Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):283-290

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Extraction and Characterization of Pectin from some selected non-citrus agricultural food wastes

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ABSTRACT

This research study aimed at extracting pectin using 1NHNO_3, at different temperatures (60, 90 and 100^{0}C) and times (60, 90 and 120 min) from five different non-citrus agricultural wastes namely: Telfairia occidentalis (Pumpkin seed peel-psp), Telfaria occidentalis (Pumpkin seed white pod-pwp,) Artocarpus camanis (Breadfruit seed peel-bsp), Artocarpus camanis (Breadfruit seed peel-bsp), Artocarpus camanis (Breadfruit creamy peel-bcp) and Mucuna urens (Horse eye bean peel-hbp). Preliminary results showed that optimum condition for extraction of pectin was at a temperature of 100^{0}C for 90 min with horse eye bean peel recording the highest pectin yield (4.40%), while psp recorded the least (2.81%) on dry weight basis. Pectin yield increased with increase in temperature, however there was decrease in yield after 90mins for all samples. The pectin obtained was characterized using both qualitative and quantitative analysis. The result for qualitative analysis showed that the pectin from the five samples was found to be brownish in colour. As for the solubility, the samples were all soluble in hot water and partially soluble in cold water. Quantitatively, the equivalent weight (1471-1923mg/mol), methoxyl content (1.48-2.48%), total anhydrouronic acid content (14.08-20.78%), degree of esterification (55.48-71.47%) and neutral sugar (0.193-0.769%) were significantly ($p \leq 0.05$) different among the samples. The overall results showed that the pectin from these non-citrus agricultural food wastes will be suitable for industrial use.

Key words: Pectin, Non-citrus, Agricultural food wastes, Qualitative analysis, Quantitative analysis.

INTRODUCTION

Pectin (pectic polysaccharide) is a family of variable mixtures of polysaccharides imbedded in the primary cell wall and middle lamella of higher plants. Pectin is responsible for different physiological processes such as structural stability and cell to cell adhesion [1] and may be linked covalently to other polymers [2]. The main constituent of pectin is D-galactouronic acid polymers whose subunits are connected by D-α-(1→4) glycosidic linkages. These uronic acids have carboxyl groups some of which are present as esterified methyl (methyl esters) and others are treated with ammonia commercially to produce carboxamide groups. Their hydroxyl groups are partially acetylated [3,4]. Rhamnose (Rha) is a minor component of the pectin side chain linked to arabinogalactan and other neutral sugars such as D-xylose, D- glucose, and L- fructose which are sometimes present in the side chain. The concentration of pectin as well as other properties such as chemical composition and structure varies from one plant to another and in the different parts of the plant where it is located [5]. Pectin is majorly found in citrus fruits; for instance as fruit ripens the pectin content reduces drastically because pectinesterase and pectinases rapidly breakdown the pectin molecules leading to softening of the fruit [6]. In the presence of acids and sugars, a component of pectin (High Methoxyl Pectin -HM-Pectin) can form gel which constitutes major function in food

industries [7]. Pectin has applications in the food, pharmaceutical and biotechnology industry. It is popularly used as stabilizers, water binders, thickeners in yoghurts and fruit juices. It reduces blood cholesterol levels, detoxifies the system and used in the treatment of diarrhea in children [6, 8, 9,10,11,12]. Commercial pectin is primarily extracted from citrus peels and apple pomace [13]. Other sources of pectin include sugar beet pulp [14] and sunflower head residue [15].

Wastes arising from agricultural produce, have constituted enormous challenges to a clean and safe environment and thus need to recover food wastes into useful product such as pectin has always occupied a prime position in nutritional research. The current research looks at pectin extraction from five food wastes namely; pumpkin seed peel (psp), pumpkin seed white pod (pwp), breadfruit seed peel (bsp), breadfruit creamy pulp (bcp) and horse eye bean peel (hbp) with the aim of sourcing for the new potential utilization of these agro food wastes

EXPERIMENTAL SECTION

Materials

Five agro food wastes were used as the source of pectin namely; *Telfairia occidentalis* (Pumpkin seed peel-psp), *Telfeiria occidentalis* (Pumpkin seed white pod- pwp), *Artocapus camanis* (Bread fruit seed peel- bsp), *Artocapus camanis* (Bread fruit creamy pulp -bcp) and *Mucuna urens* (Horse eye bean peel -hbp) were bought from Creek road market in Port Harcourt, Rivers State, Nigeria. Samples were identified in the Herbarium of the department of Plant Science and Biotechnology, University of Port Harcourt. All chemical and reagents used were of analytical grade.

Sample preparation

The pumpkin pods were washed and cut open; the seeds were collected and the edible portion removed while the peels were reserved for analysis. The waste pods were diced into pieces. The breadfruits were cut open, the edible portions in the seed were removed leaving behind the seed peels and the creamy pulp which formed part of the peels. The horse eye beans were also cut open the seed peels collected after removing the edible parts. All the reserved wastes were dried at 50^0C to obtain a constant weight. The dried samples were crushed to fine powder and stored in a sample bottle at room temperature (25-27^0C) until ready for use.

Pectin extraction

Pectin extraction for all dried samples was carried out using the method of Azad et al., [16]. The extraction was carried out using a solution of 1 N HNO_3 at varying temperatures (60, 80^0C 100^0C) and times (60, 90, 120 min). Sample weight of 20g each was transferred into a 250ml beaker containing 100ml of deionized water and 2.5ml HNO_3 with a pH of 1.76. The mixture was placed in a water bath (at a particular temperature) with a constant shaking until the desired extraction time elapsed. The sample was cooled, thereafter the residue was removed and the filtrate collected through filtering with Whatman No1 filter paper. The filtered solution was combined with twice the volume of ethanol (i.e. 1 parts of the filtrate to 2 parts of 95% ethanol) and kept at room temperature overnight for the pectin to form precipitate. The precipitated pectin was separated from ethanol solution using a double layer cheese cloth and the samples were washed three times with 70% ethanol (%), 85% ethanol (%) and absolute ethanol to remove soluble impurities. The resulting pectin was dried at a temperature of 40^oC-50^0C in an aluminum sample dish until a constant weight was obtained. Samples were cooled in desiccators and the weight measured after cooling. The weighted sample was further ground using a laboratory mortar with pestle. Ground sample was stored in a sample bottle until ready for analysis.

Qualitative test for pectin

Colour

Colour was identified by visual observation.

Solubility of dry pectin in cold and hot water

An amount (0.1g/100g) of the dry pectin from each sample was measured into a conical flask containing 5ml of 95% ethanol and 25ml distilled water. The mixture was thoroughly shaken to form a suspension which was heated for 15 min [17].

Quantitative test for pectin

Determination of equivalent weight (EqW):

Equivalent Weight was determined according to the method of Ranganna [18]. Pectin sample (0.25g) was weighed into a 250ml conical flask; followed by the addition of 5ml Ethanol, 0.5g sodium chloride and 100ml of distilled water. The mixture was thoroughly shaken and 3 drops of phenol red were added and the solution was titrated against 0.1 N NaOH to a purple colour at the end point. This neutralization solution was stored for determination of methoxyl content. The result expressed as:

$$\text{Equivalent weight} = \frac{\text{Weight of pectin sample X 1000}}{\text{Vol. of alkali X Normality of Alkali}}$$

Determination of Methoxyl content (MeO)

Determination of Methoxyl content was carried out using Ranganna's method [18]. To neutral solution above was added 12.5ml of 0.25N NaOH. The mixture was thoroughly stirred and kept at room temperature for 30min. Thereafter 12.5ml of 0.25NHCl was added and the solution titrated against 0.1 N NaOH. Methoxyl content calculated as;

$$\text{Methoxyl content (\%)} = \frac{\text{Vol. of alkali X Normality of Alkali X 3.1}}{\text{Weight of pectin sample}}$$

Determination of Total Anhydrouronic Acid content (AUA)

Total AUA of pectin was obtained by the method of Mohammed and Hassan [19] using the formula;

$$\%\ \text{AUA} = \frac{176 \text{ X } 0.1Z \text{ X } 100}{\text{Wt of Pectin Sample X } 1000} - \frac{176 \text{ x } 0.1Y \text{ x } 100}{\text{Wt of Pectin Sample X } 1000}$$

As;

Molecular Unit of AUA (1 unit) = 176g

Z= ml (titre) of NaOH from equivalent weight determination

Y= ml (titre) of NaOH from Methoxyl content determination

Determination of Degree of Esterification (DE)

The DE of pectin was measured in accordance to the method of Owens et al; 1952[20]. Values of MeO and AUA were used in the calculation as:

$$\%\ \text{DE} = \frac{176 \text{ X \% MeO X } 100}{31 \text{ X \% AUA}}$$

Determination of Neutral sugar

Neutral sugar was determined according to the method of Miller [21] with a slight modification. The dried pectin (0.25g) sample from each waste sample was measured into a 50ml volumetric flask and made up to 10ml by adding distilled water. The mixture was kept at room temperature for 20 min and later filtered through filter paper (Whatman No. 540). Thereafter 1ml of the filtrate from each sample waste was transferred into different test tubes and 1ml of Dinitrosalycyclic acid (DNS) reagent was added to the tubes; then the samples in the tubes were heated in a boiling water bath for 10 min. While still warm, 1ml of 40% Rochelle salt solution (Potassium-sodium tartrate) was added to each of the tubes. The tubes were cooled and absorbance of samples read at 540nm against a blank. Serial dilutions of standard maltose were prepared and a graph of absorbance against concentration was plotted.

Statistical analysis

All measurements were carried out in triplicate for each of the sample. Results are expressed as mean value standard deviation. Data were statistically analyzed using SPSS. Version 20.0.

RESULTS AND DISCUSSION

Qualitative analysis

Table 1 show the result for the qualitative analysis of the five non-citrus agro food wastes. Pectin extracted from these samples was brown in colour. However, IPPA [22] reported that standard pectin are usually light because light colours represents quality gel. Aina et al., [17] reported same for some citrus peels. They also suggested that factors

such as surface contamination, environmental factors types of agricultural material used and human error may have contributed to the variation in colour. As with solubility, the extracted dry pectin were all soluble in hot water and insoluble in cold water except for psp and pwp which were slightly soluble in cold water. Sriamonsak [6] reported that viscosity, solubility and gelation are generally related; for instance factors that increase tendency to gel, decrease solubility and increase viscosity and vice versa.

Table 1: Qualitative test for the five samples

Parameter	psp	pwp	bsp	bcp	hbp
Colour	Brown	Brown	Brown	Brown	Brown
Solubility in cold water	Dissolved slightly and form suspension after a while.	Dissolved slightly and form suspension after a while.	Insoluble	Insoluble	Insoluble swells after vigorous shaking to form suspension.
Solubility at 85-90°C for 10 min.	Soluble	Soluble	Soluble	Soluble	Soluble

Quantitative analysis

Figures 1-5 below shows the percentage pectin yield of the different agro food wastes using 1 N HNO_3 at pH 1.76 under different temperatures (60^0C, 80^0C, and 100^0C) and times (60, 90, 120 min).

The percentage pectin yield is temperature and time dependent. However there was a decline in pectin yield after 90 min at 100^0C. The range of percentage pectin yield for psp, pwp, bsp, bcp and hbp at different temperatures and times include 1.56-2.81%, 1.10-3.98%, 1.06-2.98%, 1.00-2.80% and 1.09-4.40% respectively. The optimum yield of pectin was obtained at 100^0C for 90 minutes for all samples. The value for hbp (4.40%) is significantly ($p \leq 0.05$) higher than the rest of the samples (Figure 6); while bcp had the lowest content (2.83%). Azad et al., [16] reported that percentage pectin yield is dependent on the raw material and extraction solvent used. Pectin yields obtained in this study are similar to the pectin of citrus tankan (2.75%), guava press cake (3.49%), calamanis skin 93.50%) and carambola press cake (2.24%) as reported by Tamaki et al,. [2] and Normah and Itasnah, [23]. The values of pectin obtained in this study suggest their industrial influences even when these samples are not citrus based.

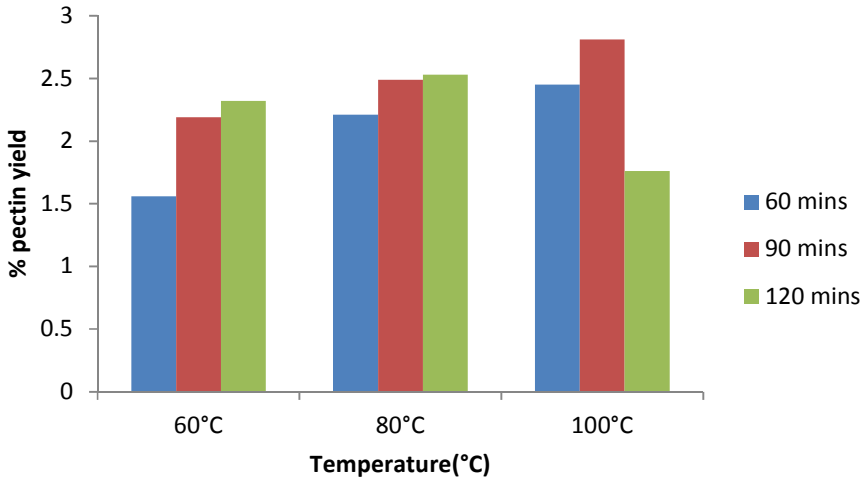

Figure 1: Percentage pectin yield obtained from pumpkin seed peel (psp) at different times (min) and temperatures (°C)

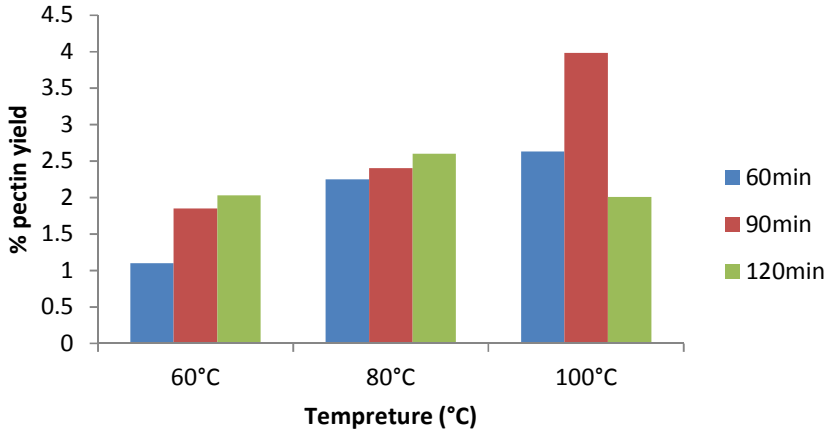

Figure 2: Percentage pectin yield obtained from pumpkin white pod peel (pwp) at different times (min) and temperatures (°C)

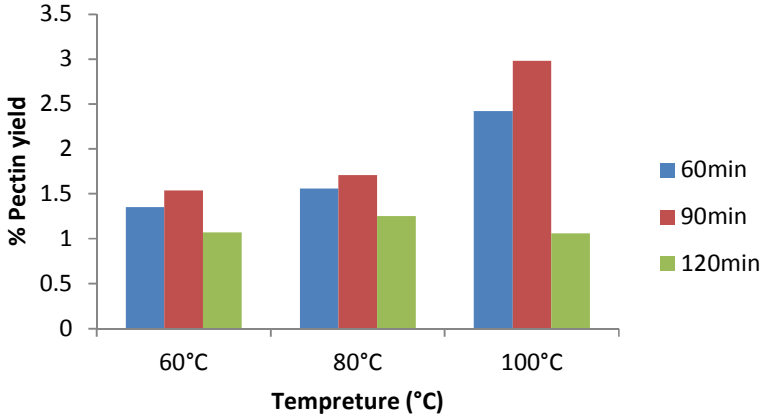

Figure 3: Percentage pectin yield obtained from breadfruit seed peel (bsp)) at different times (min) and temperatures (°C)

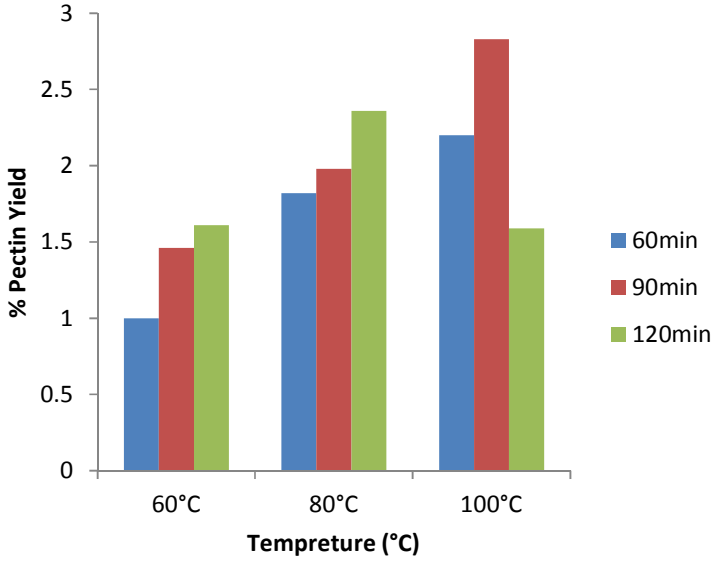

Figure 4: Percentage pectin yield obtained from breadfruit creamy pop (bcp)) at different times (min) and temperatures (°C)

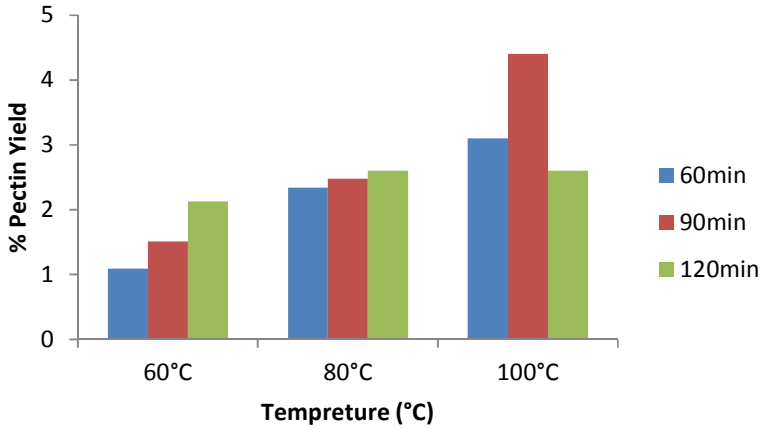

Figure 5: Percentage pectin yield obtained from horse eye bean peel (hbp) at different times (min) and temperatures (°C)

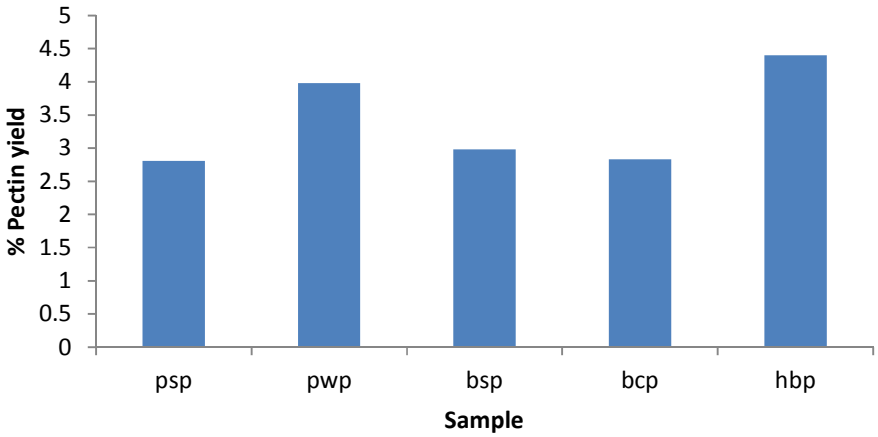

Figure 6: Optimum percentage pectin yield of the various samples at 100°C for 90minutes

Table 2 below shows that the equivalent weight for psp, pwp, bcp, bcp and hbp was found to be 1923, 1563, 1667, 1786 and 1471mg/mol respectively. From the results, the value for psp is significantly ($p<0.05$) higher compared to the values for pwp and hbp. Similar results were reported by Kuman and Chauhan [24] for apple pomace (833.33-1666.67) and matured lemon pomace (1175) as reported by Azad et al., [16]. The high equivalent weight obtained in this study could be attributed to lower partial degradation of pectin, nature of the extraction process or it may also depend on the amount of free acid [16, 17, 25]. The percentage Methoxyl content determined from the dry pectin of the five agro food wastes are 2.48, 2.24, 2.48, 1.48 and 1.720% for psp, pwp, bsp, bcp, hbp respectively. Ismail et al., [26], also reported similar values (2.98-4.34%) for dragon fruit. content of extracted pectin. Studies have shown the methoxyl content of extracted pectin vary from 0.2-12% depending on the source and mode of extraction [17]. Methoxyl content is an important factor in controlling the setting time of pectin and the ability of the pectin to form gels. The partial solubility noticed with these extracted pectin confirms the ability of pectin to form gel easily [18,24,27,28].

Results of AUA (Table 2) showed that pwp had the highest value (20.78%) which was significantly ($p\leq 0.05$) higher than the value for bcp (14.08%) results obtained do not agree with already existing data[26] on dragon fruit (59.52-70.50%) and apple pomace (74.1%) as reported by Kuman and Chauhan[24] . Low values of AUA indicate that the extracted pectin might have a high amount of protein [26]. This is because AUA determines the purity of the extracted pectin and the value of AUA must not be < 65%[29].

The degree of esterification of the pectin extracted from these five samples range from 55.48-71.47% (Table 2). The ratio of esterified galactouronic Acid (Gal A) groups to total galactouronic acid group (Gal A) is termed as the degree of esterification (DE). Based on the DE, pectin can be classified as low methoxyl pectin (ranging from 29 to 40%) and high methoxyl pectin ranging from (60-75%). The level of degree of esterification reported in this study correspond to the report by Azad et al.,[16] for lemon pomace (33.59-79.51%). Rha et al., [30] reported that DE depends on species or type of agricultural material used.

Table 2: Physicochemical characteristics of pectin

Parameter	psp	pwp	bsp	bcp	hbp
Equivalent Weight(mg/mol)	1923±0.04	1563±0.07	1667±0.03	1786±0.03	1471±1.40
Methoxyl Content (%)	2.48±0.02	2.24±0.03	2.48±0.09	1.48±0.02	1.72±0.01
AUA (%)	19.71±0.12	20.78±0.11	20.18±0.06	14.08±0.01	17.60±0.07
DE (%)	71.47±0.08	61.52±0.07	66.67±0.05	59.68±0.04	55.48±0.03

Value is expressed as mean ± standard deviation. Mean value with the same superscript along the same row are not significantly different (p≤0.05)

Table 3: Neutral Sugar

Composition (%w/w)	psp	pwp	pwp	psp	hbp
Maltose	0.769±0.13[a]	0.318±0.12[b]	0.480±0.09[b]	0.193±0.03[c]	0.295±0.11[c]

Value is expressed as mean ± standard deviation. Mean value with the same superscript along the same row are not significantly different (p≤0.05)

The only neutral sugar determined (Table 3) in this study was maltose ranging from 0.193-0.769% with psp having a significant (p≤0.05) higher value (0.769%) when compared to the rest samples. Georgeiv et al; [31] reported low glucose and galactose content of some citrus plants. Some other authors [32, 33] reported that low sugar content and higher molecular weight showed greater gelling ability and vice versa.

CONCLUSION

Pectin was extracted from five non-citrus based agricultural food wastes. The extraction of pectin with nitric acid had its optimum yield at 100^0C for 90 min. The physicochemical parameters of the extracted pectin are dependent on solvent used for extraction, nature of sample material as well as extraction process. The overall result implies that the pectin obtained from these wastes has potential for use in food and pharmaceutical industry.

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