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RESEARCH ARTICLE

EXTRACTION, PHYSICOCHEMICAL ANALYSIS AND CHARACTERIZATION OF PECTIN FROM SWEET ORANGE (*Citrus sinensis*), LIME (*Citrus aurantifolia*) AND LEMON (*Citrus limon*)

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ABSTRACT

Pectin was extracted by a modified McGready (1996) method from *Sinensis*, *Aurantifolia* and *Limon* species of citrus and then precipitated by 95% acetone. Furthermore, the obtained pectin was qualitatively and quantitatively characterized to compare certain physical and chemical properties. Obtained results indicated that the three species have the same colour, brown and are insoluble in both cold water and cold alkali but soluble at elevated temperature. The equivalent weight in mg/mL was found to be 1849.62, 1388.89 and 1720.20 for *C sinensis*, *C aurantifolia* and *C limon* respectively, while the % methoxyl content determined gave the value of 4.19, 5.60 and 4.51% in the same order. The percentage moisture content obtained from the 3 fruits was 60.53, 59.80 and 78.00%, for *C sinensis*, *C aurantifolia* and *C limon* respectively. The percentage yields of the pectin on wet basis are 35.44, 36.48 and 30.09% while on dry basis were 3.74, 3.85 and 4.97%, in the same order with *C limon* having the highest percentage yield of pectin on dry basis. The pH determinations for the pectins were found to be *C sinensis* 4.3, *C aurantifolia* 4.9 and *C limon* 5.0.

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INTRODUCTION

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 beet 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). Commercially, pectins are extracted from citrus peel (Chakraborty and Ray, 2011) but other sources of pectin include cocoa husk (Mollea *et al.,* 1995), sunflower head (Matora *et al.,* 1995), beet and potato pulp (Turquois *et al.,* 1999), soy hull, (Kalapathy and Proctor, 2001) and duckweed (Golovchenko *et al.,* 2002). In a broad sense two types of pectin are available in nature including high methoxyl pectin (greater than 50% degree of esterification) and low methoxyl pectin (Zhang and Taihua, 2011).

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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, anhydrouronic acid content, methoxyl content, degree of esterification and acetyl values. Hence, it is an unavoidable aspect that pectin should be described properly for its biochemical characters. Citrus fruits are among the most important tree fruits crops in the world. Citrus is widely grown in Nigeria and many other tropical and sub-tropical regions. Although oranges are the major fruit in the citrus group accounting for about 70% of citrus output which includes lime, lemons and grape fruits (Lacirignola and D'onghia 2009). They are small to large leathering and yellow to orange in colour when ripe. The pulp and juice may vary in taste from sweet to acid and the segment may vary from 8 – 18 but usually 10 – 14. Seeds may be many, few or none at all as in tangelo (Okpeke, 2005). The aim of this research is to extract pectin from three species of Citrus namely *Citrus sinensis*, *Citrus aurantifolia* and *Citrus limon*. The objectives are to characterize the extracted pectin and comparing the characters of the pectin from these three sources.

MATERIALS AND METHODS

Metler Toledo digital balance, Laboratory oven by Surgifield, England, pH meter, Vacuum extractor. Acetone, Ethanol, HCl, NaCl, NaOH all manufactured by BDH England.

Sample preparation

Mature citrus fruits (*Citrus sinensis, Citrus aurantifolia* and *Citrus limon)* were purchased from Anyigba central Market, Kogi State Nigeria. The fruits were examined to ascertain their wholesomeness. Each of the fruits 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 plenty of water to remove the Glycosides (the bitter taste of the peels) and then weighed with a digital weighing balance before being air dried to constant weight in the oven.

Extraction of Pectin from Citrus samples

Extraction was carried out according to McGready (1996) with slight modification. The dried peels were transferred into a beaker (1000 mL) containing 650 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. Thereafter, the peels were removed from the extracts by filtering through a whatman No. 1 filter study. The extracted pectin was washed with 250 mL boiled water and the combined filter allowed to cool to 25°C to minimize heat degradation. The extracted pectin was precipitated by adding 200 mL 95% acetone to 100 mL of the extracted pectin with thorough stirring and 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. 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.

- **Colour:** This was done by visual observation
- **Solubility of dry pectin in cold and hot water:** This was carried out by the method of Fishman *et al*., 1984. Briefly, (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.
- **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).
- **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 was read off.
- **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: Equivalent Weight = (Weight of Pectin Sample / Volume of Alkali (cm^3) × Molarity of Alkali) × 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:

Methoxyl content % = (Volume of alkali (cm^3) × Alkali × Weight / Weight of Pectin Sample (mg)) × 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:

Ash Content (%) = (Weight of Ash / Weight of Sample) × 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:

Moisture Content (%) = (Weight of the Residue / Weight of the Sample) × 100%

Statistical analysis

Mean values ±SD of replicate determinations were taken for each analysis. Significantly, different results were established at $p = 0.05$ confidence level.

RESULTS AND DISCUSSION

The characteristic colour of pectin obtained from the three samples were dark brown for *Citrus sinensis,* dark brown for *Citrus aurantifolia* and light brown for *Citrus limon* while according to IPPA (2009), pectin are usually light in color.

Table 1. Results for the physicochemical analysis of the samples

Parameters	*Citrus sinensis*	*Citrus aurantifolia*	*Citrus limon*
Colour	Dark brown	Dark brown	light brown
Solubility in cold water	insoluble suspension	insoluble suspension	insoluble suspension
Solubility in hot water	mixture dissolves	mixture dissolves	mixture Dissolves
Solubility in cold alkali	Yellow ppt formed	Yellow ppt formed	Yellow ppt formed
Solubility in hot alkali	Suspension dissolves	Suspension dissolves	Suspension dissolves

Table 2. Results showing Characterized parameters of the samples

Parameters	*Citrus sinensis*	*Citrus aurantifolia*	*Citrus limon*
% Yield of pectin (wet)	35.44±1.08	36.48±0.09	30.09±0.23
% Yield of pectin (dry)	3.74±0.01	3.58±0.29	4.97±0.01
Equivalent weight (mg/mol)	1849.62±10.43	1388.89±6.63	1720.20±6.82
Methoxyl content	4.19±0.02	5.60±0.03	4.51±0.02
Moisture content	60.53±0.42	59.80±1.59	78.00±2.00
Ash content	1.83±0.76	1.83±0.29	1.00±0.50
pH	4.3	4.9	5.0

The reasons for this difference could be due to factors such as surface contamination, environmental factors and types of fruits used might have contributed to the discrepancy in color. It could also be due to the amount of acetone used for precipitation and purification during the experiment (McGready, 1996). Table 1 summerizes the results of the qualitative analysis of the samples. In cold alkali, (NaOH), just like in cold water, the pectin suspension obtained from the fruits gave a yellow gelatinuous precipitate which turned white on activation for 15 min. This shows the unstable nature of pectin in alkaline solutions. The obtained result is in agreement with literature (Fishman *et al*., 1993). From Table 2, the equivalent weight in mg/mL was found to be 1849.62, 1388.89 and 1720.20 for *C sinensis*, *C aurantifolia* and *C limon* respectively, while the % methoxyl content determined gave the value of 4.19, 5.60 and 4.51% in the same order. These values are fall within the agreed limit as confirmed from literature. Kumar and Chauchan (2010) showed that equivalent weight range from 368 – 1632 for citrus pectin and Aina et al., (2012) demonstrated that methoxyl content varies from 0.2 – 12%. Since all the values obtained experimentally were below 7%, it therefore means that the pectin extracted were of low ester characterization indicating that it is good in terms of quality. The slight difference could be due to geographical location. The percentage moisture content obtained from the 3 fruits was 60.53, 59.80 and 78.00%, for *C sinensis*, *C aurantifolia* and *C limon* respectively. The percentage yields of the pectin on wet basis are 16.71, 15.92 and 15.70% while on dry basis were 3.74, 3.85 and 4.97%, in the same order with *C limon* having the highest percentage yield of pectin. The pH determinations for the pectins were found to be *C sinensis* 4.3, *C aurantifolia* 4.9 and *C limon* 5.0. According to Axelos *et al*., (1991) degree of methylation affects stability of pectin and since low pectin is stable at pH 5 – 6 it may mean that there is a greater degree of methylation here. Also, added is the fact that aqueous solution of pectin is slightly acidic (Fishman *et al.*, 1984).

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