, solubility of pectin in cold and hot water, solubility of pectin in cold and hot alkali. The color of the pectin extracts were consistently brown and coarse in texture for all the citrus peels samples analyzed. They were observed to be insoluble in cold water and cold alkali but soluble in hot water and hot alkali.

Table 1: Quantitative Tests for the three citrus peels samples

Quantitative test	*C. paradisi*	*C.sinesis*	*C. acida*
Moisture content (%)	6.96	7.60	5.80
Ash content (%)	8.20	9.80	6.40
Equivalent weight (mg/mol.)	295.85	531.42	684.90
Methoxyl content (MeO) (%)	4.087	4.717	4.498
Anhydrouronic Acid content	68.64	43.29	36.26
Degree of esterification (%)	33.85	61.86	70.43

Table 2: Quantitative Test for the three citrus peels Samples

Parameters	*C.paradisi*	*C. sinesis*	*C.acida*
Colour	Brown	Brown	Brown
Texture	Coarse	Coarse	Coarse
Solubility of dry pectin in cold water	Insoluble	Insoluble	Insoluble
Solubility of dry pectin in hot water	Soluble	Soluble	Soluble
Solubility of dry pectin in cold alkali	Forms yellow ppt.	Forms yellow ppt.	Forms yellow ppt.
Solubility of dry pectin in hot alkali	Soluble turns milky	Soluble turns milky	Soluble turns milky

Discussion

From Table 1, the percentage moisture content obtained from *C. paradisi, C. sinesis and C. acida* samples were 6.96 %, 7.60 % and 5.8 % respectively. High moisture content could enhance the growth of micro-organisms and production of pectinase enzymes that can further affect the pectin quality (Muhamadzadeh *et al.*, 2010).

The percentage ash content obtained was 8.2 % for C. *paradisi*, 9.8 % for *C. sinesis* and 6.4 % for *C. acida*. *C. sinesis* had the highest percentage yield of pectin. Low ash content (below 10%) and maximum limit of ash content (10%) are one of the good criteria for gel formation. Therefore, the ash content in this experiment indicates the purity of the pectin. The equivalent weight in mg/mol. were found to be 295.85 for *C. paradisi*, 531.92 for *C. sinesis* and 684.90 for *C. acida*, the lower equivalent weight could be due to the higher partial degradation of pectin. The decrease or increase of the equivalent weight might be also dependent on the amount of free acid (Ismail *et al*., 2012).

The methoxyl content is an important factor in controlling the setting time of pectin and the ability of the pectin to form gels. The methoxyl content of pectin usually varies from 0.2-12 % depending on the source and mode of extraction (Ismail *et al*., 2012). Among the pectin extracts from the three sources studied, the methoxyl content varied from 4.087 % (*C. paradisi)*, 4.717 % *(C. sinesis)* and 4.498 % *(C. acida)*, the values thus falling within range. All the values obtained experimentally were below 7 % thus the pectin samples were of low ester indicating that the pectin is good in terms of quality. The degree of esterification was determined to be 33.85 % in *C. paradisi*, 61.86 % in *C sinesis* and 70.4 % in *C. acida*. From literature, pectin samples with high DE values for commercial HM pectin ranges from 60-75% and those of LM pectin ranges from 20-40 % (Georgiev *et al*., 2012). Anhydrouronic acid content indicates the purity of extracted pectin the values of which should not be less than 65 % (FAO. 1969). The AUA obtained from *C. paradisi* pectin was above 65 %, indicating its purity. *C. sinesis* and *C. acida* were however below 65 %. Low values of AUA means that the extracted pectin might have a high amount of protein (Hwang et al., 1993).

From Table 2, the characteristic color and texture of pectin obtained from the three samples were all brown. According to literature, pectin is usually light in color and coarse, factors such as surface contamination, environmental factors and types of fruits used as well as human error might have contributed to the discrepancy in color. This could be due to the amount of ethanol used for precipitation and purification during the experiment not been enough (Owens *et al*., 1952). In cold alkali (NaOH), the pectin suspension obtained from the samples gave a yellow gelatinous color which turned white when heated at 90 ^{o} C for 15 min. This is in agreement with literature where pectin extracts are reported to be unstable under alkaline solution which corresponded with what was obtained from this research (Georgiev *et al*., 2012).

Conclusion

From this study, extraction and characterization of crude pectin from green citrus peels was successfully carried out. Pectin was extracted from the peels of *C. paradisi, C. sinesis* and *C. acida*, their characteristics were compared, with *C. sinesis* having the highest yield. Series of qualitative and quantitative tests were carried out on the pectin produced and it was found that pectin obtained from lime had higher equivalent weight compared to those of sweet orange and grape. Grape had higher degree of esterification and anhydrouronic acid content. Methoxyl content, ash content and moisture content was high in grape and lime but was found to be more in sweet orange. The pectin was observed based on the texture and color; they were all brown in color and coarse. Thus this work has facilitated the optimized production of pectin from different citrus peels and their characterizations, with the pectin, especially the one from *C. sinesis* exhibiting properties for industrial applications in food industries, pharmaceuticals, health care, personal care etc. Pectin from these citrus peels could help solve two major problems within Makurdi, this conversion and utilization of citrus peels waste generated as well as reduce the cost of production for pectin manufacturers

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