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EXTRACTION AND CHARACTERISATION OF PECTIN FROM BANANA PEEL

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ABSTRACT

Pectin is extracted from peel of banana (Musa species). Its structural, rheological and textural features are studied. Optimum conditions of extraction upon yield was also examined. There was significant yield under the given set of experimental conditions. Degree of methylation was about 62 while percentage of anhydrouronic acid was slightly above 70%. Galactose, galacturonic acid, rhamnose, mannose and other sugars were found to be present in it. This pectin was found to be more effective in increasing the viscosity of sugar solutions compared with citrus pectin. Also, banana pectin was used to prepare pineapple jam. Pineapple jam made with banana pectin was found to have more shear modulus compared with the pineapple jam made with citrus pectin.

1.Introduction

A vast majority of the people in India depend upon agriculture as their major source of income. Increasing cost of production, lack of deserving price of the products, crop failure due to biotic and abiotic stress, natural calamities etc. are making agriculture in India a loss for the common farmer. Therefore, educated youth do not consider agriculture as a viable employment. To make agriculture more attractive, production of alternate and non-conventional value-added products from the waste materials thrown away in the farm is a solution for this problem.

According to the data published by the Horticulture Statistics Division, Ministry of Agriculture & farmers' Welfare, Govt. of India, India is the largest producer of banana in the world, with an annual production of over 2,91,63,000 metric tonnes in 2016-17 from a total of about 8,58,000 hectare of land area spread all over the country(Pattanayak 2017). In banana cultivation rain, wind and other natural calamities cause loss for the farmer even before harvesting. Pest infestation is another factor for the loss. After proper harvesting, the bulk of the plant body is left in the farm as waste while only the fruit bunch is commonly collected. From that fruits also, peel is thrown away as waste. The overall quantity of waste left over is much more than the economically used parts in banana cultivation.

Pectin is a plant-derived heteropolysaccharide, widely used as a gelling/stabilising agent in food and pharmaceutical industries. Owing to its versatile structure and composition which ultimately determine its applications, thousands of tonnes of this soluble dietary fibre is produced globally every year and used for a variety of purposes(Willats, Knox, and Mikkelsen 2006). Being a component of cell wall of almost every land plant, it can be extracted from different types and parts of plants at different developmental stages. The structure and composition of the extracted pectin depends upon conditions used for extraction, source

plant, part of the plant used for extraction and even developmental stage of the plant part used(Atmodjo, Hao, and Mohnen 2013). Thus, different pectin samples obtained from different plant sources are different in their structural features and therefore have tremendous potentiality in their applications. Since new areas are identified for the application of pectin every day, it is essential to explore the possibility of new sources of this novel biopolymer. The waste materials from the of banana farm is one such source for pectin.

Traditionally pectin is used as gelling/thickening/stabilizing agents in different industries. While pectin with low degree of methylation forms thermo-reversible gels in presence of calcium ions at acidic pH, pectin with high degree of methylation forms thermo-irreversible gels in presence of sugars at acidic pH(Srivastava and Malviya 2011).

One of the oldest uses of pectin is in the manufacture of fruit jams and jellies[5]. With a soluble sugar content of about 60%, pectin is added at acidic pH in order to regulate the flow behaviour, gel strength and other rheological properties of the jams and jellies so as to increase the jam qualities up to the consumer satisfaction(May 1990).

Pectin, being a natural molecule with infinite structural diversity, offers a wide variety of uses and applications to humanity. It was found in a study that intake of pectin along with diet helps in the lowering of blood cholesterol level(Brouns et al. 2012). It was also found to be reducing the rise of blood level after meals. Pectin is thought to bind with cholesterol and bile acids in the alimentary canal thereby preventing their absorption and promoting excretion(Mudgil and Barak 2013). Different types of pectin obtained from kiwi fruits were found to be efficient in promoting beneficial health effects in alimentary canal(Parkar et al. 2010). Hydrolysates obtained from citrus pectin were reported to be beneficial for the survival of probiotic populations(Ho, Lin, and Wu 2017).

The anticancer role of pectin is well established. It is suggested that some fragments formed from chemically or enzymatically modified pectin may bind to galectin-3, a protein associated with development of cancer, thereby preventing cancer(Maxwell et al. 2012). Pectic oligomers, comprising repeating alternate galacturonic acid and rhamnose residues, extracted from tomato were reported to be inhibiting the activity of galectin-3(Kapoor and Dharmesh 2017). A galactan, having a terminal galactose at the non-reducing end of the chain is capable of binding with human recombinant galectin-3(Gunning, Bongaerts, and Morris 2008).

A matrix consisting of multi particulate calcium pectinate is an effective carrier of drugs for the treatment of colon cancer(Wong, Colombo, and Sonvico 2011) as it moves more slowly down the alimentary canal and therefore gets more contact time for the action of drugs. 5-aminosalicyic acid, after being incorporated in to a matrix of chitosan and coated with pectin, could be used for demonstration of controlled drug delivery in simulated gut in in vitro experiments(Ribeiro et al. 2014). Pectin was useful in this environment as it offers resistance against the acidic conditions of the gut.

Specific strategies for the digestion and removal of pectin from the cell wall of biomass is mooted for the efficient production of biofuels from them(Xiao and Anderson 2013). Experiments indicate that pectin based hydrogels could be used for tissue engineering in mammals(Neves et al. 2015). Some experiments indicate that pectin along with calcium carbonate could be used for the preparation of hydrogels, which could be injected in to the body for the delivery of drugs or even cells for implantation by surgery(Moreira et al. 2014). Experiments already have demonstrated that pectin along with polyvinyl pyrrolidone and glycine, may be used for the preparation of hydrogel membranes(Kumar, Mishra, and Banthia 2011), which may then be used for biomedical applications.

Films prepared using pectin, polyvinyl alcohol and chitosan exhibited good antimicrobial activity and other structural and functional properties thereby offering a potential for use in the food – packaging

applications(Tripathi, Mehrotra, and Dutta 2010). When enzymes used for the treatment of skin injuries were loaded in to a cryogel made up of polyvinyl alcohol and pectin, the gel was found to be retaining the enzymes more effectively for a longer period of time(Martínez et al. 2014). Also, it was observed that the enzymes were bound to pectin rather than to polyvinyl alcohol of the cryogel.

Since more applications are developed for this unique molecule every day, it is essential to look for alternate, abundant and easily available sources so that humanity can get maximum benefits from it. Extraction and characterization of pectin from the peel of banana fruit bunch is discussed in this communication.

2.Materials and methods

2.1. Materials

All reagents are purchased from Merck, India unless otherwise specified.

2.2. Preparation of plant material

Ripe fruit bunch of Nenthran variety of banana/plantain (*Musa sp*.) was collected from a local farmer in Palakkad district, Kerala, India. Peel was separated, washed with mild acid, chopped in to small pieces, pulverised using a food processor, sun dried to constant weight, powdered and kept in an air tight vessel for further use.

2.3. Preparation of extractant solution

Distilled water is mixed with hydrochloric acid to attain a particular range of pH as is shown in table- 1.

2.3.1. Extraction of crude pectin

10g of the powdered peel was weighed, tied up in a bag made of cotton cloth, immersed in a particular volume of water maintained at a pH and heated at a constant temperature (table 1) in a 500 – ml Erlenmeyer flask (Borosil) for a certain duration of time (table 1). After heating, the flask is cooled to room temperature, the bag containing powdered peel was taken out and squeezed to release any drop of liquid. Then pH is brought above 6.0 using Barium Carbonate, centrifuged at 5000 rpm for 10 minutes and the supernatant is collected. It was then evaporated under vacuum to a volume of 50 ml, double volume of isopropyl alcohol was added, shaken well and kept at room temperature for 24 hours. It was then centrifuged at 5000 rpm for 10 minutes, and the supernatant was discarded. The precipitated crude pectin was dried under a stream of air to constant weight, and transferred to air-tight vials for further analysis(Yapo 2009).

2.4. Characterisation

2.4.1. Estimation as calcium pectate

The method originally described by Ranganna S.(Ranganna 1977) was used with further modifications.

1 g of the powdered pectin was dispersed in 10 ml of 0.01 N HCl, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected. The residue was mixed with 10 ml of 0.05 N HCl, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected. The residue was mixed with 10 ml of 0.3 N HCl, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected. The residue was mixed with 10 ml of water, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected.

All supernatants were pooled together and made up to 100 ml with water. 30 ml of this solution was pipetted out in to a 500 ml Erlenmeyer flask, 1 drop of phenolphthalein were added as indicator, neutralised with 1 N NaOH. An excess of 3.0 ml of 1N NaOH were added for saponification, shaken well and kept at room temperature for 48 hours. Then 3.0 ml of 1 N acetic acid was added to it, shaken well. After 15 minutes, 4.0 ml of 1N $CaCl_2$ was added with constant shaking and was allowed to stand for 4 hours. Then it was boiled for 2 minutes, filtered using a pre-weighed filter paper (HiMedia Laboratories) under vacuum, washed with hot water, tested with silver nitrate for the presence

of chloride, dried in a vacuum desiccator for constant weight.

$$\% \text{ of calcium pectate} = \frac{W \times V1 \times 100}{V2 \times P}$$

where,

W = weight of calcium pectate,

V1 = total volume of solution prepared,

V2 = volume of solution used for precipitation

P = amount of pectin used

2.4.2. Equivalent mass,(Suman R Yadav, ZH Khan, SS Kunjwani 2015)

0.2 g of the powdered pectin was moistened with 5 ml of ethanol, then dissolved in water (HPLC), and made up to 100 ml. 10 ml of this solution was pipetted out in to a conical flask, 2 drops of phenolphthalein added as indicator, titrated against 0.01 N NaOH. Value noted as V1 and is the measure of the unesterified galacturonic acids.

$$\text{Equivalent mass} = \frac{W \text{ x } 1000}{V1 \text{ x } N1}$$

Where, W = weight of pectin (g) used

V1 = volume of alkali used

N1 = normality of alkali used

2.4.3. Estimation of Degree of Esterification (D.E.),

The method described in Food Chemicals Codex(Birch 2003) 3rd edition, is used with some modifications. 5.0 ml of 0.1 N NaOH is added to the above solution (used for calculation of equivalent mass), shaken well, and kept at room temperature for 3 hours for saponification. Then 5.0 ml of 0.1 N HCl is added to it to neutralise the NaOH, 2 drops of phenolphthalein added as indicator, and titrated against 0.01 N NaOH, value noted as V2. This is the measure of the esterified galacturonic acid.

$$DE = \frac{V2}{V1+V2} 100$$

2.4.4. Estimation Percentage of Anhydrouronic acid (%AUA)*(Joel et al. 2018)*

%AUA is calculated using the above values used for the estimation of equivalent mass and degree of esterification.

$$\%AUA = \frac{176 \text{ x } 0.1 \text{ x } Z \text{ x } 100}{W \text{ x } 1000} + \frac{176 \text{ x } 0.1 \text{ x } Y \text{ x } 100}{W \text{ x } 1000}$$

Where, 176 = molecular mass of AUA,

V1, V2 = volumes of alkali mentioned above

W = weight of pectin used

2.4.5.Sugar Profile Analysis (Corradini, Cavazza, and Bignardi 2012),

50 µg of the pectin sample was hydrolysed using 2N TFA at 100^0C for 5 hours, followed by removal of the acid under a stream of nitrogen gas. The sample was co-evaporated with 50% isopropyl alcohol for the complete removal of the acid. Finally, the sample was dissolved in Milli-Q water and 10 µg was injected on HPAEC-PAD. Dionex ICS-3000 was used for monosaccharide profiling using CarboPacPA-1 column (4mm x 250mm) with 100 mM NaOH and NaOAc gradient.

2.4.5. IR analysis (Kyomugasho et al. 2015),

The powdered peel was mixed with KBr (1,100) and pressed in to pellets. Then it is analysed with a Perkin Elmer (USA) machine and FTIR spectra were collected at the transmission mode in the frequency range of 400-4000 cm^{-1}, resolution = 2 cm^{-1}, No. of scans = 8.

2.4.6 NMR analysis

1D ^{1}H and ^{13}C spectra were obtained at 400MHz, using liquid state NMR spectrometer (Bruker) with D_2O as solvent for the analysis. 2D NMR spectra – HSQC and TOCSY- were obtained at 500MHz, using liquid state NMR spectrometer (Bruker AvansIII 500) with D_2O as solvent for the analysis.

2.4.7. Viscosity measurements,

Weighed amounts of pectin were mixed with water at pH = 3.2 and 16.25 g of sucrose. A series of experiments were set up (in triplicates) according to the table given below (Table 1).

All the above sets were boiled to 105^0C till volume is reduced to 25 ml, and poured in to a test tube, allowed to cool down to room temperature. They were then analyzed using a Rheometer (Anton Paar, MCR52, SN81174546; FW3.65; Slot (2,-1); Adj (1993,0)d, Application RHEOPLUS/32 V3.61 21006273-33024, Accessories TU1=P-PTD200/AIR-SN81174614, Measuring system PP75-SN16019; [d=1 mm], at constant temperature of 25^0C.

2.4.8. Rheological analysis

Ripened pineapple is chopped in to small pieces after removal of outer skin and is made in to a juice in a blender. It is then evaporated, with continuous stirring in a pan placed over a stove to remove water to a certain extent. Then 25g of this juice is weighed in to a beaker, 25g of sucrose is added and further boiled with continuous stirring. Then a powdered mixture of 1g sucrose, and 0.25g pectin is added and boiled again, a small amount of citric acid is added and boiled with continuous stirring to jam of final pH of 3.6 and brix 60%. Different sets of this jam are prepared in triplicates as per the following scheme (table 2),

The Control did not contain any pectin while citrus pectin purchase from Sigma -Aldrich was used in the Standard. The Test contained pectin extracted from banana peel. Strain sweep (shear-strain-amplitude sweep, with controlled-shear deformation CSD) experiments were conducted using Rheometer (Aanton Paar) model MCR 52, plate-plate method, sample thickness of 1mm, at 25^0C, frequency 1Hz, and shear strain range of 0.0001-100%(Dorohovich, Dorohovich, and Kambulova 2016).

Table 1. Preparation of gel using sugar and pectin

Sl. No.	Name of set up	Vol. of acidified water (pH = 3.2)	Amount of sucrose (g)	Amount of pectin (sigma)	Amount of banana pectin (g)	Final volume of gel before pouring (ml)
1	Control	30 ml	16.25	Nil	Nil	25
2	Standard	30 ml	16.25	0.5g	Nil	25
3	Test 1	30 ml	16.25	Nil	0.1	25
4	Test 2	30 ml	16.25	Nil	0.2	25
5	Test 3	30 ml	16.25	Nil	0.3	25
6	Test 4	30 ml	16.25	Nil	0.4	25
7	Test 5	30 ml	16.25	Nil	0.5	25

Table 2. Preparation of Pineapple Jam

Sl. No.	Name	Amt. of sugar(g)	Amt. of pectin(g)	% brix	pH
1	Control	26	nil	60	3.6
2	Standard	26	0.25	60	3.6
5	Test	26	0.25	60	3.6

2.4.9. Texture Profile Analysis(Banaś, Korus, and Korus 2018)

Texture Profile Analysis of the above jams were carried out using UTM-Lloyd instrument, model LR-5k, at a speed of 50mm/min, using a circular probe with a diameter of 80mm.

3. Results and Discussion

3.1. Effect of various extraction conditions upon pectin yield

Table - 3 shows the yield under specified conditions of extraction (Values are averages of six independent analysis ± SEM)

a. Provided all other conditions of extraction being identical, yield has been increased as the duration of heating increased. This is evident from the following pairs of sets, 1 and 16, 6 and 7, 28 and 20, and 24 and 10 (Table 4). In all the above-mentioned cases, the percentage of increase in yield of pectin upon increased duration of heating is noticeable.

Table 3. Yield of pectin (% of dry weight) from peel of Banana.

Set	SLR	pH	Time(min)	Temp(^{0}C)	Yield (%)
1	30	1.5	52.5	54	1.77±0.34
2	40	2	75	68	3.7±0.91
3	30	2.5	52.5	82	3.73±0.27
4	40	2	75	68	2.82±0.13
5	60	2	75	68	4.33±0.4
6	30	1.5	52.5	82	16.4±1.64
7	30	1.5	97.5	82	27.5±0.7
8	20	2	75	68	1.62±0.22
9	50	2.5	97.5	54	1.23±0.16
10	50	2.5	97.5	82	4.97±0.86
11	40	2	75	40	0.97±0.18
12	40	2	75	68	3.43±0.84
13	50	1.5	97.5	54	15.1±0.67
14	50	2.5	52.5	54	1.7±0.09
15	50	1.5	52.5	54	2.68±0.47
16	30	1.5	97.5	54	3.78±0.52
17	40	2	75	68	7.7±1.37
18	50	1.5	52.5	82	28±2.25
19	40	1	75	68	5.47±0.78
20	30	2.5	97.5	54	2.07±0.11
21	50	1.5	97.5	82	24.6±2.46
22	40	3	75	68	3.12±0.28
23	40	2	120	68	8.05±1.53
24	50	2.5	52.5	82	3.57±0.64
25	30	2.5	97.5	82	2.93±0.06
26	40	2	30	68	2.97±0.47
27	40	2	75	68	5.23±0.79
28	30	2.5	52.5	54	2.5±0.39
29	40	2	75	68	7.48±1.16
30	40	2	75	96	17.5±1.87

Table 4. Effect of duration of heating upon yield of pectin

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
1	30	1.5	52.5	54	0.8 (a)	200%
16	30	1.5	97.5	54	1.6 (b)	
6	30	1.5	52.5	82	10.6 (a)	233.02%
7	30	1.5	97.5	82	24.7 (b)	
28	30	2.5	52.5	54	0.7 (a)	242.86%
20	30	2.5	97.5	54	1.7 (b)	
24	50	2.5	52.5	82	1 (a)	240%
10	50	2.5	97.5	82	2.4 (b)	

b. As the temperature of extraction increases, yield also increases. This is evident from the relevant values of the following pairs of sets, 1 and 6, 16 and 7, 11 and 2, 29 and 30, 15 and 18, 13 and 21, and HA9 and 10 (Table 5). At a pH of 1.5 and for a less time period of heating, temperature was a very important limiting factor. This is evident from the sets 1 and 6, 15 and 18 all of which heated for 52.5 minutes. The first pair demonstrated an increase of 926 % while the second one, an increase of 1044.8 % because of the increase of temperature from 54^{0}C to 82^{0}C. However, when the mixtures were heated for a longer period of duration (97.5 min), the increase in percentage of yield decreased to 163 % (13 and 21) or 728 % (16 and 7).

Table 5. Increase in yield presumably due to increased temperature

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
1	30	1.5	52.5	54	1.77 (a)	926%
6	30	1.5	52.5	82	16.4 (b)	
16	30	1.5	97.5	54	3.78 (a)	727.5%
7	30	1.5	97.5	82	27.5 (b)	
15	50	1.5	52.5	54	2.68 (a)	1044.8%
18	50	1.5	52.5	82	28 (b)	
13	50	1.5	97.5	54	15.1 (a)	162.9%
21	50	1.5	97.5	82	24.6 (b)	
11	40	2	75	40	0.3 (a)	233.33%
2	40	2	75	68	0.7 (b)	

29	40	2	75	68	1.8 (a)	466.67%
30	40	2	75	96	8.4 (b)	

c. Yield drastically increases when pH decreases from 2.5 to 1.5. This is clear from the analysis of the following pairs of sets as is shown in tables 6 and 7, 6 and 3, 16 and 20, 7 and 25, 15 and 14, 18 and 24, 13 and 9, 21 and 10. This may be because at the higher pH, the extractant may have lower penetrability in to the cell wall materials and also because the chemical bonds between pectin and other cell wall components become weaker at the lower pH. At pH = 1, the yield was very little (Set 19), probably because the other factors (duration of heating and temperature) might not be in their optimal levels. Also, the higher concentration of the acid might have disintegrated the pectin released. Increase in yield with decrease in pH was noticeable in the sets in table 6. As is evident from the table, a feature common to all sets with increased yield is that they are maintained at a temperature of 82^0C. Even though Set-13 demonstrated an incredible increase of 1227.6% in yield over Set 9, the absolute yield of Set 13 was lower (only 15.1%) probably because of its lower temperature of 54^0C.

Table 6. Noticeably increased yield due to lowered pH

Set	**SLR**	**pH**	**Time (min)**	**Temp (^{0}C)**	**Yield (%)**	**Increase in yield = (b/a) ×100 = c**
3	30	2.5	52.5	82	3.73 (a)	439.7%
6	30	1.5	52.5	82	16.4 (b)	
25	30	2.5	97.5	82	2.93 (a)	938.6%
7	30	1.5	97.5	82	27.5 (b)	
24	50	2.5	52.5	82	3.57 (a)	784.3%
18	50	1.5	52.5	82	28 (b)	
9	50	2.5	97.5	54	1.23 (a)	1227.6 %
13	50	1.5	97.5	54	15.1 (b)	
10	50	2.5	97.5	82	4.97 (a)	495%
21	50	1.5	97.5	82	24.6 (b)	

Table 7. Influence of pH upon yield of pectin from banana peel

Set	**SLR**	**pH**	**Time (min)**	**Temp (^{0}C)**	**Yield (%)**	**Increase in yield = (b/a) ×100 = c**
16	30	1.5	97.5	54	3.78 (b)	182.6%
20	30	2.5	97.5	54	2.07 (a)	
15	50	1.5	52.5	54	2.68 (b)	157.7%
14	50	2.5	52.5	54	1.7 (a)	

d. The effect of SLR on the pectin yield was found to vary with pH. At a pH of 1.5, increase in SLR from 30 to 50 (ml of extractant solution per gram of powered peel) was found to be increasing the yield slightly (table 8). The increase in yield when the SLR is increased to 50 from 30 is not as high as in the case of the other conditions such as pH, duration of heating, temperature etc. because those conditions are more limiting than SLR (within the range studied). The increase in yield of Set -18 may be because of the higher temperature (82^0C) at which the experiment was carried out. Also, the reason for the higher yield (15.1%) of Set-13 (at SLR = 50) may be the increased time period of heating of the extraction medium.

At a pH of 2 or above, it was found that the yield decreases slightly as the SLR increases. This is evident from the analysis in table 9. This must be because of the dilution of protons in the extractant medium. At a higher pH the concentration of protons in the extractant decreases. This, when coupled with increased volume of the extractant solution, results in still lesser concentration of protons affecting the removal of pectin from other cell wall components. But this effect of dilution of protons at higher pH and SLR is solved to a certain extent when both the time of heating and temperature are increased as is evident from the following comparison in table 10. This is also the reason for the result of comparison between Set-3 and Set-24 in table 9. These observations clearly indicate that the effect of dilution of the solution can be overcome by heating the extractant medium for a prolonged time at a higher temperature.

The solid, liquid ratio should be as high as possible because as the volume of the liquid increases, more of it should be evaporated or more alcohol should be used to precipitate the pectin present in it. Also, it consumes more acid to prepare the liquid of desired pH. Therefore, it is essential to know the minimum possible volume of the liquid extractant which can extract maximum amount of pectin from the raw material.

Table 8. Influence of SLR on pectin yield at pH = 1.5

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
1	30	1.5	52.5	54	1.77 (a)	151.4 %
15	50	1.5	52.5	54	2.68 (b)	
6	30	1.5	52.5	82	16.4 (a)	170.7 %
18	50	1.5	52.5	82	28 (b)	
16	30	1.5	97.5	54	3.78 (a)	399.5 %
13	50	1.5	97.5	54	15.1 (b)	

Table 9. decreased yield due to increased SLR

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
29	40	2	75	68	7.48 (a)	57.89%
5	60	2	75	68	4.33 (b)	
28	30	2.5	52.5	54	2.5 (a)	68%
14	50	2.5	52.5	54	1.7 (b)	

3	30	2.5	52.5	82	3.73(a)	95.7%
24	50	2.5	52.5	82	3.57 (b)	
20	30	2.5	97.5	54	2.07 (a)	59.4%
9	50	2.5	97.5	54	1.23 (b)	

Table 10. Effect of SLR upon yield of pectin from banana peel

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
25	30	2.5	97.5	82	2.93 (a)	b/a = 169.6%
10	50	2.5	97.5	82	4.97 (b)	

3.2. Estimation as calcium pectate

The percentage of calcium pectate obtained is 56.871 %. During the formation of calcium pectate, adjacent polygalacturonic chains are cross-linked by Ca^{2+} ions(Caffall and Mohnen 2009). In this work, the percentage of calcium pectate obtained is far less than the value given for purified galacturonic acid (about 110%)(Ranganna 1977), probably because many of the galacturonic acid residues in pectin may be methyl esterified and not available for cross linking. Also, the crude pectin extract may contain not only galacturonic acid but other components such as galactose, arabinose, rhamnose etc. all of which are not taking part in the formation of calcium pectate.

3.3. Equivalent Mass,

The equivalent mass of the pectin extracted from the peel of banana was estimated to be 6666.6.

3.4. Degree of esterification,

The DE was found to be 62.5 % by the titrimetric method. Therefore, pectin obtained from underground stem of banana is high methyl pectin.

3.5. % of Anhydrouronic acid,

The % of AUA was estimated to be70.4

3.6. Sugar Profile Analysis,

Pectin extracted from peel of banana was found to contain the following types of sugars (Table 11).

The chromatogram is shown in figure 1.

Table 11. Sugar profile of banana pectin obtained by HPAEC - PAD

Monosaccharide	Quantity in µg
Fucose	0.095
Rhamnose	1.115
Arabinose	0.204
Glucosamine	0.048
Galactose	0.824
Glucose	0.615

Mannose	0.464
Xylose	0.069
Galacturonic acid	0.086
Glucuronic acid	0.315

3.7. IR analysis

The peak at 3398 cm^{-1} indicates that it contains –OH groups, commonly present in carbohydrates(Coates 2004). The peak at 2929 indicates C-H stretch, while 1794 indicates a C=O stretch. Peak at 1641 indicates N-H bend of an amide group and 1419 shows a C-H bend. The peak at 1079 represents the stretching of the bond between C and O in a methoxyl group (CH_3-O-)(Coates 2004). It was already reported that the region with strong absorption between 1200 and 950 cm^{-1}, called finger print region is characteristic for each type of polysaccharide and even though difficult to interpret, is independent of the source of pectin and may be instrumental in the identification of galacturonic acid(Kyomugasho et al. 2015)(M. A. Monsoor, U. Kalapathy 2001)·(Gnanasambandam and Proctor 2000). Presence of these functional groups indicates that the crude extract contains pectin-like substances.

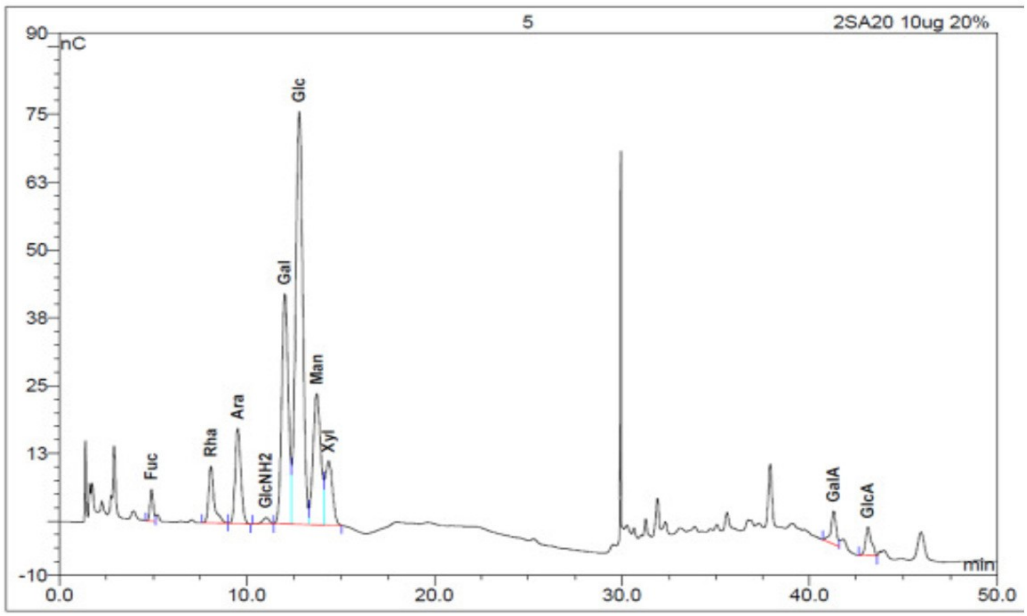

Figure 1. HPAEC – PAD chromatogram of pectin from peel of banana

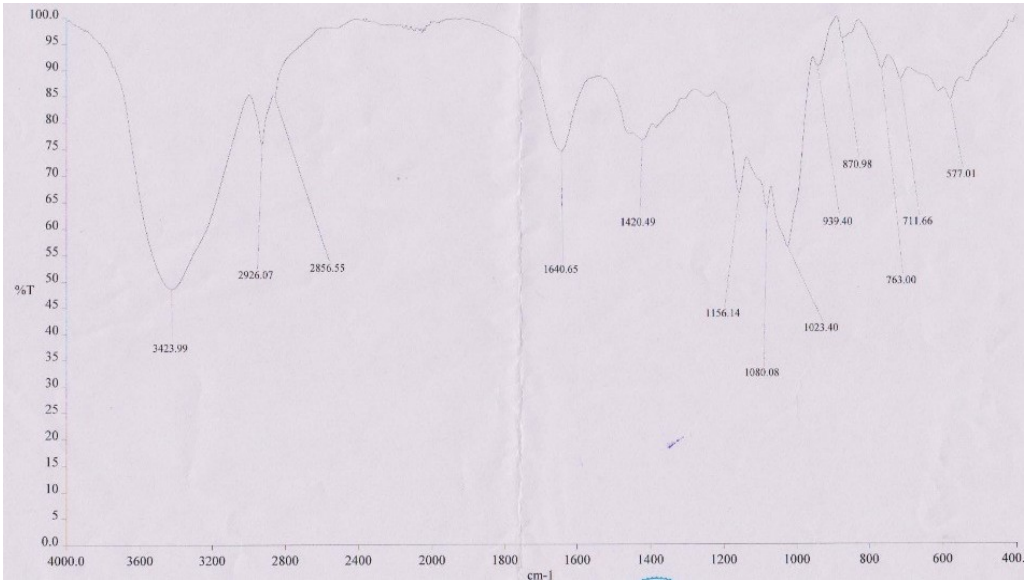

Figure 2. FT IR spectrum of pectin extracted from peel of banana

3.8. NMR Analysis

The ^{1}H NMR spectrum is shown n figure 3.

The ^{13}C spectrum is shown in figure 4.

The HSQC spectrum is given in figure 5

TOCSY spectrum is given in figure 6

Various groups assigned to the chemical shifts are given in table 12(Golovchenko et al. 2007),(BUSH 2016).

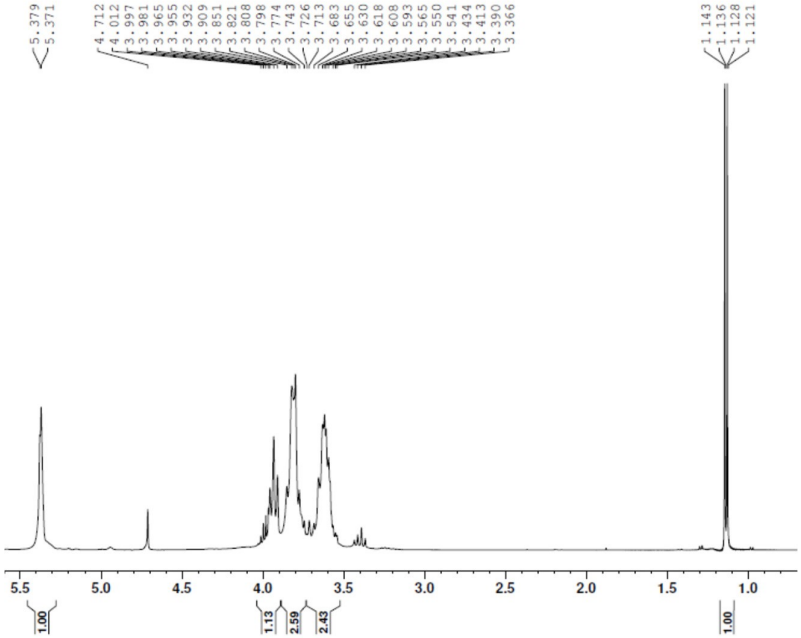

Figure 3. ^{1}H NMR Spectrum of pectin from peel of banana

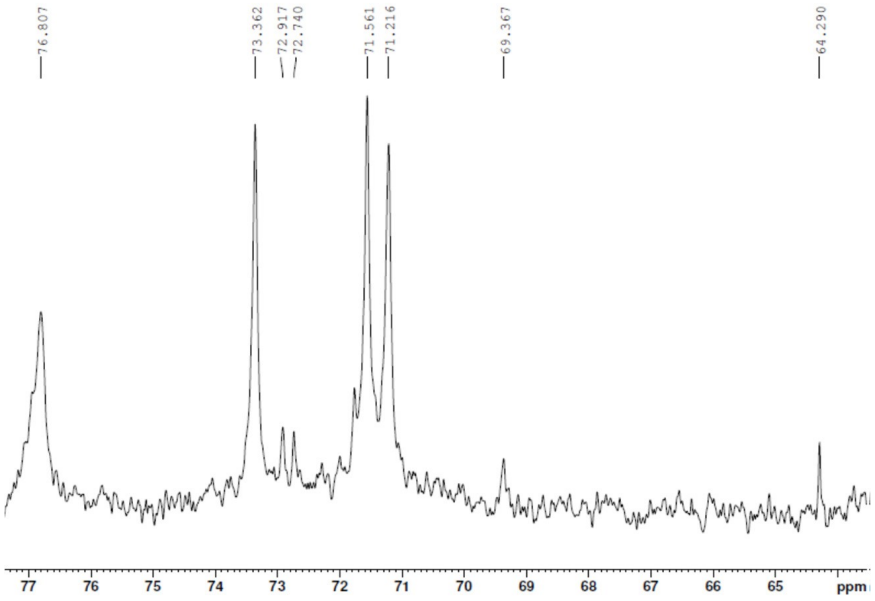

Figure 4. ^{13}C NMR spectrum of pectin from peel of banana

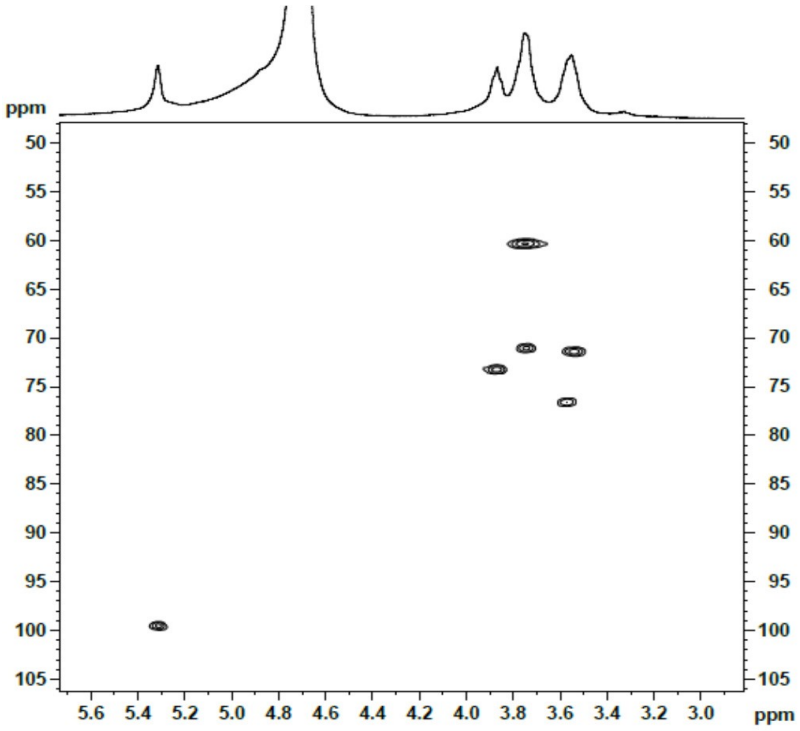

Figure 5. HSQC spectrum of pectin isolated from peel of banana

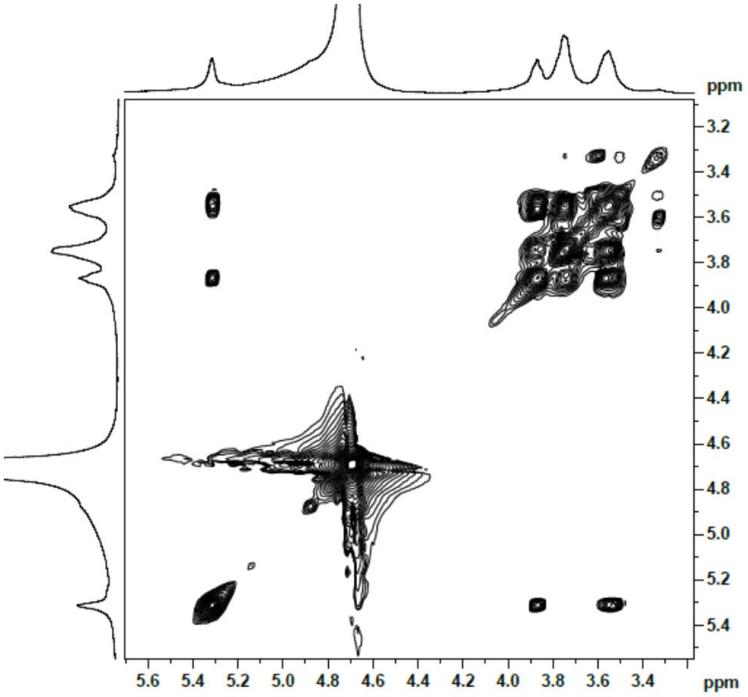

Figure 6. TOCSY spectrum of banana pectin

Table 12. Assignment of chemical shifts of 1H and 13 C NMR spectra

1H Chemical shift	Group identified
3.413	H-4 of →2) - α-L- Rhap-(1→(Rha)
3.43	H4 of → 2) -α - Rhap-(1 →
3.65	H3 of β-Galp-(1→
3.68	H-5 of β-Dgalp - (1→4)-β-Dgalp
3.71	H-5" of α-L-Araf-(1→(Ara)
3.72	H-5 of → 4)- β-DGalpOH
3.74	H-3 of →3)-β-D-Galp-(1→(G)
3.77	H-2 of →4)α-D-GalpA-(1→2) - α-L-Rhap-(1→ (GA)
3.82	H-5 of α-L-Araf-(1→(Ara)
3.85	H-3 of →2) - α-L- Rhap-(1→(Rha)
3.9	H-6" of → 4)- β-DGalpOH
3.93	H4 of β-Galp-(1→
3.96	H-3 of α-L-Araf-(1→(Ara)
4.01	H3 of → 4)-α-GalpA(Ome)-(1→
3.54	H-2 of β-Dgalp - (1→6)-β-Dgalp
3.56	H-2 of β-Dgalp - (1→4)-β-Dgalp
3.59	H-2 of → 4)- β-DGalpOH

3.65	H-3 of β-Dgalp - (1→6)-β-Dgalp
3.72	H6 of β-Galp-(1→
3.77	H-3 of → 4)- β-DGalpOH
3.79	H-6 of β-Dgalp - (1→6)-β-Dgalp
3.8	H-5" of →5)-α-L-Araf-(1→(Ara)
3.9	H-6" of β-Dgalp - (1→6)-β-Dgalp
3.95	H-4 of β-Dgalp - (1→6)-β-Dgalp
3.98	H-3 of →4)α-D-GalpA-(1→GA
13C Chemical shift	Group identified
69.3	C-2 of →4)α-D-GalpA-(1→2) - α-L-Rhap-(1→ (GA)
71.2	C-5 of →2) - α-L- Rhap-(1→(Rha)
71.5	C-3 of →4)α-D-GalpA-(1→2) - α-L-Rhap-(1→ (GA)
72.7	C-2 of →3)-β-D-Galp-(1→(G)
73.3	C2 of →4)-β-D-Galp-(1→4 (G)
76.8	C-5 of →3)-β-D-Galp-(1→(G)
72.9	C-5 of →4)α-D-GalpA-(1→GA
76.8	C-5 of β-Dgalp - (1→4)-β-Dgalp
99.6	non-esterified α-D-GalA

3.9. Viscosity measurements

Results of viscosity measurements are given in table 13.

Test 5, which contains the same concentration of banana as standard, has a viscosity much more than that of the standard which contains citrus pectin. Thus, banana pectin is efficient in increasing viscosity of sugar solutions under the given range of experimental conditions.

3.10. Rheological analysis

Result of Rheological analysis of pineapple jams made using banana pectin (test) is compared with that made using citrus pectin (standard) and that containing no pectin (control)(WINTER 2000).

a. Shear modulus, ratio of stress to strain indicates how strong is the material or how much is the ability of the material to resist a stress. In table 14, shear modulus at a shear strain of about 1.01% is given. Pineapple jam prepared with banana pectin has more shear modulus compared with citrus pectin.

b. Critical stress, also called yield stress, is the minimum stress that must be applied to initiate flow. Pineapple jam prepared with banana pectin has more shear modulus compared with citrus pectin. Jam prepared from banana pectin has more critical stress as is evident from figure 7.

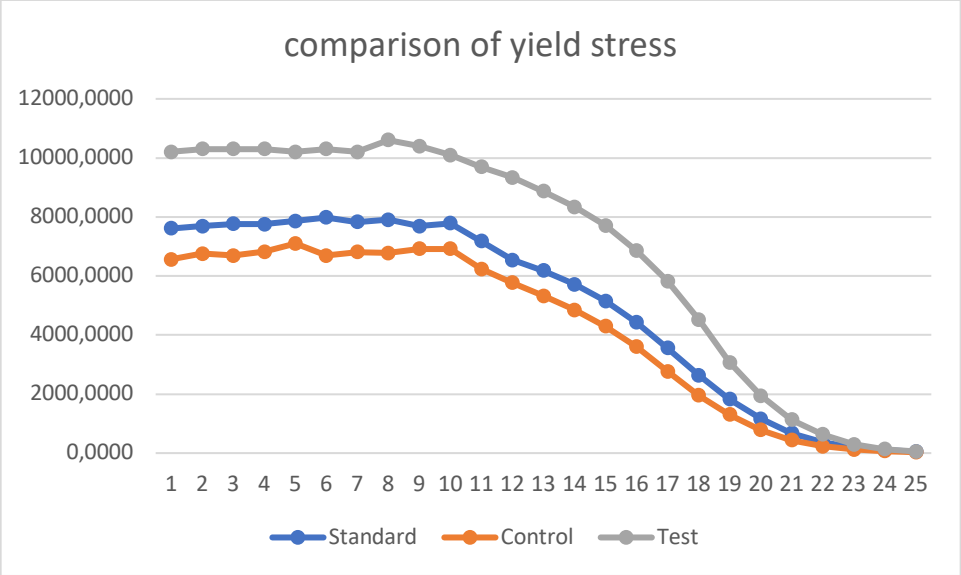

Figure 7. Comparison of yield stress of pineapple jams prepared with banana pectin (test), citrus pectin(standard) and no pectin(control)

3.11. Texture Profile Analysis

Parameters such as hardness, cohesiveness, springiness, gumminess and chewiness are measured. No significant different difference was observed between standard and test in these parameters, as is seen in table 15.

Table 13. Viscosity of sugar solutions containing pectin. All tests contain banana pectin.

Sl. No.	Name of set up	Viscosity at shear rate 1.0 (approx.) Pa.S
1	Control	1.67
2	Standard	11.5
3	Test 1	1.22
4	Test 2	1.99
5	Test 3	2.24
6	Test 4	4.03
7	Test 5	>148

Table 14. Rheological analysis of Pineapple jam prepared with pectin

Sl. No.	Parameter	Control	Standard	Test
1	Shear modulus	30.3000	37.9208	61.2621
2	Critical stress (Pa)	1.2100	1.2400	1.4000

Table 15. TPA of pineapple jams prepared with pectin

	Hardness1 (N)	Hardness2 (N)	Cohesiveness	Springiness (mm)	Gummine ss (kgf)	Chewiness (kgf.mm)
Control	0.617980957	0.531471723	0.768089198	6.244282726	0.04838578	0.302134488

Standard	1.171696864	1.016980649	0.649277941	7.547172493	0.077549126	0.585276632
Test	1.182243837	1.106384146	0.731643238	7.382928507	0.088173365	0.650977649

4. Conclusions

Pectin can be produced from banana peel. Up to 28% of pectin could be extracted under experimental conditions. Different conditions of extraction were found to be affecting the yield. The extracted pectin was found to be high methyl pectin with a %AUA of more than 70% and had a chemical composition similar to pectin from other reported sources. It was also found to be having better rheological properties. India, being the largest producer of banana in the world, has the potential to be the largest exporter as well, if the waste materials such as peel are properly used for the production of pectin. Thus, while increasing productivity and utility of agricultural activities, will also contribute more agro-based industries, employment opportunities and promotes sustainable agriculture practices in the rural and semi-urban India.

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