

Research Article

Effect of Ripening and Extraction Method on the Physicochemical Properties of Pectin Extracted from Peels of Apem and Apantu Plantain Cultivars in Ghana

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Fruit waste has emerged as a significant environmental issue in recent years, adding to the global burden. In Ghana, the extensive use of plantains at various stages of ripeness results in substantial waste during each processing stage, leading to disposal challenges. Interestingly, these wastes often hold considerable economic value and can be repurposed as a source of valuable raw materials or products. Given the broad range of uses for plantains and the significant pollution problems associated with their processing, finding new uses for this organic waste has become a pressing necessity. This study investigated the impact of the method of extraction and ripening stages on the physicochemical characteristics of pectin obtained from two different cultivars of plantains, namely, Apem (M) and Apantu (T), which are indigenous to Ghana. Pectin samples were extracted from different stages of ripening (matured-green (G), half-ripe (H), and full-ripe (R)) by the utilization of acidic (D) and alkaline (L) extraction methods. The analyses conducted on the extracted pectin included proximate composition and moisture content determination, assessment of mineral/elemental composition, and solubility testing. Phytochemical investigations were performed to determine the phytoconstituents present in these pectin extracts. The pectin samples were characterized by determining their equivalent weight, methoxyl and anhydrouronic acid content, and the degree of esterification. The pectin yields obtained from the samples ranged from 10.01% to 46.55%. The moisture levels of all the pectins were below 20%. All pectin samples had swelling indexes between $37.5 \pm 0.01\%$ and $185.71 \pm 0.02\%$. The equivalent weight (EW) of the pectin samples ranged from 1351.00 ± 0.6 g/mol to 10000.00 ± 0.07 g/mol. The methoxyl content (MeO) fell within the range of $6.2 \pm 0.07\%$ to $14.88 \pm 0.14\%$, while the anhydrouronic acid content (AUA) varied between $38.72 \pm 0.28\%$ and $86.59 \pm 0.21\%$. The degree of esterification (DE) ranged from $81.22 \pm 0.21\%$ to $97.56 \pm 0.14\%$. Furthermore, the result of the micronutrient and mineral analysis indicated compliance with nutritional requirements. The levels of heavy metals were also within acceptable thresholds, thereby adhering to existing regulatory requirements. These findings highlight the significance of both the extraction technique and the ripening stage on the physicochemical properties of the extracted pectins. Generally, the extracted pectins possess excellent nutritional quality and physicochemical properties, rendering them a very valuable resource that holds promising potential for use in the food and pharmaceutical sectors.

1. Introduction

Plantain (*Musa paradisiaca* L.), a staple crop in many tropical regions, holds both nutritional and economic

significance [1, 2]. Plantain peel waste presents an opportunity for sustainable resource optimization. Extracting potentially valuable substances from these peels not only addresses waste management concerns but also aligns with

the principles of environmental stewardship and the emerging concept of green chemistry [3, 4].

The main types of plantains grown and used in Ghana fall into two main groups: the “Apantu” (False Horn) and the “Apem” (French Horn). It is used in several ways attributable to the ripening stage (matured-green, half-ripe, full-ripe, and over-ripe). When plantains are harvested for consumption, a significant portion of the fruit consists of these peels [5]. The process of obtaining the edible fruit involves carefully removing the peel, resulting in a surplus of discarded peels. These peels, often overlooked due to their lack of immediate culinary value, constitute a substantial proportion of agricultural waste [4, 6]. In commercial and domestic settings alike, plantain peels are typically cast aside as residual material [7]. This waste generation is not limited to a specific region or context but is a common occurrence wherever plantains are processed for consumption. Whether in local markets where plantains are manually peeled for individual sale or in large-scale processing facilities where mechanization prevails, the peels remain a ubiquitous byproduct.

The discarded plantain peels present a dual challenge—both environmental and economic [8, 9]. From an environmental standpoint, the accumulation of organic waste can contribute to landfill issues by emitting greenhouse gases and contributing to pollution [10, 11]. Economically, the potential inherent in these peels remains untapped, representing a lost opportunity for value-added utilization.

Plant cell wall polysaccharides, including pectin, celluloses, and hemicelluloses, exhibit remarkable diversity and serve as crucial structural components within the plant cell. Pectin is a polysaccharide that functions as an intercellular adhesive in plants. It is a chemical molecule made up of poly α -(1, 4)-galacturonic acid with varying degrees of methylation in its carboxylic acid sequences [12]. Pectin is commonly extracted from cell wall material using aqueous solutions, enzymes, buffers, chelating agents, diluted acids, or sodium hydroxide [13, 14]. The chemical properties of pectin, such as the concentration of galacturonic acid, methoxyl groups, degree of esterification, and acetyl value, determine its suitability for diverse use [15–17]. Pectin has a wide range of structural variations depending on the kind of plant material from which it is isolated. These structural changes may lead to variations in the physicochemical characteristics of the extracted pectin. Therefore, it is imperative to accurately describe the physicochemical properties of pectin extracted from various sources. Additionally, knowledge of these properties allows for the optimization of the extraction and purification processes to obtain pectin with desired characteristics [18, 19].

The application of pectin varies depending on the specific sector involved. Pectin has been widely used in the food and beverage industry as a thickening, gelling, and stabilizing agent for products such as jams, jellies, yoghurts, and acidified milk drinks [20–22]. Pectin also has applications in the pharmaceutical and biomedical industries as an excipient, drug delivery system, wound healing agent, and anti-cancer agent [23–25]. Obele et al. [26] extracted pectin from plantain peels and formulated it into composite films. They found that the extraction methods, form of starting material,

and analysis employed affected the physicochemical properties of the pectin films. Oyawaluya et al. [5] investigated the antioxidant activity of pectin from plantain peels. Furthermore, Owusu et al. [27] in their study utilized the intermediate French Horn (Oniaba) as a suspending agent. All these studies show the inherent potential of plantain peel pectin as a valuable raw material. Even though there have been studies on pectin extraction from plantain peels, there is a paucity of literature on the characterization of pectin from various plantain species and cultivars in Ghana. Hence, by understanding the specific physicochemical properties of each pectin extract, researchers can determine its suitability for applications in food and beverage industries as well as pharmaceutical industries.

This study aims to highlight the potential of plantain peel waste as a source of valuable raw materials or products for use in various industries by thoroughly investigating the impacts of extraction method, both acid and alkaline methods, and ripening stage on the physicochemical qualities of pectin produced from plantain peel. In a world increasingly focused on sustainability, the findings generated from this research have the potential to inspire new avenues for the integration of agricultural byproducts into value-added products and processes.

2. Materials and Methods

2.1. Materials. French Horn (Apem) and False Horn (Apantu) plantain (*Musa paradisiaca* L., Musaceae) specimens were acquired from Agogo (latitude: 6.7991°N, longitude: 1.0850°W) in the Asante Akyem North district, located in the Ashanti Region of Ghana, and authenticated by the Crop Research Institute of the Centre for Scientific and Industrial Research (CSIR), Ghana. UK Chemicals in Kumasi, Ghana, provided analytical grades of NaCl, 1 M HCl, 0.1 N NaOH, 1 M NaOH, and 95% ethanol.

2.2. Methods

2.2.1. Collection and Determination of Ripening Stage. Apantu and Apem plantains were harvested in their unripe state and subsequently divided into three distinct categories based on their level of ripeness: matured green (G), half-ripe (H), and full-ripe (R). The maturation process of the remaining two segments of the plant's fruits was closely monitored. The groups of half-ripe and full-ripe specimens were acquired after a duration of four (4) and nine (9) days [28, 29], respectively. Prior to obtaining the fruit peels, the different groups underwent a washing process. The peels were gathered and subsequently sliced into smaller segments. These segments were then subjected to the process of sun-drying before being blended using a countertop blender.

2.2.2. Extraction of Pectin from Plantain Peels (*Musa paradisiaca*). A mixture of 200 g of the powdered plantain peel and 250 mL of acidified water (prepared by adding 5 mL of 1 M HCl to 1000 mL of distilled water) was heated for 1 hour at 90°C and filtered through a cheesecloth. To

precipitate the pectin that had been extracted, 95% ethanol equivalent in volume to the filtrate was added, thoroughly mixed, and allowed to sit for approximately 30 minutes. The precipitates were then filtered, collected, and freeze-dried. The extracted pectin was subsequently stored in an airtight vessel. The same procedure was carried out for the alkaline extraction process. Alkaline water was prepared by adding 5 mL of 1 M NaOH to 1000 mL of distilled water [15, 27, 30].

2.2.3. Determination of Percentage Yield. An analytical balance (Metler, B634930296) was used to determine the mass of the dried powdered plantain peel (M2) and that of the freeze-dried plantain pectin (M1). The percentage yield was determined using the following equation:

$$\text{yield (\%)} = \frac{M1}{M2} \times 100. \quad (1)$$

2.2.4. Test for Pectin. A 1% pectin solution was formed by adding 1 g of pectin to 9 mL of distilled water. Following this, the solution underwent rapid agitation, heating, and cooling. A gelatinous substance exhibiting high degree of viscosity was generated. A 1 mL aliquot of sodium hydroxide solution was introduced into a 5 mL aliquot of the pectin solution. The mixture was undisturbed for a duration of 15 minutes, resulting in the formation of a translucent gel. A 1 mL volume of HCl was introduced to the precipitated gel. The solution was subsequently heated. A colourless gel and precipitates resembling white cotton were observed [31, 32].

2.2.5. Phytochemical Analysis of Extracted Pectin

(1) Test for Alkaloids. A quantity of 1 g of pectin was treated with 10 mL of ammoniacal alcohol, followed by filtration and subsequent drying in an oven. The residue was dissolved in 10 mL of a 1% solution of sulphuric acid, filtered, and made distinctively basic with half its volume of 5 M NH_3 . The resulting solution was shaken with chloroform and evaporated off at 28°C . Subsequently, the residue was dissolved using a 2.5 mL 1% solution of sulphuric acid, and a small amount of Mayer's reagent was introduced [33].

(2) Test for Glycosides. A 200 mg sample of pectin was heated in the presence of 5 mL of aqueous H_2SO_4 using a water bath for approximately 2 minutes. Subsequently, the solution underwent filtration, followed by the addition of 4 drops of an aqueous solution containing 20% NaOH, to alkalize the resulting filtrate. A volume of 1 mL of Fehling's solutions A and B was applied to the filtrate, which was heated in a water bath for approximately 2 minutes [33].

(3) Test for Saponins. A quantity of 5 g of pectin was placed into a test tube. Subsequently, 6 mL of distilled water was introduced into the test tube, followed by vigorous shaking. The combination underwent filtration, and the resulting filtrate was subjected to gentle agitation and subsequently left undisturbed for an estimated duration of 5 minutes [33].

(4) Test for Tannins. A solution containing 500 mg of pectin was prepared by dissolving it in 25 mL of water followed by heating for a duration 5 minutes. Following this, the solution proceeded through a cooling process and was subsequently filtered. 10 drops of a 1% solution of lead acetate in water were added to a 1 mL volume of the filtrate. An additional 1 mL of filtrate was obtained and subsequently diluted with 10 mL of distilled water. Approximately 5 drops of 1% ferric chloride aqueous solution were applied [33].

(5) Test for Flavonoids. A quantity of pectin (1 g) was immersed in 10 mL of distilled water to achieve softening. Subsequently, it underwent filtration. A filter paper was immersed in the filtrate and dried. The dried filter paper was treated with an aqueous solution of NH_3 for an approximate duration of 30 seconds [33].

(6) Test for Coumarins. Petroleum ether was used to extract pectin, and 5 mL was oven-dried. After drying, the residues were dissolved in warm distilled water, chilled, and split into two parts. The first portion was dissolved in 0.5 mL of 10% NH_3 (aq). In the second portion, ammonia solution was not added. Both solutions were then examined under UV light [33].

(7) Test for Triterpenoids. An amount of 1 g of pectin was dissolved in 2 mL of chloroform. This was followed by the addition of 1 mL of concentrated sulphuric acid [33].

(8) Test for Sterols. An amount of pectin, 1 g, was dissolved in 2 mL of chloroform. This was followed by the addition of 1 mL acetic anhydride. The extract was then treated with 5 mL of concentrated sulphuric acid [33].

2.2.6. Characterization of Extracted Pectin

(1) Equivalent Weight. A quantity of pectin weighing 0.5 g was put into a 500 mL conical flask, followed by the addition of 5 mL of ethanol. A mass of 1 g of NaCl was added to the solution to sharpen the end point. A total of 6 drops of phenol red as an indicator were applied. The resultant mixture was subsequently subjected to titration using 0.1 N solution of NaOH [15, 27]. The equivalent weight was determined using the following equation:

$$\text{equivalent weight} = \frac{\text{mass of sample (g)}}{(\text{volume of alkali used (mL)} \times \text{normality of the alkali used})} \times 1000. \quad (2)$$

(2) *Methoxyl Content*. A 25 mL volume of 0.25 N sodium hydroxide (NaOH) was introduced into the neutral solution obtained during the equivalent weight determination. The solution was subsequently subjected to heating and allowed to cool for 30 minutes. Consequently, 6 drops of phenol red

and a 25 mL volume of 0.25 N hydrochloric acid (HCl) were added and titrated against 0.1 N solution of sodium hydroxide (NaOH) [34, 35]. The methoxyl content was determined using the following equation:

$$\text{methoxyl content \%} = \frac{(\text{volume of alkali (mL)} \times \text{normality of alkali} \times 31)}{(\text{mass of sample (mg)})} \times 100. \quad (3)$$

The molecular weight of the methoxyl group is represented as 31.

(3) *Anhydrouronic Acid Analysis (AUA)*. AUA content was determined using (4), by utilizing results obtained from the equivalent weight and methoxyl content determination:

$$\text{AUA \%} = \frac{1.76 (A + B)}{M}, \quad (4)$$

where 1.76 g is the molecular unit/100 for AUA (1 unit), A = volume of NaOH determined from equivalent weight, B = volume of NaOH determined from methoxyl content, and M = sample mass (g).

(4) *Degree of Esterification (DE)*. The degree of esterification was calculated using the following equation [35]:

$$\text{degree of esterification \%} = \frac{176 \times \text{MeO \%}}{\text{AUA \%} \times 31} \times 100. \quad (5)$$

2.2.7. Physicochemical Characterization of Extracted Pectin. Official procedures were used to evaluate the moisture content, insoluble matter, swelling index, total ash, water-soluble ash, and acid-insoluble ash [36, 37].

2.2.8. Proximate and Elemental Content Analysis. The pectin samples underwent a process of dry digestion, and the resulting clear supernatant obtained after centrifugation was utilized for elemental analysis. The presence and quantities of iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), cadmium (Cd), lead (Pb), mercury (Hg), arsenic (Ar), and nickel (Ni) in each digest were determined using an atomic absorption spectrophotometer (Buck scientific model 210V GP). The hollow lamp was chosen, taking into consideration the appropriate wavelengths. The wavelengths of the atomic emission lines for various elements are as follows: iron (Fe) at 248.3 nm, copper (Cu) at 324.8 nm, zinc (Zn) at 213.9 nm, manganese (Mn) at 279.5 nm, cadmium (Cd) at 228.9 nm, lead (Pb) at 283.3 nm, mercury (Hg) at 253.7 nm, argon (Ar) at 193.7 nm, and nickel (Ni) at 341.5 nm. The stock solution, together with the produced sample solutions, was subjected to analysis for the elements using triplicate determinations, as described by Motsara and Roy [38]. The concentrations of magnesium (Mg) and calcium (Ca) were assessed using EDTA titration, as described by Motsara and Roy [38]. The

concentrations of potassium (K) and sodium (Na) were measured in triplicate for each sample using flame photometry. The flame photometer used for this analysis was the Jenway PFP7, with potassium measured at a wavelength of 766 nm and sodium measured at a wavelength of 589 nm. The phosphorus concentration was evaluated using spectrophotometry using the Jenway 6051 colorimeter at a wavelength of 430 nm, following the phosphovanadomolybdate procedure as described by Oyewale [39]. The carbon (C) and nitrogen (N) contents were assessed utilizing the modified Walkley–Black wet oxidation method [40] and the Kjeldahl method [38], respectively.

2.2.9. Fourier-Transform Infrared (FTIR) Spectroscopy Analysis. Bruker Alpha II Fourier-transform infrared spectroscopy was used to scan the pectin samples individually across a wavenumber range of 4000 to 400 cm^{-1} . The spectra were generated using OriginPro software 2023 (OriginLab Corporation, Northampton, Massachusetts, USA).

2.2.10. Statistical Analysis. GraphPad Prism version 8 (GraphPad Software, San Diego, California, USA) was used for the analysis, and the mean and standard deviation were calculated for each dataset. Analysis of variance followed by multiple comparison tests was used in the calculation with a p value set at ≤ 0.05 level of significance.

3. Results and Discussion

3.1. Yield and Identification of Pectin. The pectin yield is influenced by several parameters, including the extraction process and duration, source, maturity or ripening stage, plant species or variety, and geographical location of the plant, among other variables [29, 41]. Previous studies have indicated that pectin yield can be influenced by genetic variation within plant species [16, 35]. The yield of pectin obtained from the various cultivars at various ripening stages varied significantly ($p \leq 0.05$) (Figure 1). The yield for acid extraction ranged from $18.43 \pm 1.3\%$ to $40.52 \pm 0.6\%$, while the yield for alkaline extraction ranged from $10.01 \pm 0.54\%$ to $46.55 \pm 0.5\%$ for both varieties. According to a study conducted by Oyawaluya et al. [5], the pectin yield of plantain peels exhibited an upward trend as the ripening stages progressed. Furthermore, a further study conducted

by Happi Emaga et al. [29] demonstrated that the plantain peel pectin yield exhibited an initial increase from the matured-green stage to the half-ripe stage, followed by a subsequent drop in the full-ripe stage. The observed disparities in pectin yield among the cultivars, despite the utilization of a consistent extraction methodology, suggest variances in the pectin content and support the notion that genotypic variability within a plant might impact pectin yield. Based on the findings, it can be observed that the alkaline extraction method generally results in a higher yield compared to the acidic extraction method (Figure 1). This was also observed by Wandee et al. [42]. In the acid extraction method, the existence of protons (H^+) in the extraction media initiates the degradation of protopectin [43]. When the extraction medium is subjected to a low pH environment, the hydrogen ion concentration is elevated, resulting in the inhibition of carboxylate group ionization inside the pectin molecule. This implies that the carboxylate group in its hydrated form undergoes conversion into hydrated carboxylic acid groups. The removal of the carboxylate group in the pectin molecule results in a reduction of repulsive interactions between the groups. This reduction facilitates the gelation process of pectin, ultimately leading to its precipitation when an appropriate solvent is used [44]. Solubilization of polymeric networks, including hemicellulosic components and adsorbed pectin on the surface of cellulose microfibril, by an alkaline solution aids in pectin release [45]. Cell wall swelling is caused by the release of hydroxyl ions from an alkaline solution, which causes intermolecular hydrogen bonds between cellulose and other polysaccharides to be broken. Additionally, the breakdown of ester bonds, which presumably serve as crucial connections between cell wall polysaccharides and lignin, occurs [42]. Protopectin is a compound that is found in plantain peels during the green or unripe stage of maturation. It serves as a precursor to pectin and is responsible for the firm texture of the peels [46]. As the plantain undergoes ripening, the activity of enzymes such as polygalacturonase, pectin lyase, and pectin methyl esterase initiates the degradation of protopectin, leading to reduced yields [47]. Nevertheless, this phenomenon was not observed as the fruit matured from the half-ripe to the full-ripe form for either of the two types (Figure 2). The observation can be attributed to the presence of enzymes that degrade pectin, which may have undergone a period of delayed activity during that specific phase of fruit ripening [48]. The observed rise in yield from the half-ripe to the full-ripe can be attributed to the heightened tenderness of the fruit peel at the half-ripe state, which renders the interconnections between pectin and other cellular components more susceptible to extraction [18]. Consequently, the availability of pectin for extraction increased. Alternatively, the decline in yield could potentially be attributed to the enzymatic breakdown of pectin, facilitated by enzymes such as polygalacturonase (PG), pectin methyl esterase (PME), or pectate lyase (PL) [29, 49]. This phenomenon was noted in both varieties, although it was more pronounced in the Apantu (False Horn) variant.

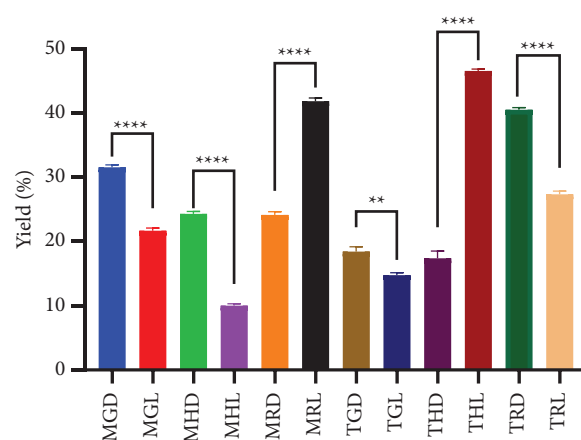


FIGURE 1: Comparative analysis of the effect of method of extraction on the percentage yield of the extracted pectin using Student's *t*-test. Values are expressed as means \pm SD ($n = 3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$.

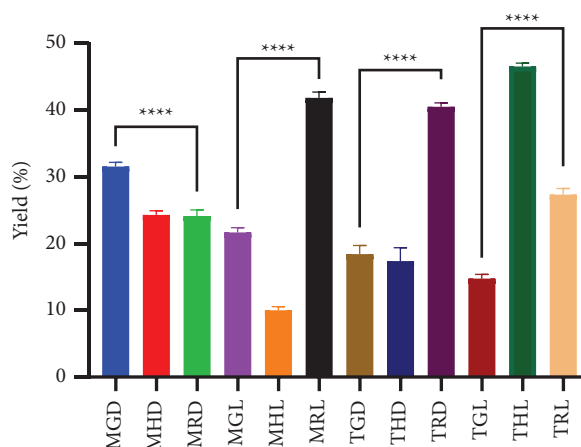


FIGURE 2: Comparative analysis of the effect of ripening stage on the percentage yield of the extracted pectin using one-way ANOVA. Values are expressed as means \pm SD ($n = 3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$.

3.2. Swelling Index, Solubility, and PH. The swelling index of a polymer is a critical parameter that reflects its ability to absorb and retain water or aqueous solutions within its structure without disintegrating or dissolving. It is a measure of the polymer's capacity to swell when exposed to water and to trap water molecules within its matrix [50]. This property is particularly important in various applications where moisture, absorption, and retention are essential. Pectin's high quantities of D-galacturonic acid in its polysaccharide backbone are responsible for its swelling capability [51]. The swelling indexes varied depending on the cultivars (Table 1). At the different methods of extraction within a specific ripening stage, the alkali-extracted pectin exhibited superior swelling indexes for the Apem variant. However, with the Apantu variant, the acid-extracted pectin had better swelling indexes, compared with those of the alkali-extracted pectin. Notably, at the matured green stage for both cultivars, acid-

extracted pectin recorded the highest swelling indexes. Hence, irrespective of the variety, acid-extracted pectin from the matured green peel exhibits superior swelling indexes with the Apem variant being the better of the two. The swelling index of acid-extracted pectin at all stages of ripening decreased from the matured green to the half-ripe stage and increased at the full-ripe stage for the Apem variant, unlike that of the Apantu variant, which decreased with ripening. On the other hand, there was an increase in the swelling indexes from the matured green to the half-ripe and a subsequent drop at the full-ripe stage for Apem alkali-extracted pectin. The Apantu variant, however, saw a decrease from the matured green to the half-ripe stage and a subsequent rise in the full-ripe stage for alkali-extracted pectin. It could be inferred that alkali-extracted and acid-extracted pectins for Apem and Apantu, respectively, are high swelling index pectins and could find use as texture modifiers, moisture retention agents to enhance moisture retention in creams and lotions, and matrix-forming agent in controlled-release formulations. However, the swelling indexes of all the varieties at different ripening stages were lower than those reported for pectin from okra [17] and *Khaya senegalensis* gum [52].

Solubility profiles differed between the two types of pectin because of differences in their chemical composition. Studies have shown that high ash content and drying method used may reduce the solubility of pectin [53]. The solubility profiles of the alkali-extracted and acid-extracted pectin were comparable at all stages of ripening for both varieties. Alkaline pH causes the methyl groups on pectin molecules to be removed in a demethylation process [54]. The demethylation process results in a more aqueous-soluble pectin. It was observed that the solubility declined along the maturation stage for both acid and alkali-extracted pectins for the Apantu variant, unlike the Apem variant, which saw an increase in the aqueous solubility from the matured green to the half-ripe stage and a subsequent decrease in the full-ripe stage for acid-extracted pectin.

There were significant differences ($p < 0.0001$) in the pH between the different methods of extraction as well as the ripening stages (Figures 3 and 4). The pH of the extracted pectin saw a drop from the matured-green stage to the half-ripe stage and subsequently increased at the full-ripe stage for the Apem variant. This was observed for the alkali-extracted pectin for the Apantu variant, unlike the acid-extracted pectin, which increased at the half-ripe stage and decreased at the full-ripe stage. It is crucial to acknowledge that the ultimate pH of the extracted pectin is influenced by an array of factors. These include the type of fruit from which the pectin is extracted, the maturity stage of said fruit, and the specific extraction process that has been employed. Consequently, while the initial pH conditions during extraction can be influenced by both acidic and alkaline extraction methods, these methods do not necessarily dictate the final pH of the extracted pectin [17].

3.3. Identification of Phytoconstituents in the Extracted Pectin.

The phytochemical screening of the pectin extracts revealed the presence or absence of the various phytoconstituents (Table 2). However, no clear trend was observed among the methods of extraction or the ripening stage. This suggests that the phytochemical composition may vary depending on several factors, such as extraction conditions and environmental conditions. The extractive processes used in obtaining pectin from its crude form can result in the loss of some phytochemical constituents [55]. However, it was observed that sterols were generally absent in the Apantu variant at all stages of ripening and even for the method of extraction but present for the acid-extracted pectin at the matured green and full-ripe stage for the Apem variant. Tannins have been found to possess antioxidant properties and have been used in traditional medicine for their anti-inflammatory effects. Saponins, on the other hand, have been reported to exhibit antimicrobial activity and may have potential therapeutic applications. The presence of alkaloids and glycosides in the extracted pectin suggests that they may possess bioactive properties that could be explored further for various pharmaceutical and industrial purposes [56]. Coumarins are known for their potential anticoagulant and anti-inflammatory effects, while sterols have been studied for their cholesterol-lowering properties. These additional compounds further highlight the potential of pectin as a versatile and valuable natural resource for various health and industrial applications [24, 57].

3.4. Characterization of Extracted Pectin.

Equivalent weight, methylation level, anhydrouronic acid content, and degree of esterification were used to characterize the pectin samples (Table 3). Titrimetric analysis revealed that the equivalent weight and methoxyl content ranging from 1351.00 ± 0.6 to 10000.00 ± 0.07 and 6.2 ± 0.07 to 14.88 ± 0.14 , respectively, were higher than those of commercial pectin [35]. Equivalent weight is the amount of free galacturonic acid (not esterified) in the pectin molecular chain [58]. This parameter indicates whether the pectin has undergone partial degradation or not. The results indicated that equivalent weight varied across the different ripening stages as well as the method of extraction. Acid-extracted pectin for both variants indicated a decrease from the matured-green to the half-ripe stage and an increase from the half-ripe stage to the full-ripe stage. The opposite was observed for the alkali-extracted pectin. As fruits and vegetables ripen, it can cause a change in the structure, composition, and concentration of pectin [46, 59]. A study by Lekhuleni et al. [60] revealed that equivalent weight varied based on fruit ripeness or maturity. Regarding the method of extraction, acid-extracted pectin generally exhibited a higher equivalent weight for the Apem variant, unlike the Apantu variant, where the alkali-extracted pectin generally showed a higher equivalent weight ($p \leq 0.05$). When pectin is extracted using a strong acid, the acid can cause the breaking of the pectin bonds leading to a higher galacturonic acid content. This could

TABLE 1: Physicochemical properties of the extracted pectin.

Parameter	MGD	MGL	MHD	MHL	MRD	MRL	TGD	TGL	THD	THL	TRD	TRL
Swelling index (%)	185.71 ± 0.02	60 ± 0.01	37.5 ± 0.01	120 ± 0.02	57.14 ± 0.1	87.5 ± 0.2	144.44 ± 0.2	69.23 ± 0.01	88.89 ± 0.03	54.55 ± 0.12	50 ± 0.01	100 ± 0.1
Solubility												
Hot water	++	+++	+++	++	++	+	+++	+++	++	++	+	+
Cold water	+	++	++	+	+	+	++	++	++	+	+	+
Ethanol	++	+++	++	+++	++	++	++	+++	++	++	++	+

+++ , very slightly soluble; ++ , slightly soluble; + , sparingly soluble.

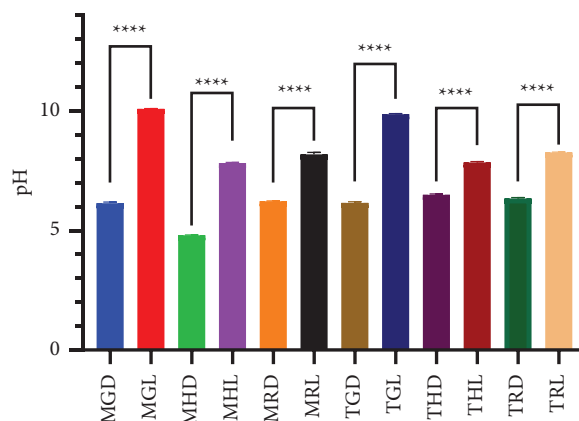


FIGURE 3: Comparative analysis of the effect of method of extraction on the pH of the extracted pectin using Student's *t*-test. Values are expressed as means \pm SD ($n=3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$.

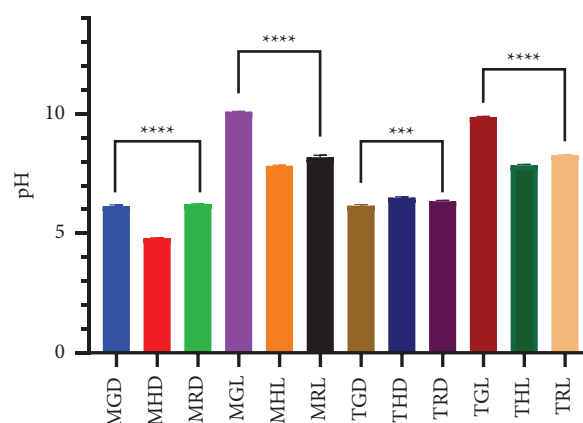


FIGURE 4: Comparative analysis of the effect of ripening stage on the pH of the extracted pectin using one-way ANOVA. Values are expressed as means \pm SD ($n=3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$.

invariably explain why acid-extracted pectin has a high equivalent weight. Although alkali-extracted pectin does not undergo as much hydrolysis as acid-extracted pectin, it can lead to demethylation, which consequently leads to a lower equivalent weight. However, the demethylation could be incomplete and may result in the extraction of pectin with a higher equivalent weight. Pectin with a high equivalent weight has better gelling ability [61].

The methoxyl content parameter is crucial since it determines the gel strength, pectin setting time, metal ion sensitivity, and other critical properties [62]. There was a general decrease in the methoxyl content along the ripening stages. As fruit ripens, the activity of pectin lyase increases, causing its sugar content to increase. The activity of the enzyme causes the hydrolysis of the methoxyl groups, which can consequently reduce the methoxyl content of the pectin. Moreover, the ripening process can increase the susceptibility of pectin to pectin lyase enzymes [59]. Considering the method of extraction, it was observed that the methoxyl content of the alkali-extracted pectin was significantly lower ($p \leq 0.05$) than that of the acid counterpart. Alkaline extraction can lead to the demethylation of pectin

chains, which can reduce the methoxyl content [30]. However, it is essential to consider that these changes can vary depending on the type of fruit and the ripening conditions employed. Pectin is classified as having a high methoxyl content if its methoxyl content is greater than 7% [63]. A pectin is considered low-methoxyl if its methoxyl level is below 7%. All the samples can thus be classified as a high methoxyl pectin because their methoxyl content was greater than 7%.

The total anhydrouronic content (AUA) determines the purity as well as the degree of esterification of the pectin molecule. It also evaluates the physical characteristics of the pectin [60]. The AUA values of the extracted pectin were similar to those of earlier studies [27, 28] and generally declined during the ripening phases as well as were lower for alkali-extracted pectin than acid-extracted pectin. AUA level of pectin may decrease due to the abundance of high amounts of protein, starch, and simple sugars, which act as contaminants [64, 65]. The proximate analysis indicated increasing levels of protein, crude fibre, and carbohydrates along the ripening stages which could have accounted for the decline in AUA during ripening.

TABLE 2: Phytochemistry of extracted pectin.

Sample ID	Test/inference						
	Tannins	Alkaloids	Saponins	Glycosides	Flavonoids	Sterols	Coumarins
MGD	—	+	+	+	+	+	—
MGL	+	+	+	+	+	—	+
MHD	+	+	+	—	+	—	—
MHL	+	+	+	+	+	—	—
MRD	+	—	+	—	+	+	+
MRL	+	+	+	+	+	—	—
TGD	+	+	+	—	+	—	+
TGL	+	—	+	+	—	—	—
THD	+	+	+	—	—	—	+
THL	+	+	+	—	—	—	—
TRD	+	+	+	+	—	—	—
TRL	+	+	+	+	+	—	—

Present (+)/absent (—).

TABLE 3: Characterization of the extracted pectin.

	EW (g/mol)	MeO (%)	AUA (%)	DE (%)
MGD	^a 8333.00 ± 0.6 ¹	^a 14.88 ± 0.14 ¹	^a 94.17 ± 0.14 ¹	^a 97.56 ± 0.14 ¹
MGL	^b 1667.00 ± 0.28 ¹	^b 11.16 ± 0.14 ¹	^b 73.92 ± 0.14 ¹	^b 88.89 ± 0.14 ¹
MHD	^a 1351.00 ± 0.6 ²	^a 10.54 ± 0.14 ²	^a 72.86 ± 0.14 ²	^a 82.13 ± 0.14 ²
MHL	^b 10000.00 ± 0.14 ²	^b 9.61 ± 0.07 ²	^b 56.32 ± 0.07 ²	^b 96.88 ± 0.07 ²
MRD	^a 4545.00 ± 0.07 ³	^a 9.92 ± 0.14 ³	^a 72.65 ± 0.14 ³	^a 94.67 ± 0.14 ³
MRL	^b 5000.00 ± 0.07 ³	^b 7.44 ± 0.07 ³	^b 45.76 ± 0.07 ³	^b 93.71 ± 0.07 ⁴
TGD	^c 3125.00 ± 0.14 ⁴	^c 12.40 ± 0.21 ⁴	^c 90.11 ± 0.21 ⁴	^c 92.59 ± 0.21 ⁵
TGL	^d 2778.00 ± 0.01 ⁴	^d 11.07 ± 0.28 ⁴	^d 69.17 ± 0.28 ⁴	^d 90.84 ± 0.28 ⁵
THD	^c 2632.00 ± 0.01 ⁵	^c 13.16 ± 0.98 ⁵	^c 81.42 ± 0.98 ⁵	^c 91.32 ± 0.98 ⁶
THL	^d 10000.00 ± 0.07 ⁵	^d 11.44 ± 0.21 ⁵	^d 66.70 ± 0.21 ⁵	^d 97.36 ± 0.21 ⁶
TRD	^c 4545.00 ± 0.07 ⁶	^c 12.50 ± 0.01 ⁶	^c 74.27 ± 0.01 ⁶	^c 95.64 ± 0.01 ⁷
TRL	^d 1563.00 ± 0.14 ⁶	^d 8.68 ± 0.14 ⁶	^d 60.54 ± 0.14 ⁶	^d 83.33 ± 0.14 ⁷

Columns with the same superscript letter show a significant difference between ripening stages and those with the same superscript number show a significant difference between the methods of extraction ($p \leq 0.5$). Each value is represented as mean ± SD ($n = 3$).

The amount of esterified carboxyl groups in relation to the total number of carboxyl groups in a pectin molecule is the measure of esterification degree. High methoxyl pectin (DE > 50%) and low methoxyl pectin (DE < 50%) are two categories of pectin that are distinguished by their respective levels of esterification. The DE of the samples ranged between 82.13 ± 0.14 and 97.56 ± 0.14 . As both varieties ripened, the DE decreased from the matured-green to the half-ripe and increased from the half-ripe to the full-ripe for the acid-extracted pectin. However, the opposite was observed for the alkali-extracted pectin. It has been reported that DE tends to decrease as fruits and vegetables ripen. This is as a result of the activity of pectin methyltransferase (PME) enzymes which can hydrolyze the methyl groups from the pectin chains [66]. Moreover, alkali-extracted pectin showed lower DE than its acid-extracted counterpart, except for MHL and THL. The differences observed could be attributed to the demethylation that occurs during alkaline extraction. Additionally, the variations observed may be due to the PME enzymes being in a lag phase during the half-ripe stage [47]. The effect of ripening on DE is complex and can depend on a number of factors, including the specific plant material, ripening stage, and extraction method. All the samples from both varieties of plantain are high-methoxyl pectin because

their degree of esterification is greater than 50%. These pectins may find use as gelling agents or viscosity modifiers in the food and pharmaceutical industries.

3.5. Proximate Composition of Extracted Pectin. The proximate composition of samples at different maturation stages is summarized in Table 4. It indicates that both varieties have similar compositions when it comes to crude fibre and fat content. The fat and crude fibre content both decreased with ripening for both varieties, irrespective of the method of extraction. Moreover, alkali-extracted pectin recorded lower fat and crude fibre content at all ripening stages. The fat content and fibre were comparable to those observed by Happi Emaga et al. [67] in their study of the dietary components of both banana and plantain peels.

The ash content for extracted pectin from various ripening stages was between 1.34 ± 0.01 and 3.23 ± 0.02 for the Apem variety and between 1.53 ± 0.01 and 2.56 ± 0.03 for the Apantu variety. Ash content is the inorganic residue remaining after either ignition or complete oxidation of the pectin [68]. As fruits ripen, the ash content of the pectin tends to increase as a result of the physiological change. The reason could be attributed to the increase in concentration of

TABLE 4: Proximate analysis of the extracted pectin.

	Fat content (%)	Crude fibre content (%)	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)	Protein content (%)	NFE carbohydrate content (%)
MGD	^a 5.05 ± 0.02 ¹	^a 1.67 ± 0.17 ¹	^a 1.87 ± 0.01 ¹	^a 0.01 ± 0.00 ¹	^a 10.68 ± 0.15 ¹	^a 3.68 ± 0.01 ¹	^a 27.85 ± 0.09 ¹
MGL	^b 3.60 ± 0.10 ¹	^b 2.02 ± 0.09 ²	^b 3.23 ± 0.02 ¹	^d 0.02 ± 0.00 ²	^b 20.96 ± 0.57 ¹	^b 4.74 ± 0.01 ¹	^b 33.26 ± 0.11 ¹
MHD	^a 4.42 ± 0.06 ²	^a 1.65 ± 0.05 ³	^a 2.84 ± 0.02 ²	^b 0.01 ± 0.00 ³	^a 16.53 ± 0.21 ²	^a 3.51 ± 0.03 ²	^a 43.26 ± 0.03 ²
MHL	^b 4.40 ± 0.16 ³	^b 1.63 ± 0.01 ⁴	^b 1.66 ± 0.03 ²	^d 0.02 ± 0.00 ⁴	^b 10.68 ± 0.38 ²	^b 4.15 ± 0.07 ²	^b 34.72 ± 0.21 ²
MRD	^a 6.94 ± 0.11 ⁴	^a 2.02 ± 0.04 ⁵	^a 2.15 ± 0.01 ³	^c 0.04 ± 0.02 ⁵	^a 13.44 ± 0.27 ³	^a 2.11 ± 0.04 ³	^a 46.90 ± 0.26 ³
MRL	^b 4.62 ± 0.13 ⁴	^b 1.01 ± 0.02 ⁶	^b 1.34 ± 0.01 ³	^d 0.05 ± 0.00 ⁶	^b 9.76 ± 0.09 ³	^b 2.66 ± 0.01 ³	^b 69.08 ± 0.17 ³
TGD	^c 5.67 ± 0.13 ⁵	^c 1.42 ± 0.03 ⁷	^c 2.34 ± 0.02 ⁴	^e 0.06 ± 0.00 ⁷	^c 14.64 ± 0.10 ⁴	^c 6.80 ± 0.12 ⁴	^c 27.40 ± 0.22 ⁴
TGL	^d 2.43 ± 0.15 ⁵	^d 1.66 ± 0.32 ⁸	^d 2.56 ± 0.03 ⁴	^f 0.05 ± 0.00 ⁸	^d 16.41 ± 0.18 ⁴	^d 6.82 ± 0.04 ⁵	^d 51.15 ± 0.23 ⁴
THD	^c 6.29 ± 0.12 ⁶	^c 2.56 ± 0.09 ⁹	^c 1.57 ± 0.01 ⁵	^e 0.04 ± 0.00 ⁹	^c 9.64 ± 0.10 ⁵	^c 3.52 ± 0.09 ⁶	^c 59.68 ± 0.50 ⁵
THL	^d 3.04 ± 0.14 ⁶	^d 1.59 ± 0.16 ¹⁰	^d 2.05 ± 0.01 ⁵	^g 0.04 ± 0.00 ¹⁰	^d 12.47 ± 0.22 ⁵	^d 3.72 ± 0.08 ⁶	^d 58.44 ± 0.25 ⁵
TRD	^c 6.32 ± 0.19 ⁷	^c 1.06 ± 0.05 ¹¹	^c 1.53 ± 0.01 ⁶	^e 0.06 ± 0.00 ¹¹	^c 10.58 ± 0.25 ⁶	^c 2.86 ± 0.06 ⁷	^c 60.89 ± 0.27 ⁶
TRL	^d 4.65 ± 0.12 ⁷	^d 1.01 ± 0.02 ¹²	^d 1.58 ± 0.02 ⁶	^h 0.04 ± 0.00 ¹²	^d 11.37 ± 0.17 ⁶	^d 3.45 ± 0.12 ⁷	^d 62.31 ± 0.21 ⁶

Columns with the same superscript letter show a significant difference between ripening stages and those with the same superscript number show a significant difference between the methods of extraction ($p \leq 0.5$). Each value is represented as mean ± SD ($n = 3$).

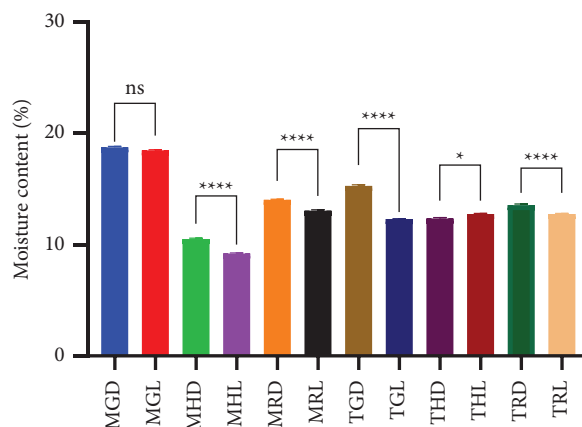


FIGURE 5: Comparative analysis of the effect of method of extraction on the moisture content of the extracted pectin using Student's *t*-test. Values are expressed as means \pm SD ($n=3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

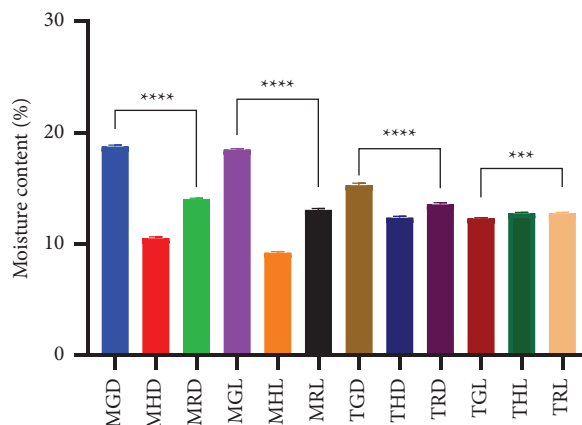


FIGURE 6: Comparative analysis of the effect of ripening stage on the moisture content of the extracted pectin using one-way ANOVA. Values are expressed as means \pm SD ($n=3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$.

nonpectin substances present in fruits. Moreover, alkaline extraction may produce pectin with a higher ash content than acid extraction due to the dissolution of more non-pectin components such as minerals and salts [69]. This was observed in the extracted pectin as there was generally an increase in the ash content along the ripening stages and for alkali-extracted pectin. Ash content determines the purity of the pectin. It is recommended that the ash content in pectin be low preferably $\leq 10\%$ because a high ash content in pectin makes the pectin insoluble in water. All the pectin samples had total ash content lower than 10% indicating their favourability for gel formation. Ash content in pectin, however, can be reduced by washing with a suitable alcohol [70].

Carbohydrate content may be defined as the simple or complex sugars found in pectin [71]. According to the present study's findings, carbohydrate is the most abundant proximate content among the two cultivars analysed. When the carbohydrate content is higher than the recommended value, it contributes to the impurity of the pectin [72]. Carbohydrate content includes simple sugars in pectin such as galactose, arabinose, mannose, glucose, and rhamnose.

During maturation, carbohydrates increase considerably from the green stage to the ripe stage [29]. This phenomenon was seen for both the False and French Horn plantains. Generally, the alkaline extraction resulted in more carbohydrates for the Apantu variety than for the Apem variety.

Similar to previous research, the moisture content of all pectin samples ranged from $9.21 \pm 0.10\%$ to $18.76 \pm 0.12\%$ [16, 34]. The moisture content of the acid-extracted pectin was generally higher than that of the alkali-extracted pectin and also declined from the matured-green stage to the half-ripe stage, increasing again at the full-ripe stage (Figures 5 and 6). Acid extraction usually results in pectin with a higher moisture content because the acidic conditions can cause the pectin to swell and absorb more water unlike alkaline extraction, which causes the pectin to precipitate and expel water [59]. During acid extraction, the acidic conditions can interact with the insoluble part of pectin and influence the hydrolysis of insoluble pectin molecules into soluble form which can lead to a potentially higher moisture content than that of the alkaline extraction process [59]. It is worth noting that regardless of the conditions of extraction, the moisture content is more likely to be influenced by the drying process

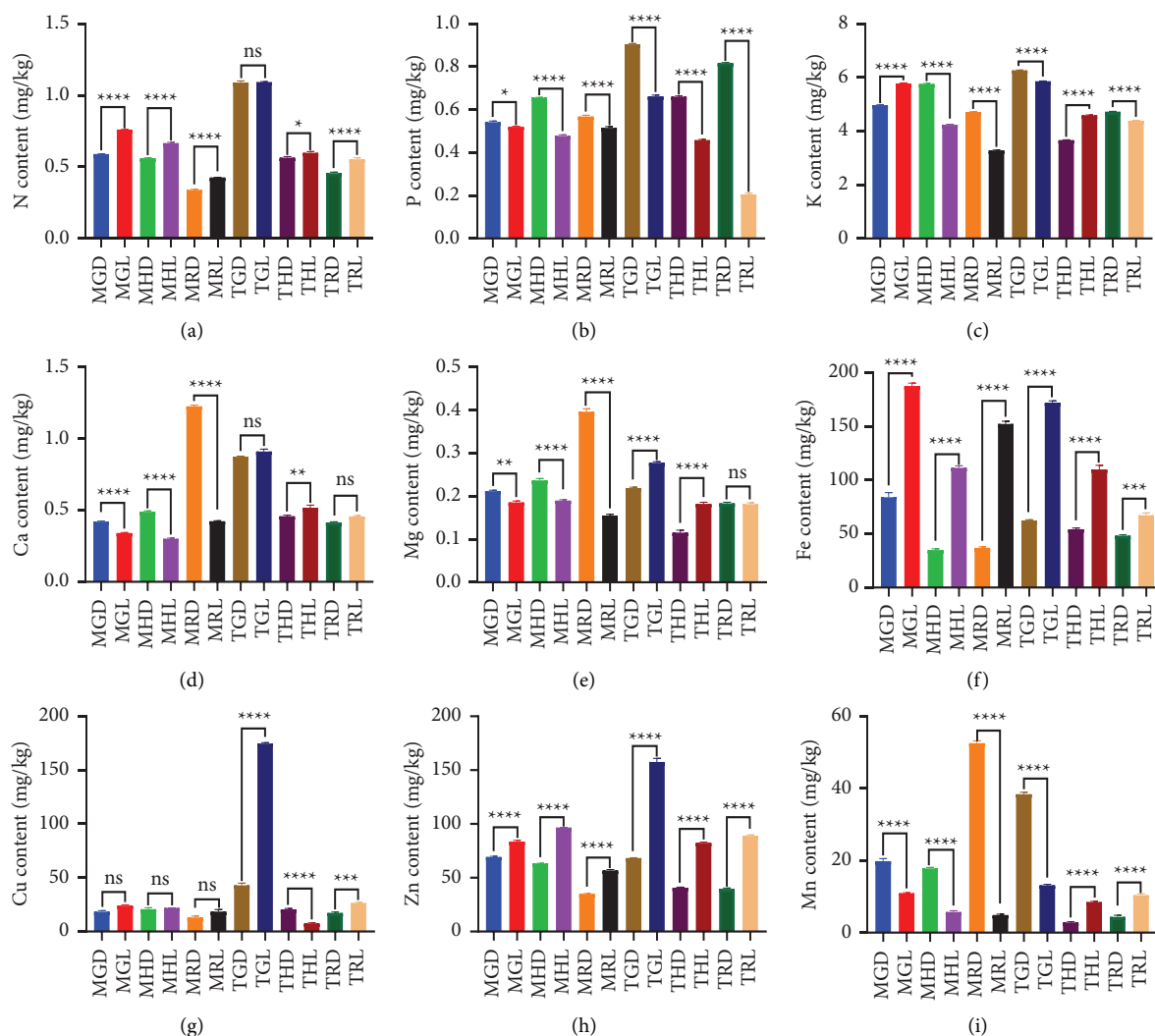


FIGURE 7: Comparative analysis of elemental/mineral composition of the extracted pectin using Student's *t*-test. (a) Nitrogen. (b) Phosphorus. (c) Potassium. (d) Calcium. (e) Magnesium. (f) Iron. (g) Copper. (h) Zinc. (i) Manganese. Values are expressed as means \pm SD ($n = 3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

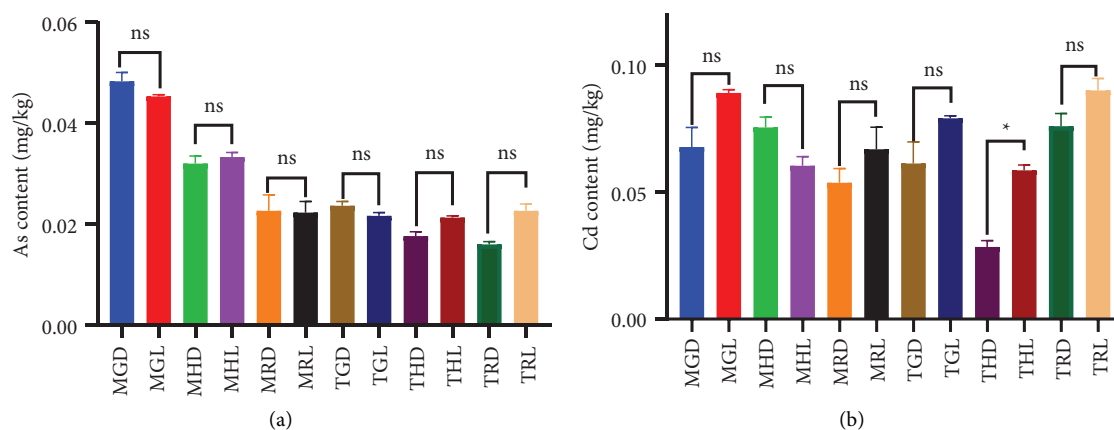


FIGURE 8: Continued.

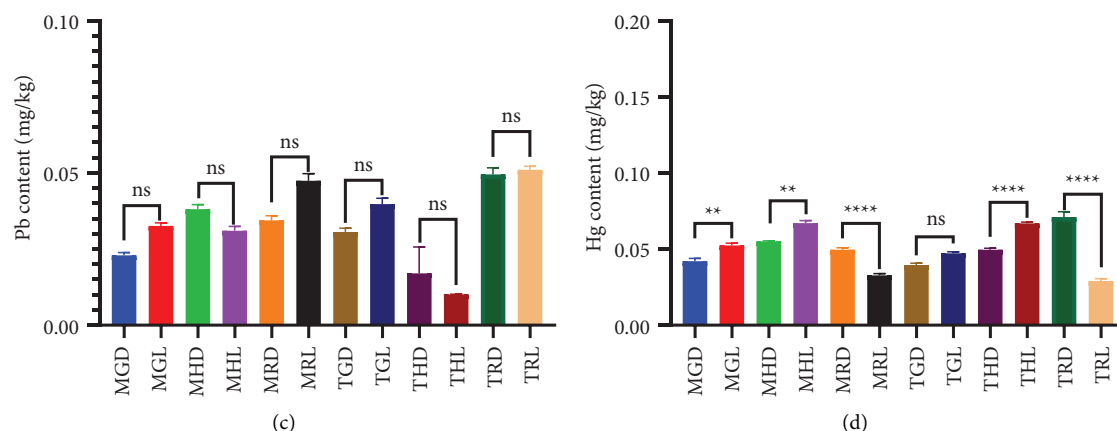


FIGURE 8: Comparative analysis of heavy metal content of the extracted pectin using Student's *t*-test. (a) Arsenic. (b) Cadmium. (c) Lead. (d) Mercury. Values are expressed as means \pm SD ($n = 3$). **** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

following extraction. A food's freshness and longevity can be gauged by measuring its moisture content; foods with a high moisture content are more vulnerable to microbial decomposition and have a shorter shelf life; hence, the drying process and duration have a significant impact [73]. Moisture levels in all samples were below 20%, and all were below the pectin moisture levels described by Obele et al. [26].

3.6. Mineral/Elemental Content. The mineral composition of the pectin was analysed and found to change significantly between the two plantain varieties with potassium being the most abundant mineral, followed by calcium, phosphorus, and nitrogen, and generally decreasing as the plantain ripens (Figure 7). Iron (Fe) was the most abundant micronutrient present in the pectin samples for both varieties of plantain followed by zinc, copper, and manganese. These values are in agreement with studies carried out by Happi Emaga et al. [67]. They all generally decreased during maturation. However, the iron (Fe) content in the French Horn variety increased from the half ripe to the full ripe stage for alkali-extracted pectin. Moreover, the alkali-extracted pectin had higher iron content than that of the acid-extracted pectin. This may be due to differences in varieties. Oxidizing and alkaline conditions promote precipitation of insoluble Fe^{3+} , whereas acidic and reducing conditions promote the solution of Fe^{2+} compounds [74]. It is worth noting that the presence of minerals in pectin can depend on several factors, including the type of plant material it is extracted from, the maturity of the fruit, and the specific extraction process. Calcium and phosphorus play crucial roles in the development of robust skeletal structures and dental health, as well as in processes such as growth, blood coagulation, cardiac performance, and cellular metabolism [75]. Potassium serves as a crucial raw ingredient in the production of soap and plays a significant role in soil neutralization. The elemental analysis of pectin derived from various cultivars revealed significantly lower concentrations of toxic metals, all of which were under the permitted thresholds (Figure 8) (USP, 2018).

This observation suggests that plantain peel pectin derived from the two cultivars at different ripening stages possesses nontoxic properties and can potentially be utilized in pharmaceutical products and the food and beverage industry.

3.7. FTIR Analysis. The chemical profile of extracted pectin was characterized by FTIR and their spectra are presented in Figures 9 and 10. The spectral range spanning from 800 cm^{-1} to 1300 cm^{-1} is well recognised as the "finger print" zone for carbohydrates. This range facilitates the identification of key chemical groups that are unique to distinct polysaccharides. The spectra of the samples extracted from plantain peels exhibit similarities in the "finger print" region to those of okra and watermelon rind pectin [16, 17] as well as to the reported spectra of pectin in previous studies [76, 77]. This suggests that the extracted samples in this study are pectin.

The absorption range spanning from 3300 cm^{-1} to 3500 cm^{-1} , characterized by a wide and intense peak, is mostly caused by the vibrational motion of O-H stretching. This phenomenon can be linked to the presence of intermolecular and intramolecular hydrogen bonds. The occurrence of O-H stretching vibrations encompasses a wide range of frequencies and provides valuable insights into various characteristics of a molecule. These vibrations can be observed in the stretching bands of "free" hydroxyl groups present in vapour phase samples, as well as in the bound O-H bands of carboxylic acids [78]. The absorbance observed in pectin samples inside the O-H region can be attributed to the vibrational modes of inter- and intramolecular hydrogen bonds present in the galacturonic acid polymer [79]. The absorption peak seen at a wavenumber of 2938 cm^{-1} (within the range of $3000\text{--}2800\text{ cm}^{-1}$) is ascribed to the vibrational modes of C-H bonds. The band corresponding to C-H stretching and bending vibrations is commonly found in pectin samples, overlapping with the larger O-H band that spans from 2500 cm^{-1} to 3600 cm^{-1} [80]. The observed peak at a wavenumber of 1727 cm^{-1} can be ascribed to the vibrational modes associated with the O-CH₃ moiety. The presence of two distinct peaks in the

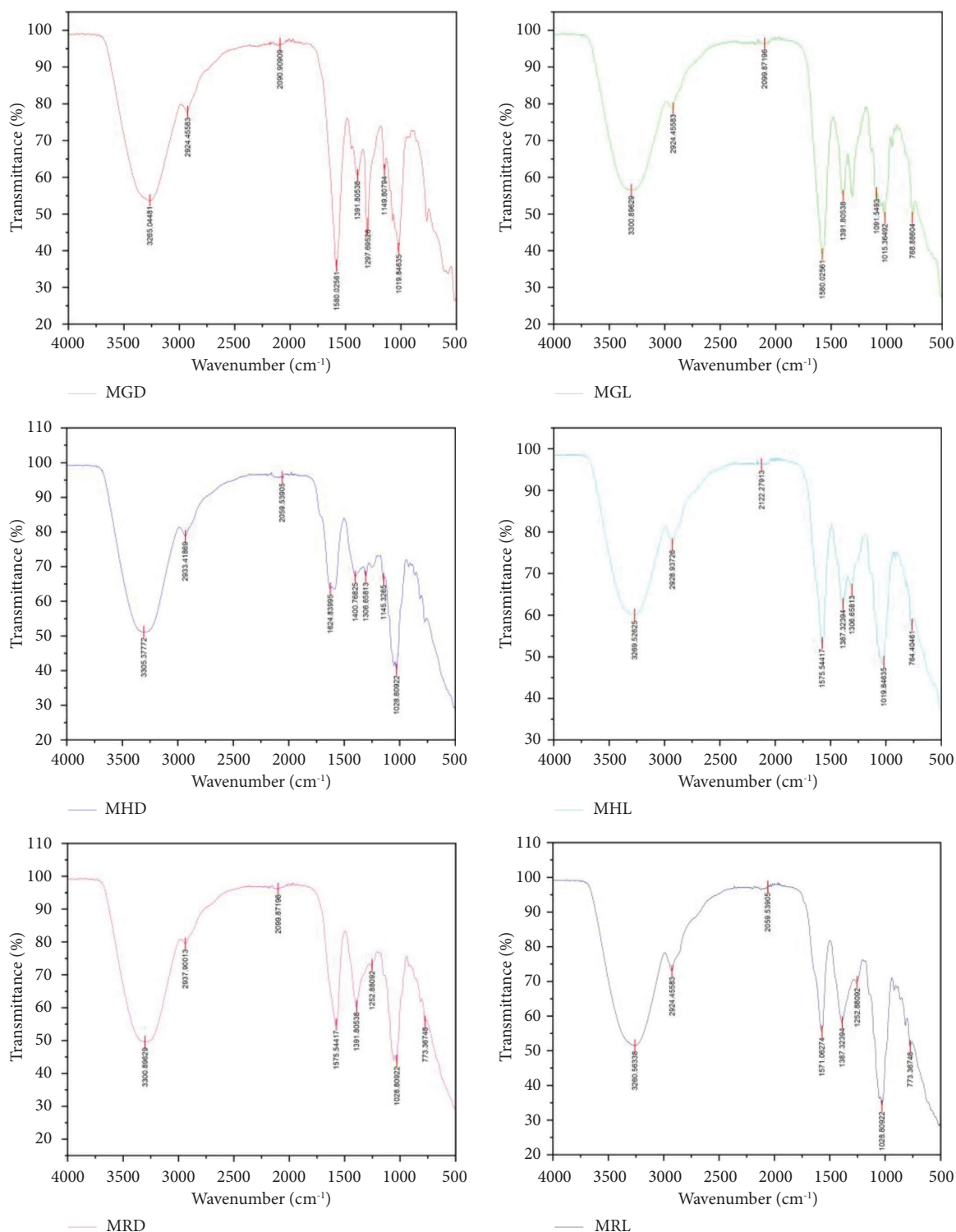


FIGURE 9: FTIR spectra of extracted pectins from Apem variety (M) at matured-green (G), half-ripe (H), and full-ripe (R) stages using acidic (D) and alkaline (L) media.

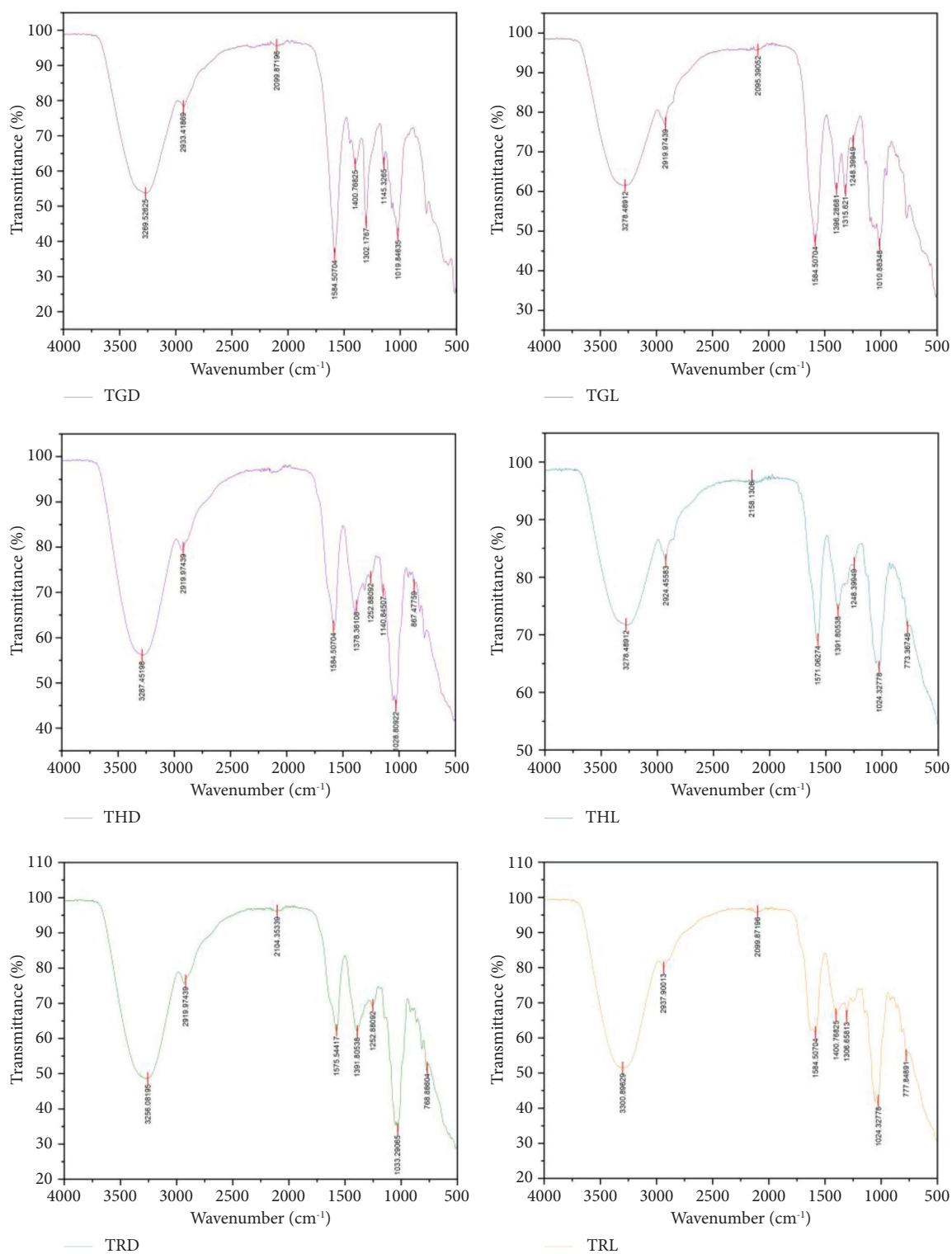


FIGURE 10: FTIR spectra of extracted pectins from Apantu variety (T) at matured-green (G), half-ripe (H), and full-ripe (R) stages using acidic (D) and alkaline (L) media.

absorbance spectrum, specifically within the range of 1074 cm^{-1} to 1043 cm^{-1} , can be attributed to the glycosidic connections that connect sugar units [77].

4. Conclusion

The primary objective of this investigation was to extract and characterize pectin from plantain peel at various stages of ripening encompassing matured green, half-ripe, and full-ripe by using acid and alkaline extraction. The findings of this investigation revealed that the extraction yield generally declines as the crop ripens, taking genotypic diversity, varying pH conditions, and a constant extraction temperature into consideration. The yield for acid extraction ranged from $18.43 \pm 1.3\%$ to $40.52 \pm 0.6\%$, while the yield for alkaline extraction ranged from $10.01 \pm 0.54\%$ to $46.55 \pm 0.5\%$ for both varieties. Based on the findings, it can be observed that the alkaline extraction method generally results in a higher yield compared to the acidic extraction method. The inherent constraint of this study is the use of plantain peels from a specific geographical location, which may affect the generalizability of the results. The pectin samples demonstrated favourable and similar physicochemical characteristics throughout all stages of ripening, suggesting their potential application in various industries. Additionally, pectin from the two plantain varieties, which were obtained at different ripening stages using both acid and alkaline extraction, could be utilized in the pharmaceutical sector as a release modifier for matrix tablets, as well as a binder and disintegrant in immediate release tablet formulations. They can also find use in the food and beverage industries as thickening agents, gelling agents, emulsifiers, and stabilizers. The aforementioned possible applications have the capacity to enhance the economic outlook of plantain peel waste and simultaneously address the issue of environmental pollution.

Data Availability

The supporting data for the results of the study are included in the article and are also available from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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