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Extraction of Pectin from Unripe Banana Peel

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Abstract - *This work aims to develop value-added product such as pectin from solid waste by extraction process from unripe banana peels. Pectin is widely used as a gelling agent, thickener, emulsifier and stabilizer in different food processing operations. Chemically it represents a polysaccharide, which is generally present in different amount in cell walls of all plants. Pectin was extracted from unripe banana peel powder using Hydrochloric acid acids at 90±5°C and at five different time period(1, 2, 3, 4,5) hour and at constant pH 2. Pectin yields varied from 7.5% to 13%. The best extraction condition using HCl acid were temperature 90°C, pH 2 and extraction time of 4 hours with a pectin yield of 13%. The structure of the product is confirmed by Fourier transform infrared spectroscopy (FTIR) analysis. Extracted pectin was characterized by the estimating parameters moisture, ash content, methoxyl content, anhydrouronic acid content, degree of esterification and equivalent weight.*

Key Words: **Anhydrouronic acid content, Extraction, FTIR, methoxyl content, Unripe banana peels.**

1. INTRODUCTION

The production of banana in India was 26509096 Metric Tones (25.58%) in 2015 (Post harvest profile of banana 2015). Main banana producing states in India are Tamil Nadu, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Bihar, Assam and Madhya Pradesh. Key varieties of banana include Dwarf Cavendish; Robusta; Rasthali; Poovan; Nendran; Red Banana; Karpurvalli; Pachanadan; Virupakshi etc. Most of the bananas grown in India are for the domestic market. Even through more than half of the bananas produced in India are Cavendish types. Bananas are considered as one of the most important tropical fruits in the world market. In India bananas is most widely processed into chips which account for 95% of the country's earnings on processed banana. Banana fruit peels compose a significant quantity of wastes produced from banana processing, which is equivalent to 40% of the total weight of fresh banana. However, the peel of banana is often thrown away. Fruit wastes, which are highly perishable is a problem to the processing industries and pollution monitoring agencies .Suitable methods have to be adopted to utilise them for the for the conversion into value-added product.To reduce the environment problems, banana peels can be uitilised in production of pectin. Pectins are complex polysaccharides consisting mainly galacturonic acid units being linked by α-(1→4) linkages (Wosiacki, 1977) which consists of chains of 300–1000 galacturonic acid units (kalapathy U., 2001). It also contains non-sugar substituents, essentially methanol, acetic acid, phenolic acids and occasionally amide groups. The presence of acetyl groups prevents gel formation with calcium ions but provides the pectin with emulsion stabilizing properties (B.R.Sharma., 2006). Following FAO regulation, pectin must contain at least 65% galacturonic acid. It was commonly found in the cell walls and middle lamella of plants. The degree of esterification (DE) is one of the properties influencing pectin application as it determines the gelling nature of pectin. The DE percentage above 50% is classified as high methyl ester (HM) pectin while those less than 50% is known as low methyl ester (LM) pectin

Pectin with DM > 50% forms gels in the presence of high sugar concentration, usually sucrose or fructose and low pH; whereas pectin with DM < 50% forms gels in the presence of divalent ions.

Pectin extracted from banana skin could find application as a gelling agent. It's use in food industry as a gelling agent for example by producing jellies, jam, marmalades, confectionary jelly products, and other food applications. The larges used of pectin is in the manufacture of jellies. About 85% of the commercial pectin in the world is used to make jelly and similar products. Pectins are widely used in the food science, nutrition, cosmetics and pharmaceutical (BeMiller,1986). The yield and DE of a pectin source need to be determined prior mass production of pectin. An extraction process is the most important operation to obtain pectin from banana peel. Pectin extraction is a multiple-stage physical–chemical process in which hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, pH and time.

The yield and quality of pectin depends mostly upon the source as well as the method employed for extraction of pectin (Rehman *et al.*, 2004). In the present study, unripe banana peel waste was utilized as the source of pectin. It aimed to optimize the conventional extraction method in terms of extraction time, and to chemically characterize the extracted pectin.

2. MATERIAL AND METHODS

2.1 Preparation of Saba banana peel powder

Banana peel wastes were soaked in 0.05% sodium metabisulfite for an hour to prevent discoloration, after which were dried in an oven at 55°C for 24 hours (Castillo-Israel, 2015). The dried peels were then cooled at ambient temperature and were made into flour using a grinding mill. The powdered banana peels were then stored in polyethylene bags.

2.2 Chemicals

In our experiments the chemicals used without further purification were: Hydrochloric Acid, 95% Ethanol, 0.1N NaOH, Absolute ethanol, phenolphthalein indicator. Sodium Metabisulphite, 0.25N HCL, 0.25N NAOH,0.1N NAOH, Phenolphthalein indicator.

2.3 Method of extraction

The sequence of the operations performed for the extraction of pectin from lemon peel is presented in figure 1.

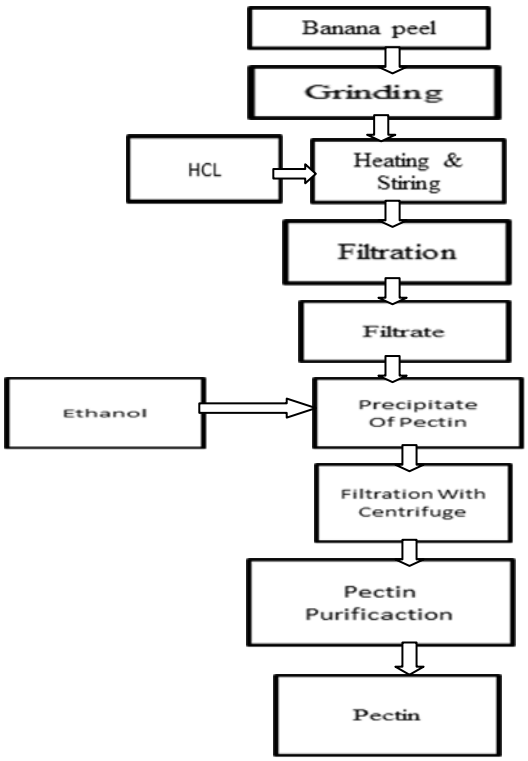

Fig -1: Flow sheet for the extraction of pectin from banana peel

A total of 40 g banana peel powder measured on a weighing balance was blended with 500 ml distilled water and acidified with hydrochloric acid to meet the designed pH of 2.0. The mixture was then stirred using a stirrer until all the banana peel powder was evenly wetted by acidified water in homogenous form. The pectin extraction procedure was continued treating the acidified samples at 90±5°C in a stirring hot plate for 1, 2, 3 and 4 hours. The solution was then cooled and filtered through an ordinary screen with 1-mm mesh size with two-layer cheesecloth. The filtrate was collected then added with twice its volume of absolute ethanol. The precipitated pectin was obtained, and then it was recovered by centrifuge at 5000 rpm for 10 minutes. The resulted pectin substance was dried in a conventional oven at 65°C until a constant weight was reached. The pectin yield was calculated using the equation:

$$\text{Pectin yield (\%)} = \frac{p}{Bi} \text{ x } 100$$

Where

p = extracted pectin in gram

Bi = weight of alcohol-insoluble-residue in gram

2.4 Pectin Purification

Pectin washed with 60% (v/v) ethanol until the filtrate gave a negative response for chloride ions with silver nitrate, indicating that no free sugars were present within the so purified samples. This treatment was carried out for removing free sugars and salts and converting all the non-methylestrified carboxyl groups of pectin macromolecules to the free acid (-COOH) form for a correct titration with 1 N NaOH.

Fig -1: Coagulated pectin after ethanol addition

Fig -2: Pectin powder extracted using Hydrochloric acid

2.5 Pectin Characterization

The extracted pectin was characterized in terms of Presence of amide, moisture content, ash content, equivalent weight, methoxyl content, and anhydrouronic acid (AUA), degree of esterification. The pectin is also analyzed by FTIR.

2.5.1 Presence of amide

Add 0.1 g of sample to the 0.1 N NaOH solution and heated strongly. Smell of NH3 indicates the formation of amide. Heat it until no ore NH3 is evolved. Add few drops of concentrated HCl. If there is absence of precipitate, then there is presence of aliphatic amide.

$CH_3CONH_2 + NaOH \rightarrow CH_3COONa + NH_3$
$CH_3COONa + HCl \rightarrow CH_3COOH + NaOH$

The 0.1 g of substance was taken in test tube and mixed with 5 ml of NaOH. Few drops of conc. HCl were also added. This resulted the presence of amide by the absence of precipitation.

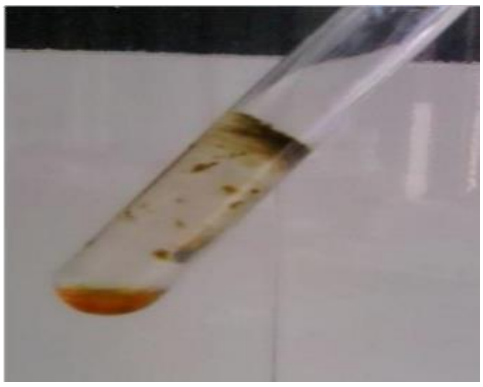

Fig -2: Presence of amide group

2.5.2 Ash content

Ash content of pectin was determined by Ranganna's method. (Ranganna., 1986) Weighed 1.2g of pectic substance (sample).The sample was ignited slowly, then heat for 3-4 hr at 600 ºC. Then cooled the crucible to room temperature in desiccators and weighted properly. The process will be weighted till constant weight come and final weight will be noticed

$$\text{Ash } \% = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.5.3 Moisture content

Ash content of pectin was determined by Ranganna's method (Ranganna., 1986)
One gram of pectin sample was weighed and placed into a metal dish. The sample was dried in an oven for 5 hours at 100°C, cooled in desiccators and then weighed. The moisture content was determined using the equation:

$$\text{Moisture content (\%)} = \frac{\text{weight of residue x 100}}{\text{Weight of Sample}}$$

2.5.4Equivalent Weight

Equivalent weight is determined by Ranganna's method (Ranganna., 1986). Take 0.5 g sample in a 250 ml conical flask and add 5 ml ethanol. Add 1 g of sodium chloride and 100 ml of distilled water. Finally add 6 drops of phenol red and titrate against 0.1 N NaOH. Titration point is indicated by purple color. This neutralized solution is stored for determination of methoxyl content & anhydrouronic acid content.

$$\textbf{Equivalent Weight} = \frac{Weight\ of\ sample \times 1000}{\text{ml of alkali} \times \text{Normality of alkali}}$$

2.5.5 Methoxyl Conten0t (MeO)

The methoxyl content is an important factor in controlling the setting time of pectins. Determination of MeO is done by using the Ranganna's method (Ranganna., 1986). Collect the neutral solution from determination of equivalent weight, and add 25 ml of sodium hydroxide (0.25 N). Stir this mixed solution thoroughly and keep it at room temperature for 30 min. After 30 min add 25 ml of 0.25N hydrochloric acid and titrate against 0.1N NaOH.
Methoxyl content is calculated by following formula

$$\textbf{Methoxyl Content\%} = \frac{ml\ of\ alkali \times Normality\ Of\ Alkali \times 3.1}{Weight\ Of\ sample}$$

2.5.6 Total Anhydrouronic Acid Content (AUA)

Estimation of anhydrouronic acid content is essential to determined the purity and degree of esterification, and to evaluate the physical properties.
Pectin, which is a partly esterified polygalacturonide, contains 10% or more of organic material composed of arabinose, galactose and other sugars. Estimation of anhydrouronic acid content is needed to determine the purity, degree of esterification, and in evaluating the physical properties of pectin.
Total AUA of pectin was obtained by the following formula (W.Elizabeth Devi, 2014)

$$(AUA)\% = \frac{176 \times 0.1z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

molecular unit of AUA (1 unit) = 176 g
Where, z = ml (titre) of NaOH from equivalent weight determination.
y = ml (titre) of NaOH from methoxyl content determination.
w = weight of sample.

2.5.7 Degree of esterification (DE)

The DE of pectin is measured on the basis methoxyl and AUA content (W.Elizabeth Devi, 2014) and
calculated by flowing formula.

$$\text{Degree of esterification (DE)}\,\% = \frac{176 \times \%MeO}{31 \times \%AUA} \times 100$$

3. RESULT & DISCUSSION

A study was carried out on the pectin extraction from unripe banana peel powder. Pectin was extracted from unripe banana peel by using extraction and characterizations of extracted pectin were done.

Table-1:the effect of extraction time on yield of pectin.

Wt. of Sample taken (g)	Wt. of pectin obtained (g)	Extraction time (h)	Yield (%)
40	3	1	7.5
40	4.2	2	10.5
40	4.8	3	12
40	5.4	4	13.5
40	4.5	5	11.2

At 4h the yield of pectin was 13.5% whereas at 5h yield was 11.2% this reveals that extension of extraction time leads to burning & yields less amount of pectin due to continuous stirring and heating. Yield of pectin increases as the extraction time increases and the yield are significantly higher at 4hr. Thus, 4h extraction time was considered as optimum for banana peel pectin extraction.

Characterization of pectin

The pectin obtained by precipitation with 95% ethanol was used for characterization in this work. The characteristics of the pectin in work have been summarized in Table 2

Table -2: Characteristics of pectin

Characteristics	Extraction Time			
	1hr	2hr	3hr	4hr
Pectin yield%	7.5	10.5	12	13
Moisture Content(%)	6	5	4.5	4
Ash(%)	9	10	11	11
MethoxyContent(MeO)%	7.44	7.75	8.06	8.3
Equaivalent Weight	1315.78	1388.88	1470.5	1515.15
AnhydrouronicAcid(AUA)%	55.61	56.67	57.72	58.77
Degree Of Esterification(DE)%	75.95	77.64	79.27	80.18

3.1Moisture content-

Moisture content of banana peel pectin was found to be in the range of 4-6%. Low moisture content is necessary for safe storage as well as to inhibit the growth of microorganisms that can affect the quality due to the production of pectinase enzymes (Muhmadzadeh, 2010). The pectin is very hygroscopic. For this reason, it must be preserved in closed dry atmosphere.

3.2Ash content

The ash content of the pectin isolated in this work is found to be as high 9-11%. The maximum limit though, for good quality gel is 10% (Norazelina, 2011). which is lower than that of unripe peels (11%). Thus, the gel quality that will be produced from these pectins would vary, with banana peel pectin expected to be of lower quality.

3.3Methoxyl content

The methoxyl contents of the extracted pectin fall within this range of 7.4-8.4%. Methoxyl content of commercial pectins generally varies from 8-11% and can form high sugar gels (>65% sugar). On the other hand, low methoxyl pectins (less than 7.0%) can form gels with lower concentrations of sugars.Therefore we can conclude that pectin obtained from banana peel has property to gel with lower concentrations of sugars (Castillo-Israel, 2015)

3.4Equivalent weight

The equivalent weight of pectin is the degree of esterification which is the indicator of its jelly-forming ability, high molecular weight pectin have better ability. Pectin from unripe bananas peels found to be higher equivalent weight (Table.2)

3.5Anhydrouronic acid content

Anhydrouronic acid AUA (%) is essential to determine the purity and degree of esterification and to evaluate physical properties (Ranganna., 1986). It indicates the purity of extracted pectin if it is not less than 65% (Food Chemical Codex IV monographs, 1996). AUA content of less than 65% may indicate impurities due to the presence of proteins, starch and sugars in the precipitated pectin (Norazelina, 2011). The pectins from banana peels have low purity with AUA contain less than 65%.

3.6 Degree of esterification

Degree of esterification (DE) values obtained was within the range of 60-90% which is generally found in tissues (Shaha, 2013). Pectins could be classified as rapid-set (DE >72%) and slow-set (DE58-65%), which describes the rate

of gel formation. Pectin from unripe peels (DE) values greater than 75% as shown in Table no.2.So pectin obtained from unripe peels is rapid-set.

3.7Analysis of Pectin FTIR Spectroscopy:

FTIR spectra of investigated Banana waste sample showed the presence of predominantly esterified pectin form. It is used to examine the functional group in pectin shown in fig. below. FTIR spectrum shows following functional groups present in pectin:

Absorption band at 2644 cm^{-1} corresponds to O-H stretching of hydroxyl group in polysaccharide. The band at 1750 cm^{-1} related to presence of carbonyl absorption of esterified carboxyl group. Band at 1517 cm^{-1} shows amide group. Band at 1235 and 1156 cm^{-1} represent –C-O-C glycoside ring bond. Band at 1078 and 1031cm^{-1} corresponds to C-O stretching in COOH and O-H bending Therefore the data from FTIR agreement, corroborating the predominant existence of esterified form of pectin in given sample.

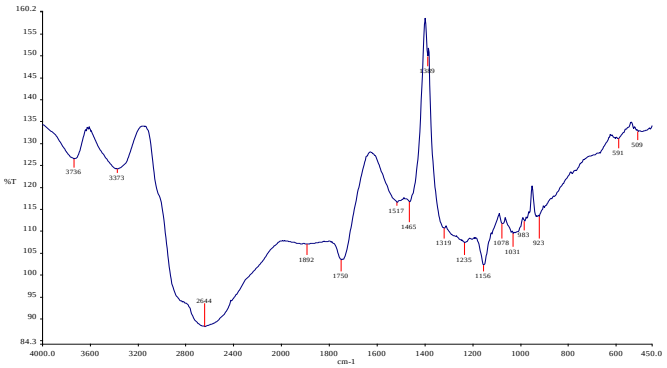

Chart -1: FTIR chart

4. CONCLUSION

This research emphasized on pectin extraction and characterizations from unripe banana peel. In this work effect of extraction time at constant temperature and pH on pectin yield and its characterization was studied. Results from this study indicated that at constant pH and extraction temperature extraction yield increased with increase in time. The best conditions were, extracting temperature at 90±5°C at pH 2 and for 4h by using hydrochloric acid as the extracting solvent. This gave a highest yield of 13%. Further increase in time leads to burning of pectin and causes decrease in yield. FTIR spectroscopy confirms the existence of predominantly esterified pectin in sample. At this extraction conditions the equivalent weight of pectin was found to be high, hence it has lower partial degradation of pectin. From the result it also concluded that pectin has low methoxyl content since it takes time to set in gel form. Extracted pectin contained higher amount of ash which affected the gel formation. Anhydrouronic acid content content is less than 65% which indicate that pectin has impurities. Thus banana peel pectin extracted in this study could be of lower quality. Though the yield from unripe peels is high, the purity is however low, thus further purification is needed to obtain a higher quality pectin for commercial use.

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