

Article

Valorization of Orange Peels for Pectin Extraction from BARI Malta-1 (Sweet Orange): A Green Approach for Sustainable Utilization of Citrus Waste

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Abstract

The agro-industrial valorization of citrus waste represents a promising avenue to employ underutilized bioresources. This research investigated the potential of the peels of BARI malta 1 (sweet orange), a widely grown variety in Bangladesh, as a viable and new source for pectin extraction. Pectin is a polysaccharide, having extensive applications in the pharmaceuticals, cosmetics, and food business as a thickening, texturizer, emulsifier, gelling agent, and stabilizer. This study investigated the optimum extraction conditions for maximum yield, characterization, and physicochemical properties of the obtained pectin and compared the results with the pectin obtained from other sources. Comprehensive characterization through Fourier-Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) spectroscopy, X-ray diffraction (XRD), thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC), and Field Emission Scanning Electron Microscopy (FESEM) confirmed the structural identity, crystallinity, thermal stability, and morphological features of the extracted pectin. Physicochemical properties, including moisture content, ash content, equivalent weight, methoxyl content, and degree of esterification, indicate the suitability and superiority of the extracted pectin for industrial applications. This research approach not only supports eco-friendly processing of citrus waste but also opens avenue for circular economy initiatives in Bangladesh.

Keywords: pectin; polysaccharides; sweet orange; methoxy; peels



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1. Introduction

Research on agro-industrial waste management has become crucial, especially in developing nations like Bangladesh, where the processing of fruit produces large volumes of biodegradable trash [1]. Citrus fruit peels, one of the many agro-wastes, are widely available byproducts of the food and juice industries, adding to the environmental load and underutilization of bioresources. One of the most extensively grown citrus fruits in Bangladesh is the sweet orange (*Citrus sinensis*), sometimes referred to as Malta locally. The Bangladesh Agricultural Research Institute (BARI) created the BARI Malta 1 variety, which is particularly well-known for its excellent yield and climate adaptability [2]. However, this cultivar's peels, which make up almost half of the fruit's mass, are frequently thrown away as garbage, even though they are a rich source of important biopolymers like pectin [3,4]. As

a complex polysaccharide that is predominantly found in the main cell walls of terrestrial plants, pectin has extensive applications in the food, pharmaceutical, and cosmetic sectors because of its thickening, gelling, and stabilizing properties [5,6]. Pectin is traditionally produced from citrus peels, especially those of lemons and oranges, as well as apple pomace, because of the favorable composition and high pectic concentration. Citrus peel-derived pectin is a sustainable and profitable resource due to the growing demand for natural and biodegradable ingredients worldwide, as well as the focus on waste valorization and the circular economy [7]. Pectin is present in the cell walls of higher plants and acts as a cementing and hydrating agent for the cellulose network. Pectin's complicated structure, which is made up of a backbone of mostly alpha- D- galactopyranosyluronic acid units (Figure 1), is interrupted by rhamnose and decorated with different neutral sugar side chains. This intricate architecture reinforces its diversified applications [6]. Orange production in Bangladesh has steadily increased due to the rising interest in commercial citrus farming, particularly BARI Malta 1. Despite this progress, orange peels are rarely used for value-added purposes, and the citrus processing sector is still in its infancy. Orange peels have significant potential for bioresource valorization since they are a rich source of galacturonic acid units and methoxylated polysaccharides, which are essential components of high-quality pectin [8]. According to studies, the dry weight of pectin in sweet orange peels ranges from 20% to 30%, depending on the variety, maturity, and extraction circumstances [9]. In addition to improving environmental sustainability, the use of this biomass has the potential to generate economic value by producing useful components for the domestic industry. There are three methods for extracting pectin from citrus peels: enzymatic treatment, acid hydrolysis, and microwave assistance. The most used method is acid hydrolysis, which utilizes citric or hydrochloric acid and is simple and economical [10–12]. The quantity and quality of pectin are greatly influenced by the optimization of extraction parameters, including pH, temperature, duration, and the ratio of solid to liquid [13]. Along with optimizing the yield of pectin, the equivalent weight, degree of esterification (DE), methoxy content, and galacturonic acid content are necessary for characterizing it since these factors affect its gelling and functional qualities [14]. Pectin is being imported in large quantities by Bangladesh for usage in pharmaceutical formulations and food processing. Therefore, the production of pectin from locally available fruit wastes might greatly lessen reliance on imports while encouraging environmental care and industrial independence. Additionally, by creating small-scale extraction facilities and increasing the profitability of orange cultivation, the growth of a regional pectin industry might help sustain rural lives. Considering the aforementioned facts, the purpose of this study is to assess BARI Malta 1 orange peels as a promising source of high-quality pectin. The aim of the study is to develop sustainable and eco-friendly extraction techniques, followed by an in-depth analysis of the physicochemical properties for characterization. Ultimately, the research findings could offer a viable and financially appealing substitute for pectin sourcing, aligning with principles of resource circularity and green chemistry.

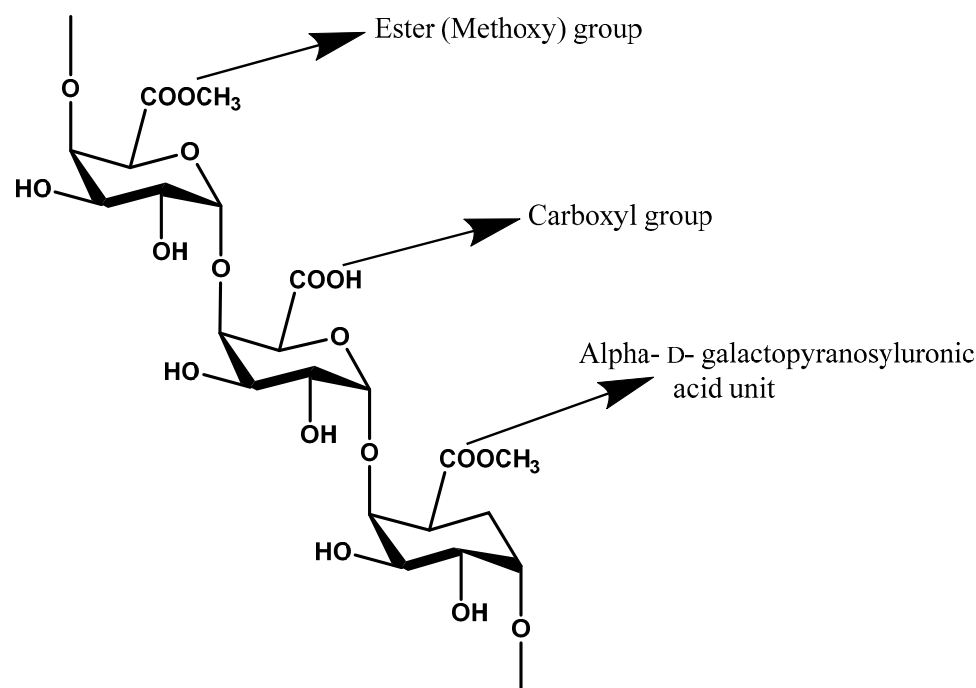


Figure 1. Structure of pectin.

2. Experimental Section

2.1. Materials

Oranges (BARI malta-1) were collected from the local market. All chemicals were purchased from commercial sources and utilized without additional purification. Citric acid, ethanol, sodium hydroxide, sodium chloride, hydrochloric acid, and phenolphthalein were used for the extraction and characterization of pectin. Citric acid monohydrate (98%) and ethanol were purchased from Merck, Darmstadt, Germany. All chemicals utilized were of analytical reagent grade. Aqueous solutions were prepared utilizing deionized (DI) water.

2.2. Extraction and Purification of Pectin

Pectin was extracted from orange peel waste using the acid hydrolysis protocol, followed by the alcohol precipitation method shown in Figure 2 [15]. First, orange peels were removed from the BARI 1 orange. After removing the inner flesh, the peels were washed with water, cut into small pieces, and dried at 50 °C in an oven for 24 h (Memmert 30-1060, Memmert GmbH & Co. KG, Schwabach, Germany) [16]. The dried peels were ground into powder, weighed, and kept in a desiccator at room temperature. To begin the pectin extraction process, 50 g of powdered peels were suspended in 300 mL of deionized (DI) water in a beaker (solid–liquid ratio: 1:6). The solution was acidified with citric acid monohydrate to pH ~2 and stirred at 90 °C for two hours using a magnetic stirrer. Afterwards, the resultant slurry was allowed to settle to ambient temperature, then filtered through two layers of cheesecloth. After collecting the filtrate, it was treated with cooled ethanol in a 1:1 ratio, constantly agitated for 20 min, and refrigerated at 4 °C overnight for complete precipitation. The precipitated pectin was filtered through four layers of cheesecloth to remove the soluble impurities, and it was then rinsed three times with cold aqueous ethanol at 75 percent (v/v), 85 percent (v/v), and 95 percent (v/v). Following washing, the precipitate was dried overnight at 40 °C in a hot air oven. Finally, the dried pectin was crushed into powder and kept in a desiccator to preserve it for future use.

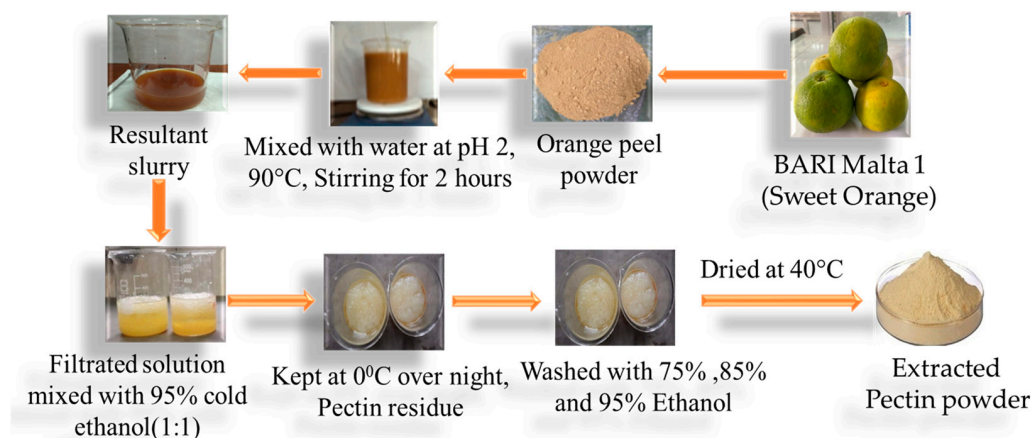


Figure 2. Flow chart of pectin extraction.

Calculation of the % yield of pectin was based on the gram of peel sample taken and was calculated by the formula given below:

$$\% \text{ Yield of pectin} = \frac{\text{Weight of dry pectin(g)}}{\text{Weight of dry orange peel(g)}} \times 100 \quad (1)$$

2.3. Physicochemical Properties

2.3.1. Determination of Moisture and Ash Content

The technique designed by the Association of Official Analytical Chemistry was used to measure the amount of moisture and ash content of the pectin [9].

2.3.2. Determination of Equivalent Weight

The equivalent weight of the extracted pectin was determined by the Ranganna method [17,18]. Here, a 500 mL conical flask was filled with distilled water (100 mL), ethanol (5 mL), and pectin powder (0.5 g). To this mixture, six drops of phenolphthalein indicator along with sodium chloride (1.0 g) were added to sharpen the titration end point. The solution was then titrated with 0.1 N sodium hydroxide until a persistent pink color was observed. The volume of required NaOH solution was recorded for calculation. The resulting neutralized solution was preserved for subsequent determination of methoxyl content.

The equivalent weight was determined using Equation (2)

$$\text{Equivalent weight} \left(\frac{\text{gm}}{\text{mol}} \right) = \frac{\text{Weight of sample(g)}}{\text{mL of alkali} \times \text{normality of alkali}} \times 100 \quad (2)$$

2.3.3. Determination of Methoxy Content

Then, the neutralized solution obtained from the equivalent weight determination study was mixed with 25 mL (0.25 N) of sodium hydroxide solution, kept at room temperature for half an hour, and then agitated to complete the reaction. Subsequently, 25 mL of 0.25 N hydrochloric acid was added to neutralize the excess sodium hydroxide and titrated again with 0.1 N NaOH solution to obtain the end point. The volume of required NaOH solution was recorded and the percentage of the methoxy group was determined using Equation (3):

$$\text{Methoxy content(\%)} = \frac{\text{mL of alkali} \times \text{normality of alkali} \times 31}{\text{Weight of sample (g)}} \times 100 \quad (3)$$

2.3.4. Determination of Anhydrouronic Acid Content

The anhydrouronic acid content (AUA) was determined using Equation (4)

$$\text{AUA}(\%) = \frac{176 \times 0.1(\text{N}) \times V_1(\text{mL}) + V_2(\text{mL})}{1000 \times \text{weight of sample}(\text{mg})} \times 100 \quad (4)$$

where V_1 is the sodium hydroxide used in the determination of equivalent weight content, and V_2 is the sodium hydroxide used in the determination of methoxy content.

2.3.5. Determination of Degree of Esterification (DE)

The titrimetric approach was used to determine the degree of esterification using a well-established literature methodology [19]. To put it briefly, 40 mL of distilled water was used to dissolve 0.4 g of dry material that had been soaked in tiny volumes of ethanol in a conical flask. Using a phenolphthalein indicator, 0.1 N sodium hydroxide solution was added to the pectin solution until a pink hue developed in order to calculate the percentage of free carboxylic acid. V_1 was the initial titration volume, which was the volume measured at the end point. To hydrolyze ester groups to carboxylic acid groups, 10 mL of 0.1 N sodium hydroxide was then added to this solution and swirled for two hours. The solution was then stirred with 10 mL of 0.1 N hydrochloric acid until the pink hue vanished. After that, 0.1 N sodium hydroxide solution was used to titrate surplus HCl until the end point appeared as pink. The final titration volume (V_2) was the titration volume that was noted [20].

Degree of esterification (DE) was calculated using Equation (5)

$$\text{DE}(\%) = \frac{\text{Final titration volume (mL)}}{\text{Initial titration volume (mL)} + \text{Final titration volume (mL)}} \times 100 \quad (5)$$

2.3.6. Solubility Test

For the solubility test, 50 mg of pectin and 5 mL of sodium hydroxide (1 M)/methanol (96%)/acetone were mixed for 2 min and their solubility was monitored [21].

2.3.7. Liquid-Holding Capacity (LHC)

In order to determine the liquid-holding capacity (LHC), 1 g of pectin was combined with 40 g of water, acetone, dimethyl sulfoxide, and acetic acid, left to stand for 2 h, centrifuged for 30 min at 3500 rpm, and weighed, and the LHC was computed using the following formula [22].

$$\begin{aligned} & \text{Liquid holding capacity}(\%) \\ &= \frac{\text{Wet weight of pectin}(\text{g}) - \text{dry weight of pectin}(\text{g})}{\text{Dry weight of pectin}(\text{g})} \times 100 \end{aligned} \quad (6)$$

2.4. Characterization of the Extracted Pectin

2.4.1. Fourier-Transformed Infrared (FTIR) Spectroscopy

The functional groups and structural features of the extracted pectin were characterized using Fourier-Transform Infrared (FTIR) Spectroscopy (Thermo Scientific iS50 ABX, Thermo Fisher Scientific Inc., Waltham, MA, United States). Before analysis, the pectin was dried for 24 h at 45 ± 2 °C in a hot air oven to remove any remaining moisture that might have affected the interpretation of the spectrum data.

2.4.2. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy (Bruker Avance Neo 600 MHz, Bruker Corporation, Billerica, Massachusetts, United States) was used to characterize the

structure of pectin. A transparent solution was prepared by dissolving around 10 mg of pectin in 0.7 mL of deuterium oxide (D₂O, 99.9%) and stirring the mixture at room temperature before analysis. Then the sample solution was transferred into a 5 mm NMR tube for measurement.

2.5. Thermal Properties and Morphological Analysis

2.5.1. X-Ray Powder Diffraction (XRD) Analysis

The crystalline structure of the sample was analyzed using an X-ray Powder Diffractometer (XRD-7000, Shimadzu Corporation, Kyoto, Japan). The measurements were carried out using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) operated at 40 kV and 30 mA. The diffraction patterns were recorded over a 2θ range of $10\text{--}80^\circ$ with a scanning speed of 2° min^{-1} and a step size of 0.02° . Before analysis, the samples were finely powdered and evenly spread on a glass sample holder to ensure a smooth surface. The crystal structures of the sample were analyzed using XRD (XRD-7000, Shimadzu Corporation, Kyoto, Japan).

2.5.2. Thermogravimetric Analysis (TGA)

The thermal stability and decomposition behavior of the extracted pectin were evaluated using a differential thermogravimetric analyzer (SHIMADZU, DTG-60H, Shimadzu Corporation, Kyoto, Japan). Approximately 5–10 mg of the extracted pectin was placed in a platinum crucible and heated from 30°C to 700°C at a constant heating rate of 10°C/min under a nitrogen atmosphere with a flow rate of 50 mL min^{-1} to stop oxidative degradation.

2.5.3. Differential Scanning Calorimetry (DSC) Analysis

The thermal transitions of the extracted pectin were analyzed using a Differential Scanning Calorimeter (DSC) (DSC-60, Shimadzu Corporation, Kyoto, Japan). Approximately 5–10 mg of the dried pectin sample was accurately weighed and sealed in an aluminum crucible, with an empty crucible used as a reference. The analysis was carried out over a temperature range of $25\text{--}500^\circ\text{C}$ at a heating rate of 10°C/min under a nitrogen atmosphere flowing at 50 mL min^{-1} to prevent oxidative reactions.

2.5.4. Field Emission Scanning Electron Microscopy (FESEM) Analysis

The surface morphology of dried pectin powder was analyzed using Field Emission Scanning Electron Microscopy (FESEM) (JSM-IT800, JEOL Ltd., Akishima, Tokyo, Japan). The extracted pectin was mounted on brass (Cu-Zn) stubs with double-sided carbon adhesive tape and sputter-coated with gold for 60 s to improve conductivity and minimize charging effects. Imaging was performed under high vacuum at an accelerating voltage of 5–10 kV, with micrographs captured at various magnifications to observe surface texture, particle size, and microstructural features.

2.6. Statistical Analysis

All reported values were the average of three replicate analyses, and results are expressed as mean $\pm$ standard deviation (SD). The Origin program was used to make the graphs and perform Tukey's test ($p < 0.05$), with a confidence level of 95% ($p < 0.05$) (LabOriginPro (version 10.15)). Principal component analysis of the dataset was carried out using RStudio (Version-2025.09.0-387). Pearson correlation analysis was performed using LabOriginPro (version 10.15).

3. Results and Discussion

3.1. Physicochemical Properties Extracted from Pectin

The physicochemical properties of the pectin extracted from BARI Malta 1 (Sweet Orange) are shown in Table 1. The moisture level of the obtained pectin is $9.30 \pm 0.32\%$, which belongs within the range that pectin manufacturers advise. The International Pectin Producer Association recommends a maximum moisture level of 12% for commercial pectin [23]. In a comparative investigation, the moisture content of pectin derived from different varieties of sweet orange peel was reported to be up to 15.03% [24]. This suggests that a moisture content of $9.30 \pm 0.32\%$ is within the acceptable range. A study on green-extracted pectin from sweet orange peels reported a moisture content of 8.21%, which closely aligns with the value observed in the present investigation [25]. Again, the moisture content of the extracted pectin closely aligns with the previously reported value of 7.81% for pectin derived from dragon fruit peel [26]. To prolong the shelf life, a lower pectin water concentration is preferred. The ash content of the extracted pectin is $6.87 \pm 0.21\%$, which is significantly less than the Worldwide Pectin Production Association's suggested value of a maximum of 10 percent [23]. Another study reported an ash content of 6.77% in pectin derived from sweet orange peel using the alcohol precipitation method. This value is close to the reported value in the current study [27]. The ash content is less than the 19.4% figure recorded for pectin derived from the peels of *Hylocereus polyrhizus*. Pectin is purer when its ash content is lower. The amount of ash in the extracted pectin is influenced by the types of fruit, the pre-treatment process, and the extraction techniques. The total amount of free galacturonic acid in pectin is shown by the equivalent weight. The equivalent weight of the extracted pectin is around 868 ± 6.08 mg/mol, exceeding the minimum threshold of 400 mg/mol recommended by the International Pectin Producer Association [26,28]. Different equivalent weights have been reported in various studies for pectin isolated from sweet orange peel, including values of 594.86 g/mol [29] and 655 g/mol [30]. This figure is less than the value found in the current investigation, indicating that the source, extraction methods, and techniques may affect the equivalent weight of pectin. The equivalent weight of the pectin extracted in this study surpasses the reported value, such as 356 g/mol for pectin extracted from watermelon rind using sulfuric acid [31], and (578 g/mol) for pectin extracted from banana peels with citric acid. The range of equivalent weights listed for pectin extracted from banana peel (43–1456 g/mol) and pectin extracted from apple pomace (833–1666 g/mol) is consistent with our results. The number of free acids on pectin, which varies depending on the kind of pectin source, and the pectin extraction method (pH, acid employed, etc.), are the primary determinants of the equivalent weight. Pectin with a greater equivalent weight has better gel-forming qualities. The degree of methylation is indicated by the methoxy content, which also establishes the gel-forming ability of the pectin and may indicate its potent cohesive and sticky properties [29,32]. Pectin is classified into two categories based on the amount of methoxyl content. While low methoxy pectin (LMP) includes 2.5–7.12% of the methoxyl group, high methoxy pectin (HMP) has higher methoxy group content ($>7.12\%$) [28]. Based on this, pectin extracted from BARI malta-1 is classified as high methoxy pectin, with a methoxy value of $7.40 \pm 0.23\%$. The methoxy value of our extracted pectin is greater than the reported values for pectin derived from apple and banana pomace, which are 2.23–6.21% and 3.86–5.97%, respectively. The percentage of galacturonic acid units that make up the polymer's backbone is reflected in the anhydrouronic acid (AUA) concentration, which is a crucial measure of pectin purity. The FAO/WHO Joint Expert Committee, 2001 on Food Additives states that food-grade pectin ought to contain at least 65% AUA. The pectin extracted from BARI malta-1 in this study has an AUA concentration of $65.15 \pm 0.64\%$, which satisfies international requirements and indicates a high-quality product. In a study,

reported AUA contents of citrus pectin exhibit 65–75%, which matches our study [33]. Overall, the study's $65.15 \pm 0.64\%$ AUA concentration shows that BARI Malta-1 peel is a potential raw material for the manufacturing of high-quality pectin. In a similar vein, pectin may be divided into two categories according to its degree of esterification (DE): high and low. One crucial characteristic that establishes the gelling nature of pectin is DE, which is the ratio of esterified galacturonic acid groups to total galacturonic acid groups in the pectin. Pectin with less than 50% DE is referred to as low methoxyl pectin (LMP), whereas high methoxyl pectin (HMP) is defined as having more than 50% esterification. At $69 \pm 1.92\%$, the obtained pectin's DE falls into the category of having a high degree of esterification. The DE for this pectin is lower than the values found for pectin derived from dragon fruit (63.74%) and bananas (63.15–72.03%). However, the present pectin's DE is greater than the documented values for pectin derived from apple pomace, which range from 22.15 to 52.51%. The degree of esterification and methoxy content are often determined by the pectin extraction parameters (such as the kind of acid employed, the pH of the solution, the extraction duration, temperature, etc.) in addition to the pectin source [34]. The solubility profile is shown in Table 2; the material is soluble only in strong alkaline media (1 M NaOH) but insoluble in organic solvents such as methanol and acetone, suggesting the presence of polar, ionizable functional groups. Strong affinity for DMSO (392% *w/w*), poor retention of acetone (57% *w/w*), and notable water absorption (478% *w/w*) are all shown by the liquid-holding capacity (LHC) results. The material's hydrophilic qualities and restricted compatibility with non-polar solvents are demonstrated by this selective affinity.

Table 1. Physicochemical parameters of extracted pectin.

Parameter	Our Work	Reference (Sweet Orange)
Moisture	$9.30 \pm 0.32\%$	15.03% [24], $13.39 \pm 0.29\%$ [28]
Ash content	$6.87 \pm 0.21\%$	5.0% [35], $3.87 \pm 0.10\%$ [36]
Equivalent weight	868 ± 6.08 mg/mol	833.33 mg/mol [37], 796.40 ± 2.07 [38]
Methoxy content	$7.40 \pm 0.23\%$	7% [39], $8.29 \pm 0.38\%$ [38]
Degree of esterification	$69 \pm 1.92\%$	70% [39], $64.66 \pm 2.08\%$ [38]
Anhydrouronic acid content	$65.15 \pm 0.64\%$	65–75% [40], 67.19% [28]

Table 2. Solubility and liquid-holding capacity of extracted pectin.

Solubility	1 M NaOH	Soluble	Soluble [12]
	Methanol Acetone	Insoluble Insoluble	Insoluble [12] Insoluble [12]
Liquid-holding capacity	Water (% <i>w/w</i>)	478	323.53 ± 2.37 [12]
	DMSO (% <i>w/w</i>)	392	290.27 ± 11.10 [12]
	Acetone (% <i>w/w</i>)	57	80.65 ± 3.08 [12]

3.2. Characterization by FTIR Spectroscopy and NMR Spectroscopy

3.2.1. Characterization by FTIR Spectroscopy

The Fourier-Transform Infrared (FTIR) spectrum of the extracted pectin is shown in Figure 3. The FTIR spectrum was taken for the sample extracted with citric acid at pH (2.0), temperature (90 °C), and time (100 min). The extracted pectin's highly hydrated polymeric network is highlighted by the broad and intense band that is centered between 3660 and 3127 cm^{-1} . This band reflects O–H stretching vibrations from hydroxyl groups that are involved in extensive intra- and intermolecular hydrogen bonding along the galacturonic acid backbone. The existence of a distinctive band at 2937 cm^{-1} is due to the sp^3 C–H stretching vibrations of the –C–H, –CH₂, and –CH₃ groups. The C=O stretching of both esterified (–COOCH₃) and free carboxylic groups (–COOH) appears as

a high intensity, mildly broad overlapping peak at 1737 cm^{-1} . The peak at 1603 cm^{-1} is attributed to the asymmetric stretching vibration of non-esterified carboxylate groups. The relative intensities of these bands directly reveal the high level of methylation in the pectin. Several overlapping absorptions trace out the polysaccharide skeleton within the fingerprint region ($1300\text{--}900\text{ cm}^{-1}$). The strong absorption band at 1014 cm^{-1} is associated with C–O stretching of the pyranose ring, and the band near 1218 cm^{-1} is attributed to C–O–C asymmetric stretching of glycosidic linkages. Together, these spectral characteristics verify that the acid-extracted pectin maintains the integrity of its polysaccharide backbone, shows a high level of methyl esterification, and has very little contamination from proteins or other polysaccharides—all of which are essential for its gelling and emulsifying properties [41,42].

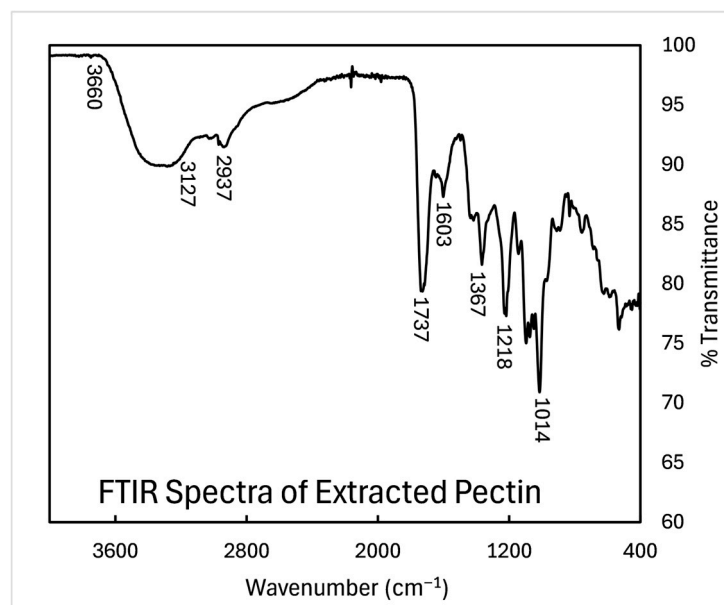


Figure 3. FTIR spectra of extracted pectin.

3.2.2. Characterization by NMR Spectroscopy

The NMR spectra of the extracted pectin shown in Figure 4 were obtained using D_2O as solvent. The ^1H NMR spectrum revealed a distinctive anomeric proton doublet at δ 5.3 ppm. The α -(1 $\rightarrow$ 4)-linked D-galacturonic acid is reported as the main repeating unit of homogalacturonan. This α -linkage is confirmed by determining the H1/H2 coupling constant of 3.0 Hz for the doublet at δ 5.3 ppm. Signals at δ 3.3–4.5 ppm arise from ring protons of galacturonic acid and neutral sugars, indicating the presence of rhamnogalacturonan regions. A singlet at δ 3.7 ppm represents methoxy protons, confirming partial methyl esterification of galacturonic acid residues. The ^{13}C NMR spectrum and ^1H – ^{13}C HSQC spectrum were analyzed for the remarkable carbon peaks present in the structure. The carbon/proton correlation at δ 95.1/ δ 5.3 ppm in the HSQC spectrum reflects anomeric carbon signal at δ 95.1 ppm (GalA units) and correlation at δ 55.8/ δ 3.7 ppm exhibits $-\text{OCH}_3$ carbon of the ester group at δ 55.8 ppm, which supports partial esterification of the pectin. Multiple resonances of δ 70–82 ppm (C2–C5 carbons), confirm homogalacturonan and rhamnogalacturonan structures. However, the absence of correlation of the carbon signal at δ 106.6 ppm in the HSQC spectrum suggests the presence of a carbonyl group in the ester or acid moiety in the pectin structure. The spectral analysis aligned with the literature [43,44]; therefore, it could be concluded that the NMR data represents the structure as partially methyl-esterified pectin comprising homogalacturonan and rhamnogalacturonan regions.

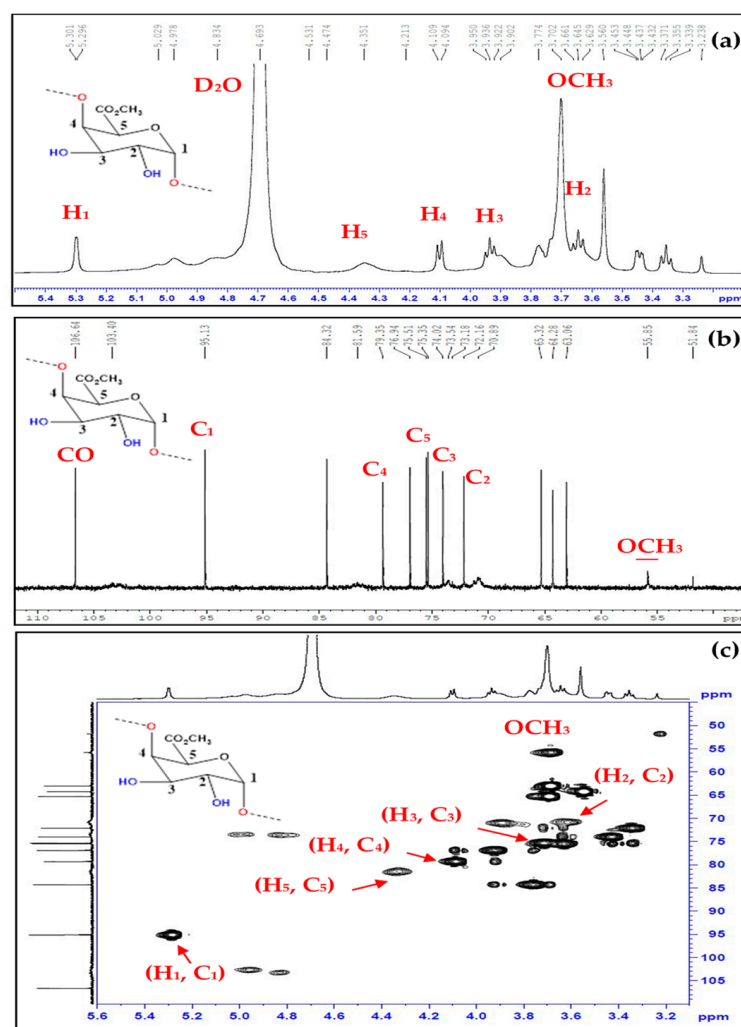


Figure 4. The NMR spectra of three purified pectins in D₂O [31–33]: (a) ¹H spectra; (b) ¹³C spectra; (c) HSQC spectra.

3.3. Thermal and Morphological Analysis

3.3.1. X-Ray Diffraction of Extracted Pectin

The acid-extracted pectin's X-ray diffraction spectrum (Figure 5) shows a broad hump, a diffused peak from about 10° to 25° 2θ, indicating that it is mostly amorphous. There are two faint but noticeable diffraction peaks at 2θ = 18.8° and 19.66° superimposed on this amorphous backdrop. The smaller feature at 11.8° most likely results from retained acetyl groups or clustered rhamnogalacturonan side chains, but the more prominent reflection at 25° indicates the presence of short-range organized domains within the galacturonic acid backbone. Despite the low overall crystallinity, these peaks demonstrate that partial chain alignment and aggregation are induced by the high temperature and low pH extraction conditions. The amorphous areas promote quick water absorption and solubility, whereas the ordered sections can strengthen gel network development, increasing gel strength and thermal stability. This microstructural heterogeneity has applications. These findings are consistent with other studies on fruit-derived pectin that underwent comparable acid-extraction procedures and saw minor improvements in crystallinity in addition to the distinctive amorphous halo [45,46].

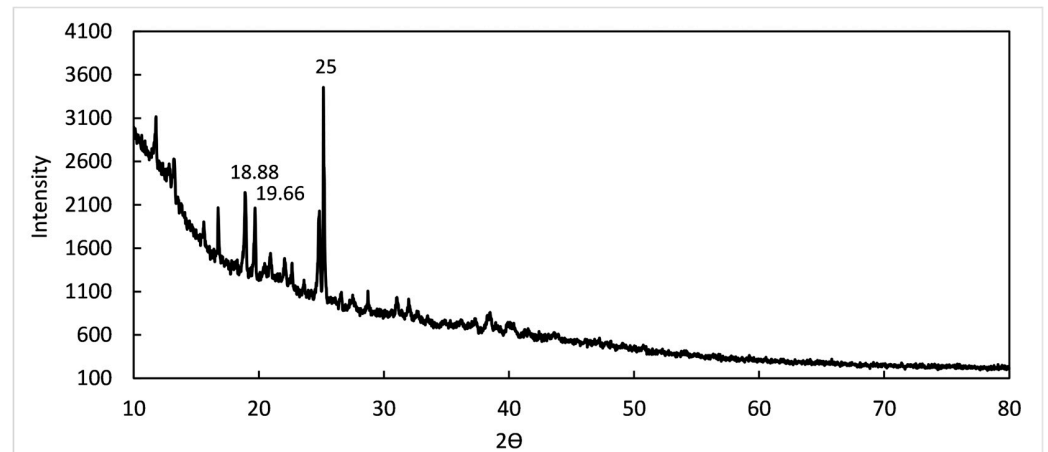


Figure 5. X-ray diffraction pattern of extracted pectin.

3.3.2. Differential Scanning Calorimetry (DSC)

Thermal analysis indicates that the behavior of polymers changes as a function of temperature [47,48]. Figure 6 displays the thermogram of extracted pectin powder, whereby the initial decrease signifies dehydration—a typical thermal phenomenon linked to moisture loss in biopolymers. The glass transition temperature (T_g) recorded at 125 °C indicates a pronounced endothermic transition, suggesting the conversion of pectin from a stiff, glassy state to a pliable, rubbery phase. This transition is essential for comprehending the functional properties of pectin in culinary and medicinal applications, as T_g affects texture, stability, and processability. It is further confirmed that the pectin isolated from BARI malta is thermally stable by the melting temperature of 204 °C and a sharp exothermic peak at ~257 °C associated with polymer backbone degradation. Given that molecular weight distribution and esterification level are known to influence thermal characteristics, a high melting point indicates a well-structured polymer network. According to Basu et al., pectin's glass transition temperature drops as moisture content rises, emphasizing how crucial it is to regulate production and storage conditions. According to earlier research on citrus-derived pectins, the observed thermal profile supports the feasibility of BARI malta pectin for high-temperature uses such as encapsulating technologies, confectionery, and jam manufacture [49].

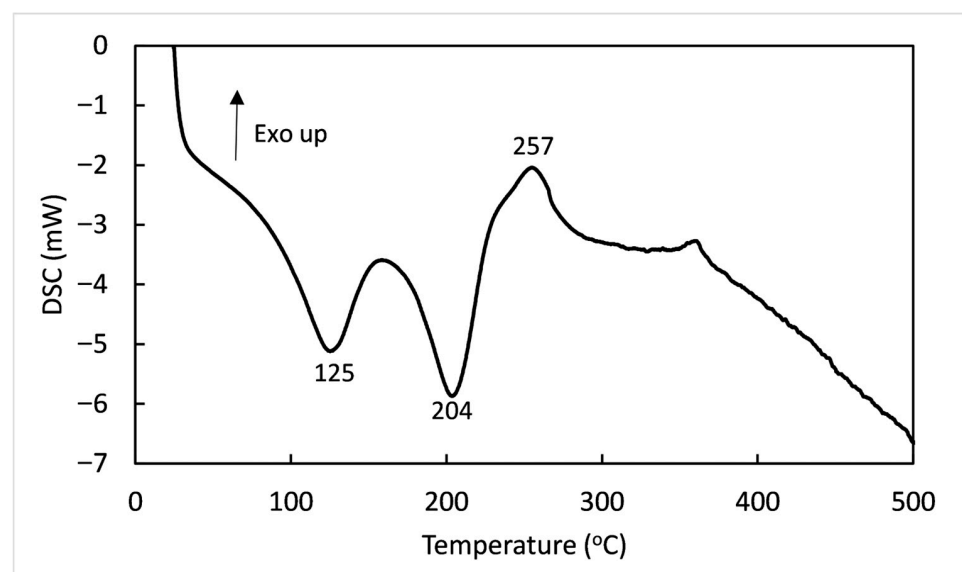


Figure 6. DSC thermogram of extracted pectin.

3.3.3. Thermogravimetric Analysis (TGA)

The thermogravimetric analysis was performed for the sample extracted with citric acid at pH (2.0), temperature (90 °C), and time (100 min). Figure 7 shows the TGA curves of the extracted pectin. The thermogravimetric analysis (TGA) results suggest that the weight loss of the extracted pectin can be divided into three regions. In the first region (up to 184 °C), most water and other volatiles are removed from the extracted pectin. This stage represents the hygroscopic nature of pectin and is consistent with previous studies. In the second region (130–184 °C), early decomposition of side chains indicates the onset of structural degradation, including the breaking of weaker bonds like ester linkages. In the third region (184–285 °C), decomposition of the main chain of the pectin occurs. This is the main pyrolytic event, where the structural integrity of pectin is lost. In the fourth step (285–600 °C), decomposition of secondary decarboxylation with the acid side group and carbon in the ring molecules occurred. Above 500 °C, thermal degradation of the carbon skeleton occurs, typical for polysaccharide chars. According to the recent literature, commercial pectin usually shows four-stage degradation with significant thermal degradation in the range of 230–260 °C [46]. Our extracted pectin, with a major degradation onset around ~185–280 °C, thus shows competitive but slightly lower stability compared to the purified commercial pectins. This suggests that while our pectin is suitable for many industrial uses, further purification or removal of residual side groups/inorganics may improve thermal performance closer to premium commercial standards.

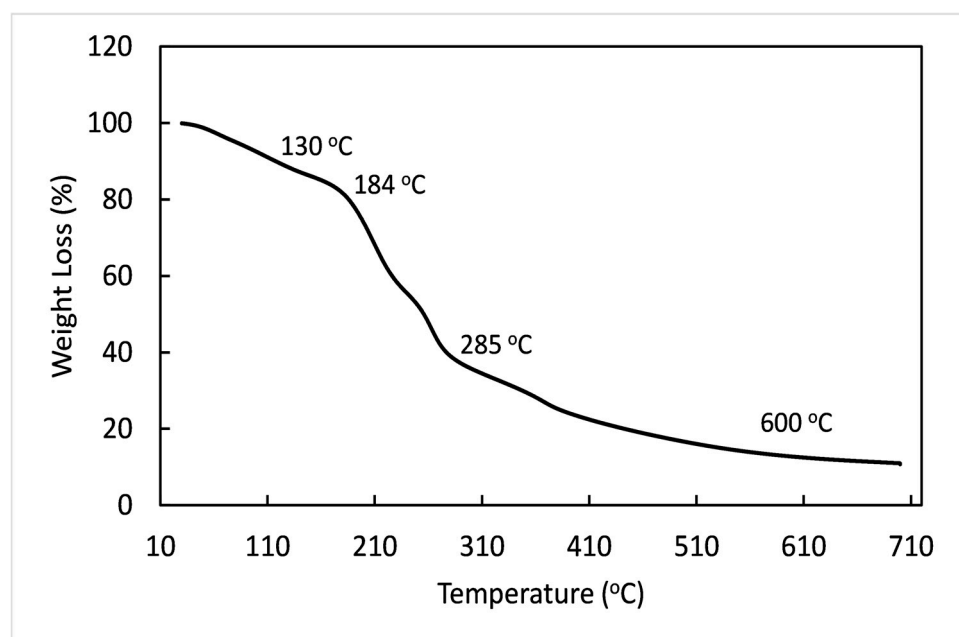


Figure 7. TGA of extracted pectin.

3.3.4. Field Emission Scanning Electron Microscopy (FESEM) Analysis

In Figure 8 shows the FESEM micrograph of the citric-acid-extracted pectin (pH 2.0, 90 °C, 100 min) shows a network of flake-like layers and microcavities that are rough, uneven, and porous. It also suggests the molecular changes that give the pectin its distinct physicochemical characteristics. The homogalacturonan chains are partially broken down by the harsh acidic environment and high temperature, which lowers the degree of esterification and produces a mosaic of amorphous areas with crystalline residues scattered throughout. By varying the extraction duration or solvent ratios, these polymer fragments may be made to create a highly open architecture with pore diameters that can be adjusted from nanometric fissures to submicron cavities when they reorganize during

ethanol precipitation. Its customizable pore shape significantly improves gel network formation and water-uptake kinetics, which makes this low-methoxy pectin a great option for controlled-release medicine encapsulants, high-viscosity food thickeners, or biodegradable scaffold material in tissue engineering. Nonetheless, the fractured, occasionally brittle look of the flake edges also highlights a trade-off: localized drying stresses brought on by either excessive acid hydrolysis or insufficient solvent clearance may jeopardize mechanical integrity. Optimizing post-extraction procedures, like spray-drying in milder conditions or adding plasticizing co-agents, can maintain the porous morphology while increasing flexibility, opening up new functional applications in eco-friendly composite films, smart packaging, and wound-healing dressings [50].

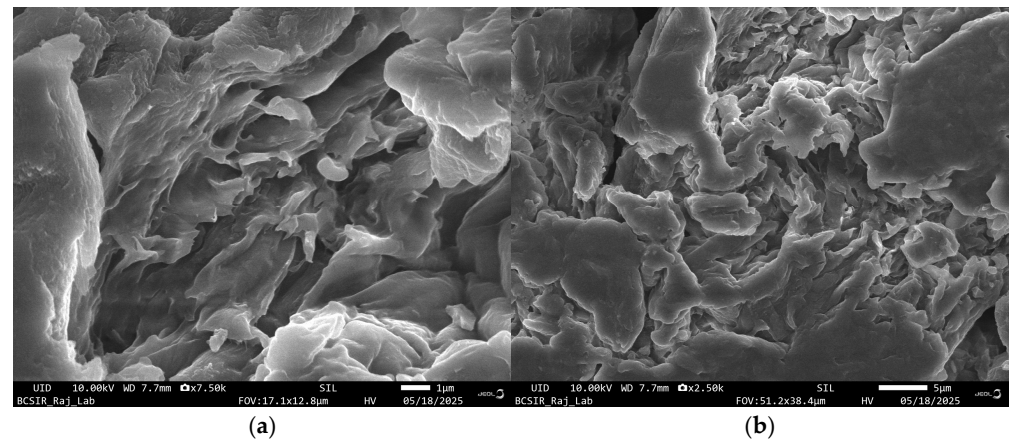


Figure 8. FESEM of extracted pectin. (a) Scale bar 1 μm . (b) Scale bar 5 μm .

3.4. Pectin Yield

In this study, pectin was extracted using conventional acid hydrolysis followed by the alcohol precipitation method. This optimization process showed that the maximum extraction yield was 17.1%. The other existing literature demonstrated that extraction yielded $28.07 \pm 0.67\%$ from sour orange peel by the ultrasound-assisted method [51], 29.1% from sour orange peel by microwave-assisted extraction [52], and $14.50 \pm 0.53\%$ from grapefruit and naggur mandarin by enzyme-assisted extraction [53]. A few parameters, including pH, temperature, extraction duration, acid types, and the kind of pectin sources, affect pectin production by the acid hydrolysis method. The effects of temperature, extraction duration, and pH were all methodically examined in this work (Figure 9). Figure 9a displays how pH affects pectin production. Pectin production grows with increasing pH from 1.0 to 2.0, reaching a maximum of 17.1% at pH 2.0. This suggests that one of the most important factors in the extraction process is pH. Lee and Choo observed a similar pattern, noting that low pH levels speed up the breakdown of hydrogen bonds and ester connections between pectin and the matrix of the plant cell wall, increasing the rate at which pectin diffuses and releases into the extraction medium [54]. But too low of a pH can also cause pectin chains to partially break down, which lowers the quality of the finished product.

Figure 9b shows the impact of extraction temperature on pectin yield. The reaction was studied at various temperatures, and it was found that at 90 $^{\circ}\text{C}$, the yield reaches the highest value (17.80%). After this, the output drops to 15.70%, with a further rise to 110 $^{\circ}\text{C}$. This decrease is explained by the depolymerization and loss of functional groups necessary for gel formation caused by the thermal breakdown of pectin molecules at high temperatures. Higher temperatures must thus be carefully managed to prevent damaging the integrity of the pectin, even if they can speed up the extraction process by increasing solubility and diffusion rates.

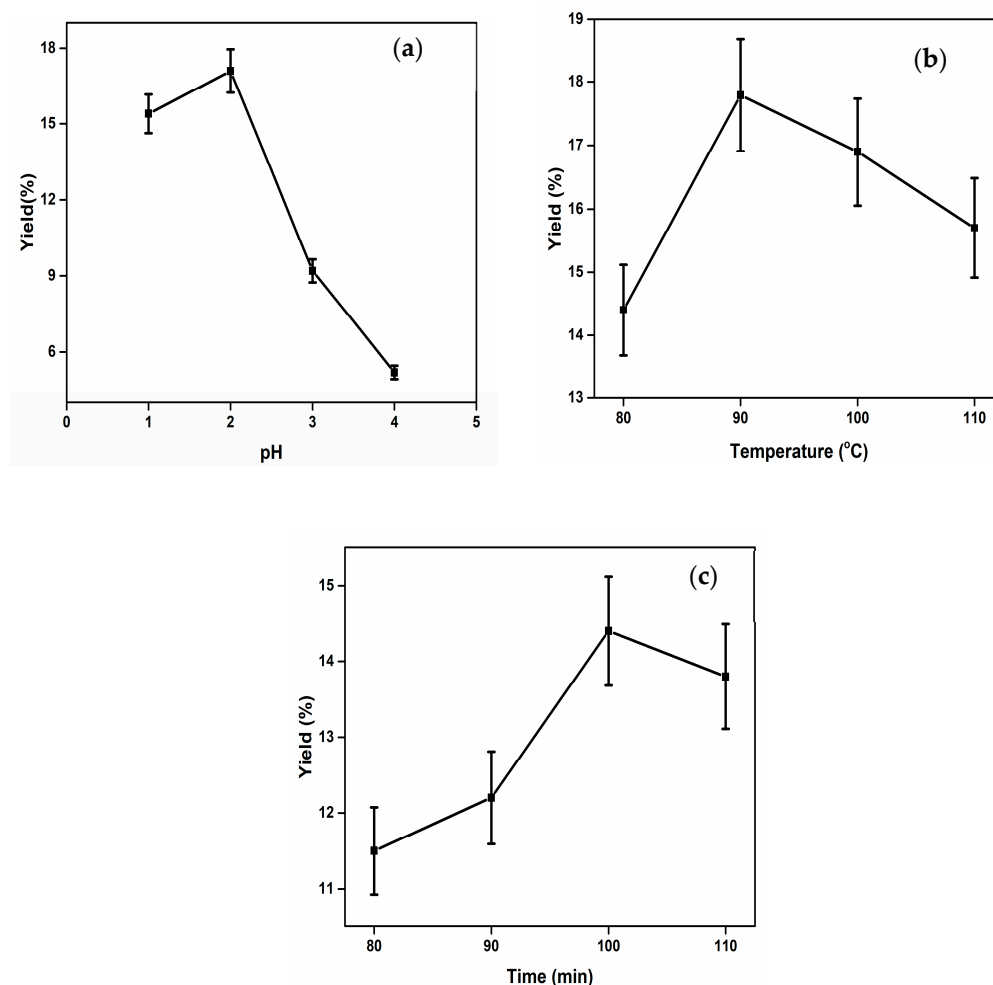


Figure 9. Effect of extraction parameters on pectin yield. (a) Effect of pH. (b) Effect of temperature. (c) Effect of extraction time.

Figure 9c illustrates how extraction time affects pectin production. As the extraction period is increased to 100 min, the yield gradually rises before declining. The extracted pectin's prolonged contact with the acidic media probably encourages hydrolytic breakdown, which breaks down the polymer chains and lowers the yield overall. This implies that before degradation processes take over, there is an ideal extraction window during which the highest yield and quality may be obtained. Overall, our results show that in order to combine effective pectin release with low degradation, pH, temperature, and extraction time optimization are critical. In addition to yield, the interaction of these variables dictates the extracted pectin's structural and functional characteristics, which are essential for its effectiveness in culinary and industrial applications [40].

3.5. Principal Component Analysis (PCA) of Pectin Based on Physiochemical Analysis

The PCA biplot in Figure 10 illustrates the connections between pectin's physicochemical characteristics. Given that the first two principal components (Dim1: 74.1%, Dim2: 20.4%) accounted for 94.5% of the variance, the data was well represented. The first component (Dim1, 74.1%) mainly separates samples based on methoxy content and anhydrouronic acid content, which are positively correlated and represent the gelling and structural integrity of pectin. The second component (Dim2, 20.4%) is influenced by equivalent weight and degree of esterification, linked to molecular density and stability. Samples positioned along the positive Dim1 axis indicate high methoxyl and high anhydrouronic acid pectin—suitable for strong gel formation—while those along the negative axis show

higher esterification and equivalent weight, implying increased viscosity and cross-linking potential. Overall, the PCA highlights that variations in methoxylation and esterification levels fundamentally govern pectin's structure–function behavior, such as gel strength, solubility, and adsorption capacity.

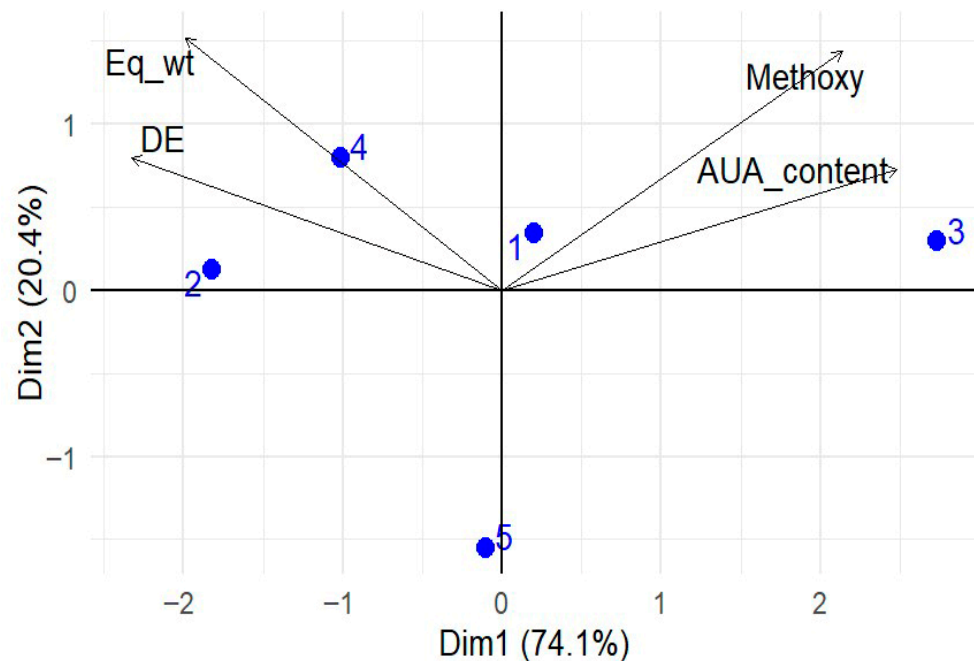


Figure 10. Biplot of principal component analysis (PCA) of equivalent weight (Eq_wt), methoxyl content, anhydrouronic acid (AUA) content, and degree of esterification (DE) of pectin from BARI malta-1 peel powder.

3.6. Pearson's Correlation Analysis

The correlations between the pectin's equivalent weight, methoxy content, degree of esterification, and anhydrouronic acid content are highlighted in the correlation matrix (Figure 11). The degree of esterification and equivalent weight had a substantial positive connection ($r = 0.769$), suggesting that higher esterification leads to higher equivalent weight. On the other hand, it showed negative correlations with the contents of anhydrouronic acid ($r = -0.557$) and methoxy ($r = -0.342$). Anhydrouronic acid content and methyl content showed a strong and substantial correlation ($r = 0.924$, $p < 0.05$), indicating that these parameters co-variate. The trade-off between esterification and free uronic acid residues was highlighted by the interesting finding that the degree of esterification was inversely correlated with both the methoxy content ($r = -0.526$) and the anhydrouronic acid content ($r = -0.771$). These associations show how the structural interdependence of pectin characteristics affects its gelling and functional properties. Overall, these correlations show that the equilibrium between esterification and methoxylation is what largely determines pectin's functional performance, including gel strength, adsorption capacity, and solubility. These characteristics' statistical interdependence makes it clear that structural changes have a direct impact on physicochemical functionality, enabling customized uses in formulations for food, medicine, and the environment.

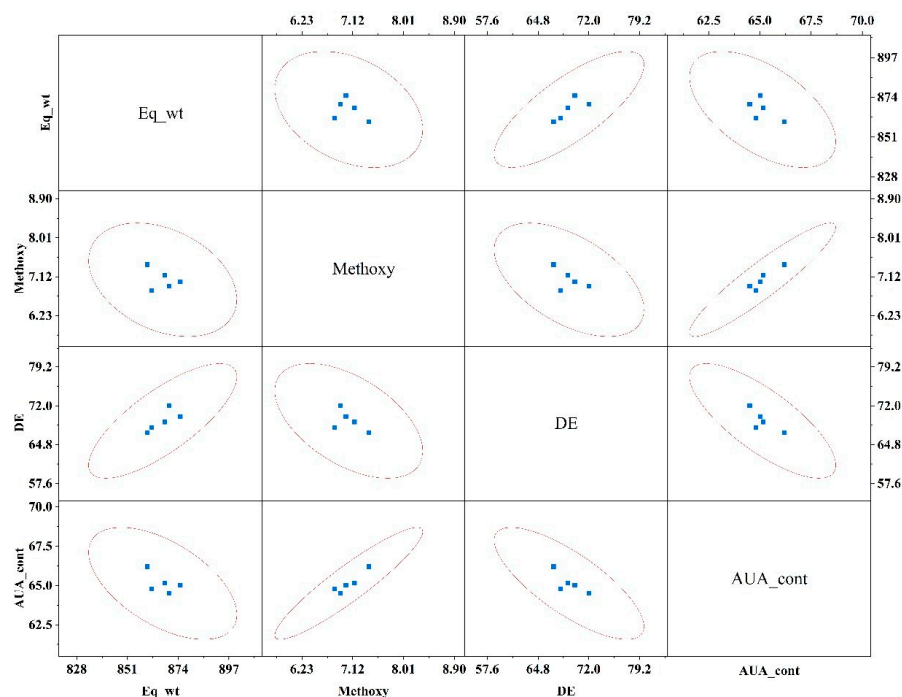


Figure 11. Pearson's correlation analysis of the pectins from BARI malta-1 peel powder equivalent weight, methoxyl content, anhydrouronic acid (AUA), and degree of esterification (DE).

4. Conclusions

In this research, pectin from BARI Malta 1 (Sweet Orange), cultivated extensively in Bangladesh, was successfully extracted and characterized, highlighting its potential as a valuable agro-industrial product. The peels, which are frequently discarded, are a rich and underappreciated source of premium pectin polysaccharides with potential uses. In order to guarantee purity and maximize production, pectin was extracted in this study using well-established acid-extraction protocols followed by alcohol precipitation. Afterwards, comprehensive characterization through FTIR, NMR, XRD, TGA, DSC, and FESEM confirmed the structural identity, crystallinity, thermal stability, and morphological features of the extracted pectin. Comprehensive characterization revealed that extracted pectin possesses favorable physicochemical and structural properties, demonstrating its appropriateness for diversified applications in food, cosmetics, and pharmaceutical industries. The process's effectiveness was demonstrated by the highest pectin yield of 17.1% that was obtained at ideal circumstances of pH 2.0, 90 °C temperature, and 100 min of extraction time. The key physicochemical characteristics controlling pectin's activity have been elucidated by combining principal component analysis (PCA) and Pearson correlation, enabling more effective selection and optimization of pectin sourcing and processing conditions. These results imply that in addition to being a wholesome fruit crop, BARI-1 Malta is a dependable and sustainable source of pectin in Bangladesh. Utilization will significantly contribute to generating value-added products, reducing waste, and advancing circular bioeconomy principles. Despite the comprehensive studies, this investigation is limited to laboratory-scale pectin production. Real-world industrial production strategy is yet to be validated. Furthermore, this study used a One-Factor-at-a-Time (OFAT) approach to optimize extraction; we acknowledge its limitation in capturing interactions between variables. As a result, it may lead to suboptimal outcomes. Future research would explore statistical methods such as Response Surface Methodology (RSM) for more efficient, precise, and scalable optimization.

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