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Extraction of Pectin from Banana Pseudo Stem

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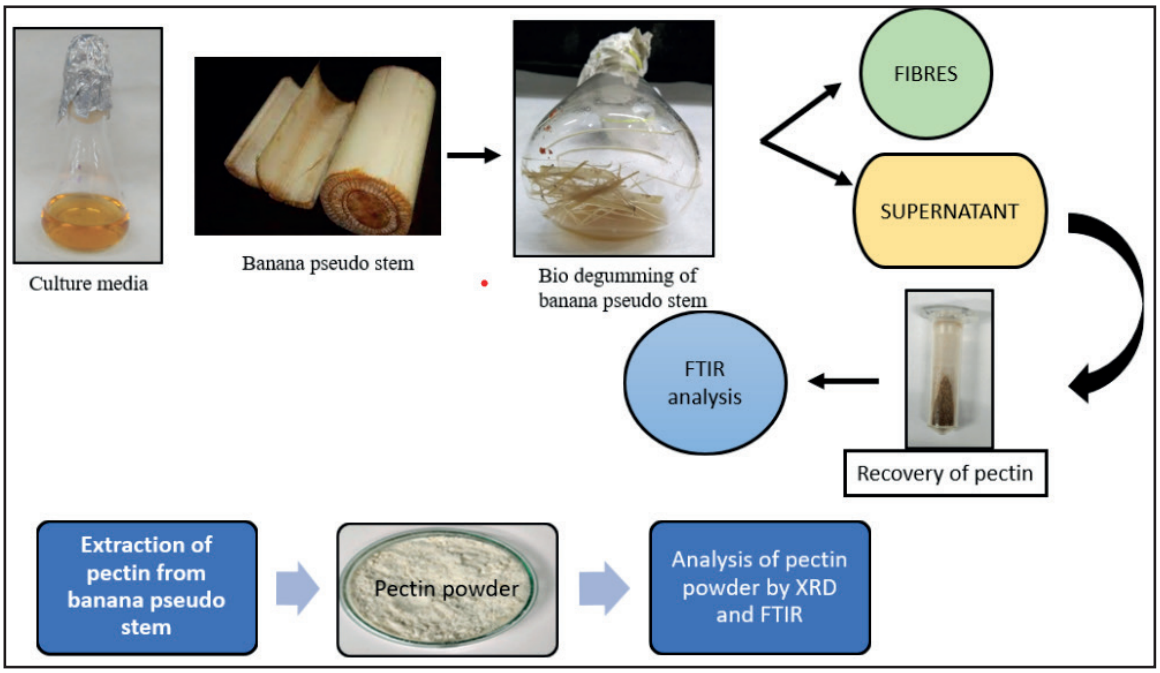

Graphical Abstract

Abstract

This study aims at to extraction of a valuable by-product, pectin, from a less-than-desirable source, namely banana pseudo stem considered to be an agro waste.. Pectin is a versatile biomolecule used in the food business as a thickening, gelling agent, emulsifier, and stabilizer. All plant cell wall include some amount of this molecule which is chemically similar to polysaccharides. Pectin was isolated by soxhlet extraction method, from banana pseudostems. In comparison to other procedures, this procedure yields 35% more pectin. The reliability of the product's construction is tested by use of ftir (fourier transform infrared spectroscopy). X-ray diffraction was utilized to evaluate the pure pectin.

Keywords- Pectin, FTIR, Pseudo stem, XRD

Introduction

Musa supientum, more often known as the banana, is a staple meal in the tropics and subtropics (1). A remarkable 72 million tons of bananas were harvested in 2004(2). In 2015,

India had a banana crop of 26509096 metric tonnes, up 25.58 percent over the previous year (Post harvest profile of banana 2015). Bananas are mostly grown in the Indian states of Tamil Nadu, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Bihar, Assam, and Madhya Pradesh. Dwarf Cavendish, Robusta, Rasthali, Poovan, Nendran, Red Banana, Karpurvalli, Pachanadan, Virupakshi, etc. are all notable banana types. The majority of India's banana crop is destined for the country's domestic market. Despite the fact that Cavendish bananas account for over 50% of India's total output. To this day, bananas remain one of the most widely traded tropical fruits in the world. Banana chips are the most common kind of banana processing in India, accounting for 95% of the country's revenue from the processed banana industry. Peels from banana fruits account for a sizable portion of the garbage generated during banana processing, over 40% of the total weight of unripe bananas.

As the banana processing industry grew, so did the amount of banana peel that was either thrown away or used as inexpensive animal feed, which was both wasteful and bad for the environment. Banana processing companies have been looking for uses for banana waste since it was found to contain useful components like pectin (3,4). Pectins are complex polysaccharides containing 300–1000 galacturonic acid units connected by -(1-4) links (5,6). Non-sugar substituents include methanol, acetic acid, phenolic acids, and amide groups. The pectin's acetyl groups prevent calcium gel formation and stabilize emulsions (7). Pectin must include 65% galacturonic acid according FAO regulation. Plant cell walls and middle lamella contained pectin and it is the degree of its esterification (DE) that affects pectin's gelling ability. Above 50% DE is high methyl ester (HM) pectin; below 50% is referred to aslow methyl ester (LM) pectin. Pectin with DM > 50% creates gels in the presence of sucrose or fructose and low pH; pectin with DM 50% generates gels in the presence of divalent ions.

Banana skin pectin can be utilized as a gelling ingredient in jellies, jams, marmalades, and other foods. Jelly and similar products use 85% of the world's pectin. Food science, nutrition, cosmetics, and pharmaceuticals industry find application of pectins in their products (8). Before mass-producing pectin, its yield and DE must be determined. Pectin extraction is a multistage physical-chemical process that comprises hydrolysis, extraction, and solubilization of pectin polymers from plant tissue.

Pectin production and quality are affected by the source and method of extraction. Some investigations studied conventional degumming technology that improved natural fibre mechanical and morphological qualities. Methods include chemical, biological, and physical. Enzymatic degumming of ramie, jute, hemp, and flax is superior to dew retting (9) Microbiological and enzymatic degumming processes are safe and environmentally benign, with negligible harm to lignocellulosic fibres(10–12).Some research revealed that alkali pre-treatment can remove lignin and other contaminants in natural fibres to improve workability (13). Lignin is the gummiest plant part and it has to be removed if cellulose is the desired end product.(14,15) Combining alkali and silane improved flax fibre processing for bio-composites (16). In the textile business, ultrasonography is utilized for degumming bast fibres. The mechanical characteristics of Apocynumvenetum fibre improved after microwave-assisted ultrasonic degumming (17). Steam explosion and alkali treatment improved hemp fibre cottonization and bamboo fibre physical and mechanical stability (18,19).

Using different degumming enzymes, enzymatic retting may be faster and more reproducible than conventional methods, creating uniform, high-quality fibres(20–24). Few commercial enzymes exist for retting bananas. Due to limited efficiency and high cost, enzymatic degumming has not been commercialized(25–27). Chemical and microbiological processes were added to a degumming process utilised on an industrial scale in China (28–30). The enzymat-

ic treatment phase in the chemical degumming process replaced the acid steeping stage. As a result, degumming time was cut in half, yield increased by 3.42 percent, and chemical use was cut in half. However, chemical contamination persisted.

With the rise of banana processing businesses and the increase in processed fruit products, a huge amount of banana peel was wasted or cheaply used as animal feed, which was wasteful and environmentally unfriendly. Banana-processing firms have been looking for uses for these by-products, which are rich in pectin (3,4).

Materials and Methods

Extraction of pectin from banana pseudo stem

Soxhlet extraction equipment was used to isolate pectin from bananas. Banana pectin and xylan were then isolated and purified (31). After 4 hours of extraction, phenethyl alcohol was utilized as a solvent to remove wax and lipids from 20 g of raw banana. After that, the treated banana was immersed in a 1:20 (m/v) solid-to-liquid solution of hydrochloric acid (pH 1.0), which was then heated to 80 C for 90 minutes. Following these procedures, flasks were allowed to cool to room temperature and their pH was then raised to 4.5 by adding 1 mol/L of sodium hydroxide solution. The solids were extracted by creating a viscous mass and centrifuging it at 6000 rpm for 10 minutes. The pectin was precipitated by adding three times as much 100% alcohol to the supernatant and letting it sit at 4 C for a full day. Centrifugation at 4000 rpm for 10 minutes and three alcohol-washing cycles were employed to recover the pectin. Pectin pellets were freeze-dried and stored at 4 degrees Celsius.

Pectin extraction rate

The yield of pectin, the focus of this investigation, was determined using the following formulas:

$$\text{Pectin yield} = m0 * 100/m \text{ (g)}$$

The weight of the dried product is denoted by m0 (g), and the weight of the dry input is denoted by m (g).

Retting of banana pseudostem

Banana fibre was retted using a culture of *Staphylococcus Sciuri* in a 60litre plastic tank containing 5 kilograms of raw banana, 40 litres of tap water, and 10 litres of inoculum. The tank was then placed in an incubator at 42 degrees Celsius with the pH left at neutral. The microbes were replaced with an equal volume of tap water, and the banana fibres were sampled and analysed every 8 hours. Once the fibres had untangled and taken on the appearance of cotton, the retting process was complete. After 30 minutes in a 0.2% NaOH solution, degumed banana fibres were rinsed in running water and dried in an oven. In order to investigate the possibility of over-retting damaging the banana fibres, we left the separated fibres in the retting solution for an extra three days.

Analysis of enzyme activity

The effectiveness of the main enzymes in the retting process was analyzed.at 8-hour intervals, samples of liquor were obtained from the retting tank and centrifuged to remove the cells and fibre debris (8000 rpm). Supernatants were collected and employed as a basic enzyme solution. Using the DNS approach, pectinase, xylanase, and cellulase activity was measured (2).One unit (U) of pectinase, xylanase, or cellulase activity was required to produce 1 mol of glucose, xylose, or galacturonic acid equivalent per minute at pH 7.0 and 42 C, respectively.

Pectin recovery from retting waste

Banana pseudo stem fibers were removed from the retting tank. ten liters of retting residual liquor (including sediment) were filtered through double-layer gauze to remove fibre debris. The filtrate was concentrated by about 10 times using microfiltration using a hollow fibre microfiltration membrane (pore size range of 0.2-75 lm). The concentrated liquor was centri-

fuged at 4000 rpm for 15 minutes. The precipitates were re-suspended in a 1 mol/L solution of hydrochloric acid and centrifuged at 6000 rpm to separate them (HCl).

Precipitates that had been treated with HCl were put onto medium-speed filter paper with pores that ranged from 30 to 50 lm. The remaining bacteria were then removed by vacuum filtration twice, rinsing with sterile water in between. Vacuum freeze-drying was used to dry filter residues and filter paper, and pectin blocks and xylan slices were mostly used to combine the powders that were collected.

FT-IR evaluation

The sample powder was dried in the oven and pulverized in a mortar and pestle using banana pseudo-stemor pseudo-stem retting leftover liquid. After that, 1 mg of the material was combined with 150 mg of KBr, and a pellet was formed for Fourier transform infrared (FT-IR) analysis. A spectrum 2infraredfrom PerkinElmer FTIR spectrometer was used to determine the sample's chemical makeup A spectral resolution of 4 cm-1 and 64 scans were used to record spectra in the 400–4000 cm^{-1} range.

Characterization of pectin powder by XRD

The X-ray diffraction patterns of the pectins were obtained using an X-ray diffractometer (XRD 6000, Shimadzu, Japan). CuK radiation was used to scan pectins in powder form from 5° to 90° of the diffraction angles (2) at a wavelength of 0.154 nm and a voltage of 40 kV and 40 mA. The step scan mode had a counting time of 0.1 seconds and was 0.02/min.

Result and Discussion

Pectin's yield and qualities are strongly influenced not only by the source from which it isderived but also by the nature of the extraction method that is applied. The amount of pectin that can be obtained via the use of the Soxhlet method is compared in table1 with the amount of pectin that can be obtained through the use of other techniques, such as the citric acid extraction method and the hydrochloric acid extractionmethod(32)

Table 31: Yield of pectin - PMp-HCl, PMp-CA, PSd-HCl and PSd-CA,

pectin	yield
P_{soxlet}	35%
P_{Mp-HCl}	14.55
P_{Mp-CA}	16.65
P_{Sd-HCl}	16.75
P_{Sd-CA}	18.79

where P, Mp, HCl, CA and Sd stands for pectin, *Malus pumila*, hydrochloric acid, citric acid, and *Spondias dulcis*, respectively.(32)

FTIR analysis

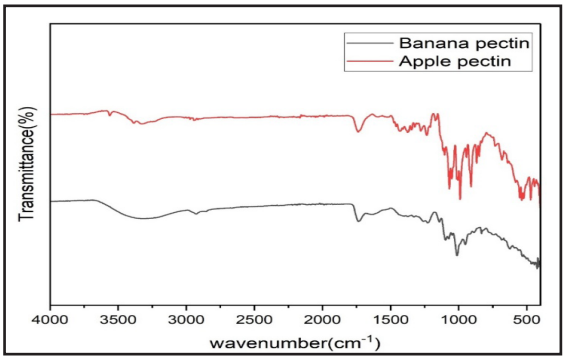

Figure 1 Comparison of FTIR spectrum of apple pectinand banana pectin

40g raw banana pseudo stem yielded 13.8g pectin. One milligrammeof extracted pectin and apple pectin were utilised for FT-IR analysis. Absorption band at 3383 cm-1 in apple pectin FT-IR spectr a (Fig. 1) is due to hydroxyl (–OH) group stretching (33). Absorptions at 1736 cm-1 are due to C=O stretch carboxylic acids, esters, and aldehydes (34), and bands at 1233 are due to C–O stretch alcohols, carboxylic acids, esters, ethers, and C–N stretch aliphatic amines. C–H wag (–CH2X) alkyl halides, C–O stretch alcohols, carboxylic acids, esters, ethers, C–N stretch aliphatic amines cause the

1235 band (35). C–O stretch alcohols, carboxylic acids, esters, ethers, and =C–H bend alkenes absorb at 1068 and 909(36,37). Also, b glycosidic bond absorption was detected at 942. Similar absorption bands were identified at 3337 cm^{-1}, 1736cm^{-1}, 1225 cm^{-1}, 1011 cm^{-1}, 951 cm^{-1} (Fig. 1).

XRD analysis

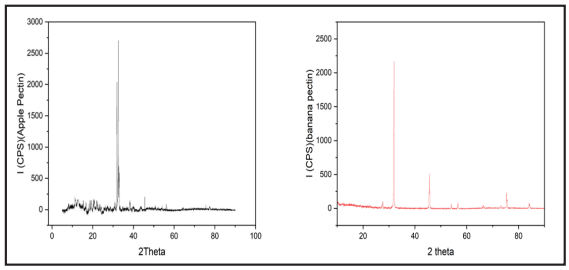

Figure 2 Comparison of XRD spectrum of apple and banana pectin

X-ray diffraction (XRD) described banana pectin's structure (crystallinity or amorphous). Sharp and powerful signals emerged at 27.5°, 31.94°,45.62°,54.12°,56.72°,75.38°,84.34° on the XRD pattern (Fig. 2). The data suggested banana pectin crystallization. Milad Kazemi (2019)(38) described the crystalline form of egg plant pectin. Sharma et al. (2015)(39) suggested *Tamarindus indica* L. pulp pectin is crystalline. Pectin crystallinity depends on raw material source and extraction method.

Analysis of enzyme activity

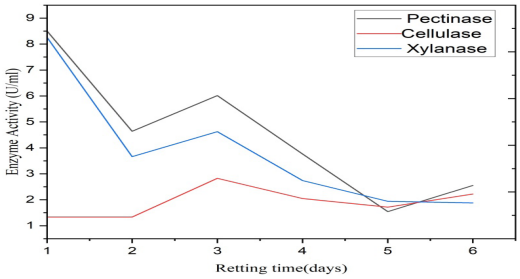

Figure 3: Enzyme activity analysis during retting process

Enzyme activity varying patterns, including those of pectinase, xylanase, and cellulase,examined to evaluate the enzymatic mechanism of *Staphylococcus sciuri's* degumming of banana pseudo stems (Fig. 3). Activity of pectinase and xylanase displayed a somewhat comparable trend. At the end of the third day, pectinase and xylanase activity rapidly increased to 6 U/mL and 4.6 U/ml, respectively, before declining to about 3.76 U/mL for pectinase and 2.7 U/ml for xylanase. Xylanase activity drops to 1.94 U/ml, and pectinase activity drops to 1.7 U/ml. After the sixth day, it further climbed to 2.5 U/mL while xylanase reached 1.8 U/ml. Similar to this, cellulase activity peaked at 2.8 U/mL on the third day and subsequently increased to 1.9 U/mL at the end of the fifth day.

Analysis of recovery pellet

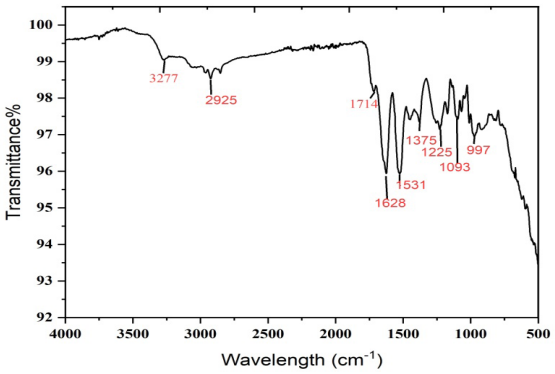

Figure 4: FTIR analysis of recovery pellet

At 3277, there is a tiny peak brought on by the OH stretch. Alkaline medium caused a band to be visible at 2925(40) . Carbonyl (vC=O) in COOH is the cause of the band at 1714 cm1 (41). O-H stretching and H-O-H bending of adsorbed water molecules are responsible for 1628 cm-1 (42). Amide II (N-H) bending has been linked to peaks at 1531 cm-1 (43) widely used in pain and inflammatory diseases. The present study aimed to evaluate the impact of biofield treatment on spectral properties of paracetamol and piroxicam. The study was performed in two groups (control and treatment.

C-H deformation in cellulose and hemicellulose is 1375 cm1 (44). Due to C-C stretch, C-H rock, C-O, and N-H bend, the band at 1225 is present (45). The peak at 1093 cm-1 revealed the vibration of the C-O stretching (46)which can be blended easily with cotton fibre or synthetic fibre to produce composite material. In the fiber extraction process, a substantial amount of lignocellulosic wastes are generated, disposal of which creates problem in the adjacent area. In this paper, extracted banana fiber (EBF. Cellulose makes a significant contribution to the band at 997 cm1 (37).

Conclusion

It was found that using *Staphylococcus sciuri* as an efficient microbial block, which could be easily collected from the remaining retting liquor, could significantly boost the overall economic advantage of banana pseudo-stem retting. The pseudo-stem of the banana was used to extract pectin. The largest percentage of waste biomass that is left over after fruit harvesting is the banana pseudo-stem, which can serve as an alternative supply for companies that rely on fibre. It was discovered that the soxhlet extraction method for removing pectin was a process that was both more effective and less disruptive to the polymer chain. The nature of the source as well as the extraction procedure are both important factors that determine the physicochemical properties of the pectin that is extracted.

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Conflicts of Interest:

The authors declare no conflict of interest

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