Comparative Extraction Performance of Solvent Systems in the Extraction and Characterization of Pectin from *Prosopis Africana* Seed Gum

Adie[1]*, P.A., Ikese[1], C.O. and Otache[2], E. A.
[1]Department of Chemistry, Benue State University, Makurdi-Nigeria
[2]Center for Food Technology and Research, Benue State University, Makurdi-Nigeria
Corresponding Author: padie@bsum.edu.ng

Abstract
Pectin was separately extracted in warm water bath from sun-dried and crushed *Prosopis africana* seed gum, using 0.05 M solutions of HCl, H_2SO_4, CH_3COOH, $C_6H_8O_7$ and a 3:2 blend of (H_2SO_4: $C_6H_8O_7$). The physicochemical properties of pectin extracted from the *Prosopis africana* seed gum shows the following: colour varied from beige-brown to wheat brown, equivalent weight (1,818.18 mg/mol to 25,000 mg/mol), methoxyl content (0.124 % to 1.705 %), moisture content (12 % to 38 %) and ash content (2 %) for all the solvent system used. Also, the comparative solvent performance shows that the H_2SO_4:$C_6H_8O_7$ solvent blend was the most effective for pectin extraction followed by the solvent HCl and H_2SO_4, while $C_6H_8O_7$ was the least effective. The comparative pectin yield of *Prosopis africana* seed gum using selected acid solvent ranged from 6.94 % to 15.28 %. Among the solvents under study, the H_2SO_4:$C_6H_8O_7$ solvent blend gave the highest pectin yield (15.28 %) with beige-brown, low methoxyl pectin of high purity and could be used as a functional food ingredient domestically and industrially.

Introduction

Prosopis africana is a flowering plant from the family *Leguminosae mimosoideae* and genus *Prosopis* found in Africa. It is commonly called African mesquite or iron tree. Its fermented seeds are used to prepare a food spice that is popular across many ethnic groups in Nigeria (Maier *et al*., 1993), while the husks and gums (endosperms) are discarded as wastes. The endosperm can be separated from the wastes, washed and used for pectin extraction, promoting the conversion of waste into wealth and creating job opportunities locally. Also, it will rid the environment of littered *Prosopis africana* waste, thus promoting a cleaner environment.

Pectin is a structural hetero-polysaccharide that occurs naturally in the cell walls of higher plants (Bagde *et al*., 2017), especially the fruits and seeds (Anjali, 2015). Pectin functions as a hydrating agent and as a cementing material for the cellulosic network (Bagde *et al*., 2017). The peels (rind) of citrus are good source of pectin. *Prosopis africana* seed gum, cowpea peels and apple pomace also contain pectin (Bagde *et al.,* 2017).

Pectin is gaining applications in the pharmaceutical industry as a carrier in drug delivery to the gastrointestinal tract, as tablet matrix, gel beads and film-coated dosage forms (Pranati and Rishabha, 2011). It is also used for the treatment of diarrhoea, to control blood cholesterol levels and gastrointestinal disorders, beneficial for human health. It has also been used as a haemostatic agent and is considered to be a safe food additive that can be taken daily without limits (Bagde *et al.*, 2017; Anjali, 2015). Pectin can form gels with sugar and acid under appropriate conditions (Bagde *et al.,* 2017). It is widely used in the food industry as emulsifier, texturizer, stabilizer, and as a jellying and thickening agent in the preparation of jams, jellies and marmalades. Pectin is also used as a fat replacer in different food formulations such as spreads, ice-cream and salad dressings (Anjali, 2015). It finds applications in biotechnology and beverage industries (Bagde *et al.,* 2017).

The production of pectin is a complicated and expensive process (Anjali, 2015; Peter, 1997), as it includes the preparation of raw materials involving deactivation of enzymes, removal of bitter glycosides and crude sugars, conversion of protopectin into soluble moiety, filtration of the extracted pectin, precipitation, purification and drying (Anjali, 2015). All these make the finished product very expensive. Different agricultural wastes including *Prosopis africana* seed gum among others are allowed to waste and litter the landscape in Makurdi metropolis, converting *Prosopis africana* on a commercial basis to pectin would save cost of production and make the final product relatively cheap and affordable.

The purpose of the study was to extract pectin from *Prosopis africana* seed gum using a range of solvent systems of the same concentration and compare their yields using statistical tool to determine the most efficient solvent system for recovery, and characterise the extract based on known physicochemical parameters.

Materials and Methods

Sample procurement and preparation

Prosopis africana seed gum was procured in North Bank Makurdi, the Benue State capital, Nigeria. It was thoroughly washed to remove any impurities and further sun dried for 72 hours, after which it was crushed to tiny pellets using a mortar and pestle and 1 kg of the crushed gum was weighed with a digital electronic balance and stored in an air-tight plastic container with lid, prior to pectin extraction.

Pectin extraction

Pectin was extracted in triplicates per solvent type from the dried and crushed *Prosopis africana* seed gum using a solid sample to solvent (extractant/liquid) ratio of 1:25 g/mL, which was 40 g of the sample to 1,000 mL of the solvent. The extraction solvents were; $HCl_{(aq)}$, $H_2SO_{4(aq)}$, $CH_3COOH_{(aq)}$, $C_6H_8O_{7(aq)}$ and 3:2 ($H_2SO_{4(aq)}$ and $C_6H_8O_{7(aq)}$) at a concentration of 0.050 M each. The extraction was done at a temperature of 75 ^oC using a thermostated water bath for 90 minutes in a 1000 mL beaker. The hot acid

extract was cooled to 50 ^{o}C using a thermostated water bath and filtered using a clean muslin cloth, to give a clear pectin liquor which was precipitated in 3 x 300 mL of 95 % ethanol at 5 ^{o}C for 2 hours. The pectin precipitate was filtered out and washed twice with 300 mL of 70 % ethanol, followed by 300 mL of 95 % ethanol, oven-dried at 45 ^{o}C for 10 hours and weighed. The dried pectin flakes were finely ground using porcelain mortar and stored in dry air-tight plastic containers at room temperature until use.

Physicochemical properties of pectin extract

The extracted pectin was analysed for colour, yield, equivalent weight, methoxyl content, moisture content and ash content. Colour of pectin extracted was determined visually with the naked eyes while the yield in percentage was evaluated by using the method of Uzma *et al.*, *(2015)*:

$$\% \text{ Pectin yield } = \frac{\text{Weight (g) of dried pectin}}{\text{Weight (g) of dried powder taken for extraction}} \text{ x } 100 \ \%$$

Equivalent weight

Equivalent weight was determined by the standard methods as described (Uzma *et al., 2015;* Omale *et al., 2017*). Equivalent weight was done by weighing 0.5 g of the dried pectin into a 250 mL conical flask and moistened with 5 mL of 70 % ethanol. One gram (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 to ensure all the pectin dissolved and no clumping occurred at the sides of the flask before the solution was slowly titrated, (to prevent possible de-esterification), with 0.1 M NaOH to a pink colour at the endpoint. The equivalent weight of pectin was calculated by using the expression of (Omale *et al., 2017*; Ranganna, 1991):

$$\text{Eq. Wt. (mg mol}^{-1}) = \frac{\text{Wt. of pectin sample (g)}}{\text{Vol. of alkali(cm}^{3})\text{x Molarity of alkali (mol dm}^{-3})} \text{ x } 1000$$

Methoxyl content

Methoxyl content was determined by adding 25 ml of 0.25 M NaOH to the neutral solution obtained from the equivalent weight determination, mixing thoroughly, and allowed to stand for 30 min at room temperature in a stopper flask. 25 ml of 0.25 M HCl was then added and titrated with 0.1 M NaOH to the same end point as before. Methoxyl content was calculated using the expression (Ermias and Teshone, 2016):

$$\text{Methoxyl content \% } = \frac{\text{Vol. of alkali (cm}^{3})\text{x Molarity of alkali (mol dm}^{-3}) \text{ x Molar mass of methoxyl (g mol}^{-1})}{\text{Weight of pectin sample (g) x } 1000} \text{x } 100 \ \%$$

Moisture content

Moisture content was determined by the standard method (Omale, *et al.*, 2017). Moisture content was done by drying an empty crucible in an oven at 105 ^{o}C, cooled in a desiccator and weighed. Half gram (0.5 g) of the pectin sample was transferred into the crucibles in the oven which was set at 130 °C for 1 h and thereafter, the crucible was removed, cooled in a desiccator and weighed. This process was repeated once. The moisture content in percentage was calculated using the equation:

$$\text{Moisture Content (\%)} = \frac{\text{Weight of Moisture (g)}}{\text{Weight of the Sample (g)}} \text{ x } 100 \ \%$$

Ash content

Ash content was also determined using the standard method of Omale *et al.,* (2017). Half gram (0.5 g) of the sample was weighed into a weighed empty crucible. The crucible was transferred to a muffle furnace set at 600 °C for 3 hours to rid it of all the organic matter. The carbon is charred and lost as carbon (IV) oxide, leaving a dark ash. The crucible was taken out of the furnace and placed in a desiccator to cool. The crucible after cooling was reweighed. The ash content in percentage was calculated by:

$$\text{Ash Content (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \text{ x } 100\%$$

Results and discussion

Results

Physiochemical properties of the pectin extracts for various solvents and their percentage yields are presented in Table 1.

Table 1: Physicochemical properties of pectin from *Prosopis africana* seed gum.

Solvent System	Colour	Yield (%)	Equivalent Weight (mg/mol)	Methoxyl Content (%)	Moisture Content (%)	Ash Content (%)
H_2SO_4	Beige-brown	13.71	6,250.00	0.496	38	2
HCl	Beige-brown	13.79	11, 111.11	0.279	28	2
CH_3COOH	Beige-brown	7.30	25, 000	0.124	18	2
$C_6H_8O_7$	Wheat-brown	6.94	3, 030. 30	1.023	16	2
H_2SO_4:$C_6H_8O_7$	Wheat-brown	15.28	1,818.18	1.705	12	2
Reference Standard	Beige-brown	4.98	250 – 8,410	0 – 16	≤ 12	≤ 10

Discussion

The colours of pectin extracted from *Prosopis africana* seed gum were majorly beige-brown. Except for $C_6H_8O_7$ and H_2SO_4: $C_6H_8O_7$ solvent systems, whose products gave wheat-brown colour, products of extracts of other solvents gave beige-brown colour corresponding to reference standard. The wheat-brown colour imparted, as can be inferred, by $C_6H_8O_7$ is an indication that the extract may contain polyphenols or other water-soluble pigments trapped inside the pectin during precipitation (Nguyen and Pirak, 2019). This can be removed by adding acetone in a drop-wise manner (Woo *et al.,* 2010) and allowed for 1 hour under a fume extractor (for residual acetone evaporation) (Koffi *et al.*,2013).

The comparative pectin yield for each solvent system, showed that the H_2SO_4:$C_6H_8O_7$ solvent system was the most effective of all the solvents used as it gave the highest percentage yield of 15.28 %, while $C_6H_8O_7$ was the least efficient solvent system. The comparative pectin yield for each solvent is shown in decreasing order thus: H_2SO_4:$C_6H_8O_7$ (15.28 %) > HCl (13.79 %) > H_2SO_4 (13.71 %) > CH_3COOH (7.90 %) > $C_6H_8O_7$ (6.94 %). H_2SO_4:$C_6H_8O_7$ exhibit mutual reinforcement in the extraction process, with yield several times higher than the reference standard.

All the pectin extracts in this work, Table 1, have higher equivalent weight compared to reference standard and would have a good gel-forming effect since their values were above the 250 - 8,409 mg/mol equivalent weight range. A number of previous studies on pectin extraction stated that, their outcomes were gave a range from 250-8409 mg/mol (Nguyen and Pirak, 2019). The higher the equivalent weight of the pectin, the better the gel-forming effect (Castillo-Israel *et al.*, 2019).

The methoxyl content of the extracted pectin (0.279% - 1.705%) all lie within reference standard range. Methoxy content is a very important parameter as it controls the gel strength, gel setting time, sensitivity to metal ions and determines the functional properties of pectin solutions (Nguyen and Pirak, 2019). Spreading quality and sugar binding capacity of pectin are enhanced with increase methoxyl

content (Nguyen and Pirak, 2019). The extracted pectin using the H_2SO_4: $C_6H_8O_7$ solvent system with a value of 1.705 % exhibits a better spreading quality than those extracted from the other solvent systems, Table 1.

Results presented in Table 1 indicate an inverse relationship between % methoxyl content and moisture content %. H_2SO_4 extract has the highest % moisture content (38%) while H_2SO_4: $C_6H_8O_7$ solvent system extract has the least (12 %). Studies have shown that, low moisture content enhances the shelf life of pectin (Begum *et al.*,2014), while high moisture content could enhance the growth of microorganisms and produce pectinase enzymes that vitiate pectin quality (Begum *et al.*,2014); Nguyen and Pirak, 2019). The pectin extracted in this work shown in Table 1 are of high moisture content as their values are above the reported limit of moisture content in the reference standard, ≤ 12 % and therefore prone to deterioration.

Ash content is an index of purity of the pectin. The lower the ash content, the higher the purity of a pectin and the higher the ash content, the lower the purity of a pectin (Begum *et al.*,2014); Nguyen and Pirak, 2019). The extracted pectin from *Prosopis africana* seed gum all have a constant ash content (2 %), indicating they are of high quality when compared to reference value.

Conclusion

This study reveals that, pectin can be extracted in substantial amounts from *Prosopis africana* seed gum. The H_2SO_4:$C_6H_8O_7$ solvent system was the most effective solvents for pectin extraction from this food waste, *Prosopis africana* seed gum, followed by HCl and H_2SO_4. The pectin extracts in the study all show high purity based on their ash content. The H_2SO_4:$C_6H_8O_7$ solvent system gave the highest percentage yield, the moisture content was outside the range of the reference standard, the equivalent weight and methoxyl contents are indications that the pectin would be a good gel-forming quality. The overall result shows that, the solvent system H_2SO_4:$C_6H_8O_7$ gives the best yield compared to the other solvents in the study and therefore recommended for use on a larger scale.

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