Extraction and Characterization of Pectin from Peels of Lemon (Citrus limon), Grape Fruit (Citrus paradisi) and Sweet Orange (Citrus sinensis)

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Abstract: This research study aimed at comparing the characteristics of three different pectins and to see which one is more suitable for industrial applications. Pectin, which is a family of complex polysaccharides that contains 1, 4-linked x and β galactosyluronic acid residues was extracted using alcohol precipitation method from peels of lemon, grape and sweet orange after which it was characterized using both qualitative and quantitative analysis to determine and compare the color equivalent weight methoxyl content, each content solubility in cold and hot alkali, pH as well as sugar and organic acid. The result showed that the colors of the pectin from these 3 sources were the same i.e., Brown they were all soluble in hot and cold alkali and water, the moisture content, the methoxyl content and the ash were all higher in the pectin extracted from peels of sweet orange with 95.25, 5.79 and 35%, respectively. However, the equivalent weight of the pectin extracted from peels of grape was higher with 793.6 mg/mol. The overall results showed that the pectin from these sources were suitable for industrial use.

Keywords: Citrus fruits, methoxyl content, pectins, peels, polygalacturonic acid

INTRODUCTION

Grapes, Lemon and Sweet orange are citrus fruits which consist of two parts namely the peels (rind skin) and pulp. These two parts are easily separated from each other with the pulp serving as the edible parts of the fruit while the peels as a good source of pectin (McGready, 1996).

Pectin is a purified carbohydrate product obtained from the inner portion of the rind/peels of citrus fruits. It consists chiefly of partially methoxylated polygalacturonic acid. Pectin is capable of forming gels with sugar and acid under suitable conditions. It is formed almost universally in plant cell of all species suitable for use in the production of sugar jellies and industrial production of apple pomace, citrus peels and sugar beat chips. Pectin extracted from various materials can be different in molecular structure (i.e., molecular weight degree of esterification, acetyl content) and therefore possesses different functional properties. Typically, a whole mature fruit contains 3-7% pectin substances on a dry weight basis and 0.1-1.1% on a fresh weight basis. The relatively high pectin and low caloric content of citrus fruits make them a good source of soluble dietary fiber (Joslyn, 1980).

A valuable by product that can be obtained from fruit wastes is pectin. Pectin designates those water soluble pectinic acid (colloidal polygalacturonic acids) of varying methyl ester content and degree of neutralization which is capable of forming gels with sugar and acids under suitable conditions (GITCO, 1999).

The suitability of pectins for different purposes is determined by their character viz, anhydouronic acid content, methoxyl content, degree of esterification and acetyl values. Hence, it is an unavoidable aspect that every pectin should be described properly for its biochemical characters.

The pectins have commercial uses as agglutinator in blood therapy (GITCO, 1999) and also as thickening agent in medium used for canning meats.

Aims and objectives of the research:
These include:

- The extraction of pectin from the peels of 3 chosen citrus fruits namely orange, Lemon and Grape peels.
- Characterization of the extracted pectin to determine the solubility of dry pectin in cold and hot water, solubility of pectin solution in cold and hot alkali (NaOH) sugar and organic acid, the colour of the pectin, equivalent weight, methoxyl content, ash content, moisture content and percentage yield of the pectin.
- To compare the characteristics of three different pectins and see which one is more suitable for industrial applications.
Pectin is a family of complex polysaccharides that contain 1, 4-linked α and β galactosyluronic acid residues.

MATERIALS AND METHODS

This research work was carried out between the months of March-September, 2010 in the Biochemistry laboratories of Applied Science Department, Kaduna Polytechnic, Kaduna-Nigeria.

Sample preparation: Mature Lemon, Grape and Orange fruits were purchased from Station Market, Kaduna. The fruits were physically examined to ascertain their wholesomeness. Each of the fruits (Lemon, Grape and Orange) were split/cut into four parts and the peel removed (a soft white substance inside the skin of citrus fruits), then the peels were further cut into smaller pieces for easy drying and washed with large quantity of water to remove the Glycosides the bitter taste of the peels and then weighed with a digital weighing balance and air dried.

Pectin extraction from the prepared sample: The dried peels were separately transferred into a beaker (1000 mL) containing 500 mL of water, 2.5 mL hydrochloric acid was added to give a pH of 2.2. Each of the fruits was then boiled for 45 min separately. Thereafter, the peels were removed from the extracts by filtering through a what man No. 1 filter study. The cake was washed with 250 mL boiled water and the combined filter allowed to cool to 25 °C to minimize heat degradation of the pectin. The extracted pectin was precipitated by adding 200 mL 95% acetone to 100 mL of the extracted pectin with thorough stirring, left for 30 min to allow the pectin float on the surface. The gelatinous pectin flocculants was then skimmed off. The extracted pectin was purified by washing in 200 mL acetone and then pressed on a nylon cloth to remove the residual HCl and universal salt. The resulting pectin was weighed and shredded into small pieces and was air dried (McGready, 1996). Finally, the dried pectin was further reduced into smaller pieces using a pestle and mortar and weighed using a digital weighing balance. Percentage yield of pectin from initial wet peels was then determined on both wet and dry weight basis.

Characterization of extracted pectin: The dried pectin obtained from the various peels of the three fruits was subjected to the following qualitative and quantitative test to characterize them.

Qualitative tests:

- Colour: This was done by visual observation
- Solubility of dry pectin in cold and hot water: (0.25%) of the pectin samples were separately 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 85-95°C for 15 min (Fishman et al., 1984).
- Solubility of pectin solution in cold and hot alkali (NaOH): To 1 mL and 0.1 N NaOH was added 5ml pectin solution and then heated at 85-90°C for 15 min, (Joslyn, 1980).
- Sugar and organic acids: One gram of the pectin sample was placed separately in 500 mL flask each and moisture with 5 mL ethanol, 100 mL water poured rapidly, shaken and allowed to stand for 10 min. To this solution, 100 mL ethanol containing 0.3 mL hydrochloric acid was added, mixed and filtered rapidly, 2.5 mL of the filtrate was measured into a conical flask (25 mL), the liquid has evaporated on a steam bath and the residue dried in an oven at 50°C for 2 h.
- 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 read off.

QUANTITATIVE TEST

- Equivalent weight determination: Pectin sample (0.5 g) was weighed into a 250 mL conical flask and moistened with 5 mL ethanol, 1.0 g sodium chloride was added to the mixture followed by 100 mL distilled water and few 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 to a pink colour at the endpoint. Equivalent weight was calculated using the equation below:

\[
\text{Equivalent Weight} = \left( \frac{\text{Weight of Pectin Sample}}{\text{Volume of Alkali (cm}^3) \times \text{Molarity of Alkali}} \right) \times 100\%
\]

- Methoxyl content determination: This was done by using the neutralized solution obtained during
the equivalent weight determination by the saponification of the pectin followed by titration of the liberated acid i.e., 25 mL of 0.25 M NaOH was added to the neutralized solution used in the equivalent weight determination. The mixture was stirred thoroughly and allowed to stand for 30 min at ambient temperature. The percentage content was calculated using the equation below:

\[
\text{Methoxyl content \%} = \frac{(\text{Volume of alkali (cm}^3) \times \text{Alkali} \times \text{Weight}}{\text{Weight of Pectin Sample (mg))} \times 100
\]

- **Ash content determination:** Five grams of each of the sample was accurately weighed into a weighed empty crucible separately. The crucible was transferred to a furnace set at 60°C to burn off all the organic matter. The carbon charred and then burnt off as carbon dioxide, leaving a dark ash; this process lasted for 24 h. The crucible was taken out of the furnace and placed in a desiccator to cool. The crucible after cooling was reweighed again. This was calculated using:

\[
\text{Ash Content \%} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100
\]

- **Moisture content determination:** A dried empty petri dish was dried in an oven, cooled in a dessicator and weighed. Five grams of the pectin samples was transferred into the crucibles in the oven which was set at 130°C for 1 h thereafter the petri dish was removed, cooled in a dessicator and weighed. This process was repeated once. The moisture content was calculated using:

\[
\text{Moisture Content \%} = \frac{\text{Weight of the Residue}}{\text{Weight of the Sample}} \times 100%
\]

**RESULTS AND DISCUSSION**

From Table 1, the characteristic color of pectin obtained from the three samples were all brown while according to IPPA (2009), pectin are usually light in color, factors such as surface contamination, environmental factors, types of fruits used and human error might have contributed to the discrepancy in color, this could be due to the amount of acetone used for precipitation and purification during the experiment not been enough McGready (1996). In cold alkali, (NaOH), the pectin suspension obtained from the fruits gave a yellow gelatinuous color which turned white when heated at 85-90°C for 15 min, however, (Fishman, 1993) stated that pectins are unstable under alkaline solution which corresponded with what was obtained from this research.

From Table 2, the equivalent weight in mg/mL found to be 694.44, 793.60, 534.75, respectively, while the % methoxyl content determined from the three fruits were 4.64, 3.90 and 5.79%, respectively, 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, thus, the percentage

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lemon</th>
<th>Grape</th>
<th>Sweet orange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Solubility in cold water</td>
<td>Insoluble, inubides smells after vigorous shaking and forms suspension.</td>
<td>Insoluble, inubides, swells after vigorous shaking, form suspension.</td>
<td>Dissolved slightly and form suspension after vigorous shaking</td>
</tr>
<tr>
<td>Solubility at 85-90°C for 15 min</td>
<td>The mixture dissolves</td>
<td>The mixture dissolves</td>
<td>The mixture dissolve</td>
</tr>
<tr>
<td>Solubility of pectin suspension in cold alkali</td>
<td>The pectin suspension forms yellow precipitated</td>
<td>The pectin suspension forms yellow precipitate</td>
<td>The pectin suspension forms yellow precipitate</td>
</tr>
<tr>
<td>Solubility of pectin suspension in hot alkali</td>
<td>The pectin suspension dissolve and turned milky</td>
<td>The pectin suspension dissolve and turned milky</td>
<td>The pectin suspension dissolve and turned milky</td>
</tr>
<tr>
<td>Sugar and organic acid</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S/No (%)</th>
<th>Lemon</th>
<th>Orange</th>
<th>Grape</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.71</td>
<td>15.92</td>
<td>15.70</td>
</tr>
<tr>
<td>2</td>
<td>2.760</td>
<td>1.680</td>
<td>2.310</td>
</tr>
<tr>
<td>3</td>
<td>694.44</td>
<td>534.00</td>
<td>293.60</td>
</tr>
<tr>
<td>4</td>
<td>4.460</td>
<td>5.790</td>
<td>3.900</td>
</tr>
<tr>
<td>5</td>
<td>66.60</td>
<td>95.25</td>
<td>80.95</td>
</tr>
<tr>
<td>6</td>
<td>30.00</td>
<td>35.00</td>
<td>34.50</td>
</tr>
<tr>
<td>7</td>
<td>4.100</td>
<td>3.600</td>
<td>4.000</td>
</tr>
</tbody>
</table>
methoxyl content obtained falls within the range. Since all the values obtained experimentally were below 7%, hence the pectin were of low ester characterization indicating that the pectin is good in terms of quality. The percentage moisture content obtained from the 3 fruits was 66.6, 80.95 and 95.23%, respectively. The percentage yields of the pectin on wet basis are 16.71, 15.92 and 15.70% while on dry basis were 2.7, 1.08 and 2.31%, respectively. Grape had the highest percentage yield of pectin. Joslyn (1980) The pH determination for the pectins were found to be lemon 4.1, grape 4.0 and sweet orange 3.6, aqueous solution of pectins are slightly acidic (Fishman et al., 1984).

Conclusively, the peels of lemon, grape and sweet orange are good sources of pectin which were extracted using alcohol precipitation.

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REFERENCES