

Research Article

Agrofood Waste and By-Product Valorization, Extraction, and Characterization of Pectin from the Waste Biomass Fruit Peel of *Aframomum angustifolium* Using Response Surface Methodology as Alternative Sources of a Functional Pectin

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Applications of pectin in the food industry are strongly influenced by their source, structures, and extraction methods, which affect their functionalities. This research aims to extract and assess pectin's physicochemical and functional properties from waste biomass peels of *Aframomum angustifolium* as an alternate source using acid (AAE) and microwave extraction (MAE) methods. Pectin extracted from *A. angustifolium* was compared based on yield, color, moisture, equivalent weight, methoxyl content (MC), and degree of esterification (DegE). Response surface experimental design was used to study the effect of the extraction process pectin such as the yield and the DegE. MAE had a significantly higher % yield of $4.74 \pm 0.1\%$ and a lower equivalent weight of 852.49 ± 16.59 mg/ml than AAE with $3.09 \pm 0.03\%$ and 882.1 ± 9.04 mg/ml, respectively, with light brown color. The lower moisture contents of 6.5%, MC of 33.06%, and DegE of 67.96% were obtained by MAE compared to 6.9%, 31.85%, and 66.61%, respectively, for AAE. The time and temperature had a positive significant effect ($p < 0.05$) on % pectin yield for MAE and AAE, while time and pH squared had a negative. Temperature squared had a negative significance on % DE, and pH had a positive significance using AAE and MAE. Optimal conditions for MAE obtained were the power of 555.18W, pH of 2.79, and time of 40.69 min with optimum desirability of 0.829, while for acid extraction, the temperature of 72.95°C, pH of 2.31, and time of 142.55 min with the desirability of 0.88. A highly functionalized pectin can be extracted from the peels of *A. angustifolium* as an alternate source.

1. Introduction

The importance of fruits and vegetables in human life cannot be overemphasized as they have been reported to play a crucial role in the diet of humans. This has led to a significant increase in their production and consumption, which is a result of the growing world population and changing dietary habits [1]. The highly perishable nature of

fruits and vegetables generates significant postharvest waste and losses, which have become a problem for human nutrition, the economy, and the environment [2]. It has been estimated by [3] that waste and losses in fruits and vegetables are the highest among food groups and may attend up to 60%. Processing of fruits and vegetables also generates significant wastes or by-products, which constitute about 25% to 30% of a whole commodity group. The waste

generated by fruits and vegetables is made up of mainly seed, skin, rind, and pomace, containing good sources of potentially valuable bioactive compounds, such as dietary, carotenoids, enzymes, polyphenols, fibers, and nonstarch polysaccharides [4]. Although pectin is currently extracted from citrus and apple wastes, this waste biomass represents a potential bioresource for the recovery of nonstarch polysaccharides as a promising strategy for the development of natural biopolymers [5].

Aframomum angustifolium (AA), also called “wild cardamom,” is a spice with a berry-like fruit that contains many seeds embedded within a sugary-sweet and sour edible pulp. The seeds are commonly used as a peppery spice in many African countries. This plant is native to many tropical African countries such as Nigeria, Ivory Coast, Nigeria, Togo, and Cameroon [6], while it is cultivated in other countries such as India [7]. Extracts of the seed have been reported to be used traditionally as an antidote to dysentery and diarrhea, dysmenorrhea bronchopulmonary disorders, sexual asthenia, sterility in females, and treatment of snake bites [8]. The sugary pulp is often eaten while the peels are thrown away because they are of little value. These peels could be utilized for many other purposes such as fiber production and heating [9]. Furthermore, it could be used as a bioresource for the production of other biomolecules such as pectin.

Pectin represents a group of structural heteropolysaccharides, which is made up mainly of covalently α -1,4-linked D-galacturonic acid (GalA) units, commonly present in higher plants, precisely in the primary cell walls and middle lamella. Traditionally, the primary source for pectin extraction has been the by-products of agrofoods. The production of pectin dates back to the early 1900s when producers of apple juice in Germany started to cook dried apple pomace, the main by-product of juice processing, and sold the extracted pectin as a gelling agent [10]. To date, citrus fruit peels and apple pomace have been the main sources of commercial pectin production [11]. With an increasing demand for highly functionalized pectin and the growing interest in transforming side streams to obtain value-added products, the search for alternative sources for the extraction of pectin is on the rise [12]. Also, pectin from some fruit peels using harsh extraction methods has led to the production of pectin with low functional properties [13]. As the utilization and demand for pectin with high functional properties continue to grow, a great need to explore other bioresources from which pectin can be extracted has developed. Therefore, coupled with the fact that there is a limited number of citrus fruits and their seasonal nature, the abundance and little utilization of *Aframomum angustifolium* fruit peel necessitate its exploitation as an alternate source of pectin. This research is aimed at extracting and characterizing pectin from *Aframomum angustifolium* fruit peel as an alternate bioresource of the functional pectin.

2. Materials and Methods

2.1. Sample Collection and Preparation. Matured and freshly harvested fruits of *A. angustifolium* were obtained from Batibo Village in the north-west region of Cameroon. It was then

presented for identification by Dr. Tacham Walters and compared with a specimen stored in the Cameroon National Herbarium. After confirmation, the powder was produced from the fruit peels of *A. angustifolium*, as illustrated on the block diagram in Figure 1. The fruits were cleaned using running tap water, and the peels were removed and oven-dried at approximately 60°C. After drying, the peels were ground using a cleaned mill and sieved to obtain a fine powder. The powder was stored in plastic bags for use in the extraction of pectin.

2.2. Pectin Extraction Process

2.2.1. Acid Extraction. Extraction of pectin was carried out in acidified distilled water (0.1 M nitric acid), as described by Gazala et al. [14] and is illustrated in Figure 2. The extraction media had a pH of 1.0 and a temperature of 80°C. A mass of 30 grams of the fine powder from the fruit peels of *A. angustifolium* was submerged into 400 ml of the extraction medium, and the extraction of pectin was carried out for 2 hours. Following the extraction, the mixture was filtered using cheesecloth, and the filtrate was further treated with an equal volume of 95% ethanol to precipitate the solubilized pectin. The medium was allowed to stand for a duration of 15 min before it was centrifuged for 10 min at a speed of 8000 rpm. Washing was done using 95% ethanol on Petri plates followed by drying in a hot-air oven at 50°C overnight to recover the pellets. Samples were weighed, and the pectin yield was calculated. The obtained dried pectin pellet was ground into a fine powder and preserved in aluminum-laminated pouches until further use.

2.2.2. Microwave-Assisted Extraction of Pectin from *A. angustifolium*. The extraction of pectin using a microwave was carried out, as described by Wang et al. [15] and is presented in Figure 3. A piece of domestic microwave equipment with a working frequency of 2450 MHz, a maximum power output of 900 W, adjustable microwave power, and irradiation time was used for this study. A mixture of 30 g of the dried powder from fruit peels of *Aframomum angustifolium* and 400 mL nitric acid (0.1 M) at a pH of 1.5 was heated using the microwave at a power of 540 W and a time of 30 min. The heated sample was filtered using cheesecloth, and the filtrate cooled down to room temperature. The insoluble pectin in the filtrate was coagulated using an equal volume of 95% ethanol. After standing for 15 min, the mixture was centrifuged at a speed of 8000 rpm for 10 min to recover the pectin clots. The insoluble residue was then washed three times with 95% (v/v) ethanol to remove all monosaccharides and disaccharides [16]. The coagulated pectin was dried at 50°C overnight until constant weight, and the pectin yield was calculated. The dried pectin was ground to obtain a powder and preserved in aluminum-laminated pouches until further needed.

2.3. Experimental Design for the Extraction of Pectin from *A. angustifolium* Fruit Peels. The effect of the various extraction process parameters on the physicochemical and

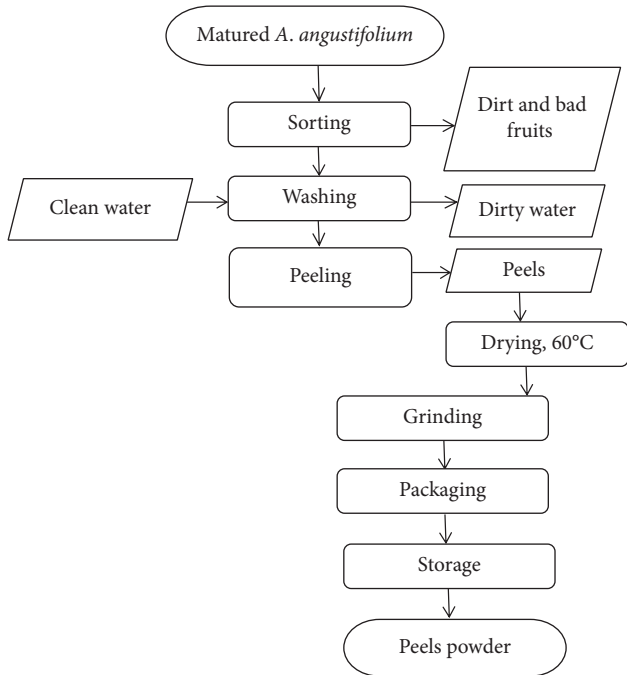


FIGURE 1: Block diagram for production of powder from fruit peels of *A. angustifolium*.

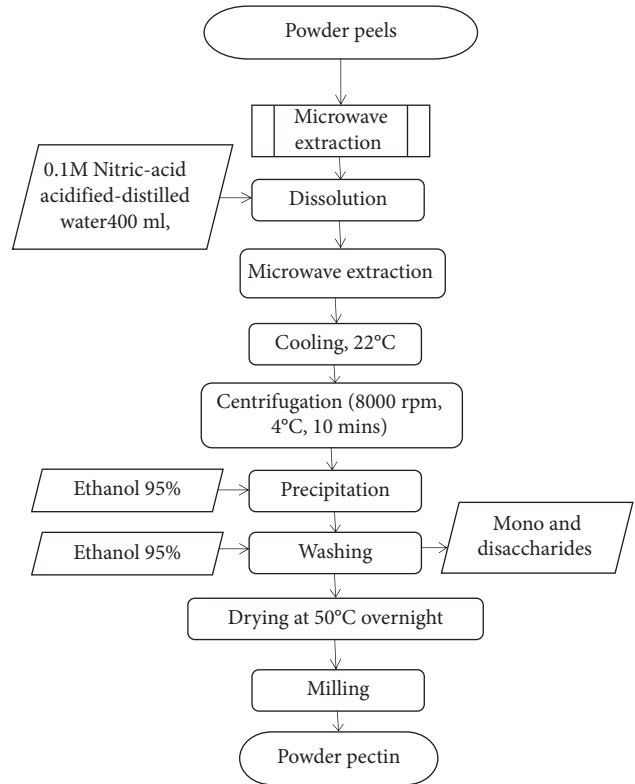


FIGURE 3: Block diagram of the extraction of pectin from the waste biomass fruit peel of *A. angustifolium* using a microwave [14].

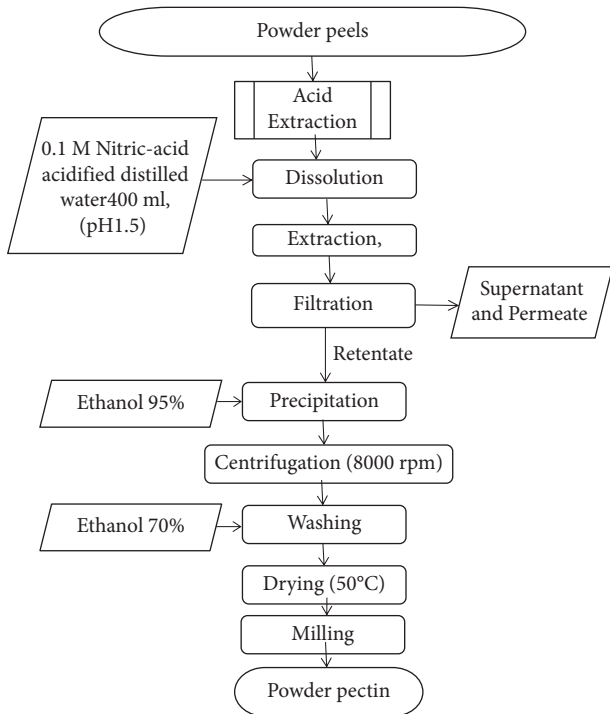


FIGURE 2: Block diagram of acid extraction of pectin from fruit peel powder of *A. angustifolium* [15].

functional properties of the extracted pectin was studied using the central composite experimental design of the response surface method, as described in previous studies [17]. The independent variables for acid extraction used were temperature (X_1), pH of acid (X_2), and time (X_3), and for microwave extraction, their variables were power (X_1), pH of

acid (X_2), and time (X_3). Tables 1 and 2 present the experimental matrix with the real and coded values for acid-assisted pectin extraction and microwave-assisted extraction processes. The levels of these variables were selected based on preliminary experiments. A total of 16 experiments with two repetitions at the center point (for calculating experimental error), six axial points and eight factorial points were conducted (Tables 1 and 2), and a quadratic polynomial model was developed with the experimental data, which in the generalized version is provided in equation (1). The experiments were performed in a random order. All analyses were performed using the software stategraphics version 14.5. After the specified treatment, the pectin extracted from both methods was then analyzed for the % yield of pectin (Y_1) and degree of esterification (Y_2). The linear, quadratic, and interaction effects of the process variables were evaluated using the mathematical model as shown in the following equation:

$$A = \beta_0 +$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2$$

$$+ \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + E, \tag{1}$$

where Y is the experimental response; X_1 , X_2 , and X_3 are the process variables; β_0 is the constant; β_1 is the linear coefficient; β_{11} is the quadratic term; and β_{12} is the coefficient of the interaction terms.

TABLE 1: Experimental matrix for acid-assisted pectin extraction process.

Experimental runs	Real values and coded values		
	Temperature (X_1) (°C)	pH of acid (X_2)	Time (X_3) (min)
1	70 (0)	3.68 (1.68)	85.0 (0)
2	53.18 (-1.68)	2.0 (0)	85.0 (0)
3	70 (0)	2.0 (0)	85.0 (0)
4	80 (1)	3.0 (1)	120.0 (1)
5	70 (0)	2.0 (0)	143.9 (1.68)
6	60 (-1)	3.0 (1)	50.0 (-1)
7	86.82 (1.68)	2.0 (0)	85.0 (0)
8	70 (0)	2.0 (0)	26.0 (-1.68)
9	70 (0)	0.32 (-1.68)	85.0 (0)
10	80 (1)	3.0 (1)	50.0 (-1)
11	60 (-1)	1.0 (-1)	50.0 (-1)
12	80 (1)	1.0 (-1)	50.0 (-1)
13	80 (1)	1.0 (-1)	120.0 (1)
14	60 (-1)	1.0 (-1)	120.0 (1)
15	70 (0)	2.0 (0)	85.0 (0)
16	60 (-1)	3.0 (1)	120.0 (1)

TABLE 2: Experimental matrix for microwave-assisted pectin extraction process.

Experimental runs	Real values and coded values		
	power (X_1) (W)	pH of acid (X_2) (ml)	Time (X_3) (min)
1	550.0 (0)	2.0 (0)	30.0 (0)
2	550.0 (0)	2.0 (0)	30.0 (0)
3	500.0 (-1)	1.0 (-1)	40.0 (1)
4	500.0 (-1)	3.0 (1)	40.0 (1)
5	600.0 (1)	1.0 (-1)	20.0 (-1)
6	600.0 (1)	3.0 (1)	40.0 (1)
7	600.0 (1)	1.0 (-1)	40.0 (1)
8	550.0 (0)	2.0 (0)	13.2 (-1.68)
9	634.1 (1.68)	2.0 (0)	30.0 (1)
10	550.0 (0)	3.68 (1.68)	30.0 (1)
11	500.0 (-1)	1.0 (-1)	20.0 (-1)
12	550.0 (0)	2.0 (0)	46.8 (1.68)
13	500.0 (-1)	3.0 (1)	20.0 (-1)
14	600.0 (1)	3.0 (1)	20.0 (-1)
15	550.0 (0)	0.32 (-1.68)	30.0 (1)
16	465.9 (-1.68)	2.0 (0)	30.0 (1)

2.4. Analytical Methods

2.4.1. Physical Properties of Extracted Pectin

(1) *Determination of the Extraction Yield of Pectin.* The extraction yield of pectin was calculated as a percentage for each batch of extraction using the following equation [18]:

$$\% \text{ Yield of pectin} = \frac{x}{y} (100), \quad (2)$$

where x stands for the weight of the dried sample of the extracted pectin and y stands for the weight of the fine dried fruit peel used for the extraction.

(2) *Determination of the Color of the Extracted Pectin.* The color of the extracted pectin was visually determined by matching the color of the powder sample against colors in

the standard color chart using a white background to know the exact color of the sample [19].

2.4.2. Determination of the Chemical Properties of the Extracted Pectin

(1) *Determination of the Moisture Content.* The moisture content of the extracted pectin was calculated using the gravimetric method [18]. Weight of one gram (W_i) of the pectin sample was dried in an oven at 100°C in the pre-weighed Petri dish to a constant weight (W_f). The plates were then cooled in a desiccator, and the moisture content of the sample was calculated using the formula presented in the following equation:

$$\text{Moisture content} = \frac{W_i - W_f}{W_i}. \quad (3)$$

(2) *Determination of the Ash Content.* The total ash content of the extracted pectin was determined using the method described by Sudhir et al. [18]. Direct analysis was carried out on the greyish-white residue remaining after the pectin sample was charred on a hot plate in a crucible and incinerated in a muffle furnace at a temperature of 550°C for 6 hours. Given that, X g represents the weight of the empty crucible, Y g is the weight of the crucible and sample, and Z g is the weight of the crucible and the ash after incineration; the ash content was then calculated using the following equation:

$$\text{Ash content (\%)} = \frac{Z - X}{Y - X} (100). \quad (4)$$

(3) *Determination of the Alkalinity of the Pectin Ash.* The alkalinity of the pectin ash was determined according to the method by Sudhir et al. [18]. The ash obtained after the complete incineration was dissolved in 25 ml of 0.1 N hydrochloric acid. The solution was heated gently until it boiled and then cooled to room temperature, followed by slow titration with 0.1 N sodium hydroxide using phenolphthalein as an indicator. The percentage alkalinity of the ash was calculated using the following equation:

$$\% \text{Alkalinity of Ash} = \frac{\text{titer value normality of NaOH} \times 60}{\text{weight of sample} \times 1000} (100). \quad (5)$$

(4) *Determination of Mineral Ion Content.* The mineral ion content of the pectin was determined as described by Kanmani et al. [20]. In doing so, 1 g of each sample was introduced into a porcelain crucible in the Carbolite Eurotherm brand muffle oven at 450°C for 2 hours then digested with 10 ml of nitric acid having a concentration of 1 N for 30 min, cooled, and filtered using a Whatman No. 1 filter paper into a 50 ml gauged vials. The total volume was then made to the gauge line using distilled water. The Na and K contents were determined using flame spectrophotometry, while Ca was determined by complexometric titration with EDTA (ethylenediaminetetraacetic acid) using 10 ml extract + 10 ml of demineralized water (pH 12.5 for the extracted mixture + water) + 1 ml of 5% KCN + a pinch of the Patton and Reeder reagent. Ca^{2+} and Mg^{2+} were determined by complexometric titration with EDTA using 10 ml extract + 10 ml of demineralized water (pH 10 for the mixture) + 1 ml of 5% KCN + 1 ml of EDTA-Mg + a pinch of the Patton and Reeder reagent. Mg was calculated by the difference method: $(\text{Ca} + \text{Mg}) - \text{Ca}$ [21].

(5) *Determination of the Equivalent Weight and the Methoxyl Content.* The equivalent weight of pectin is the most important physical property as it helps determine the

functional behaviour of pectin. The gelling ability of individual pectin is tied very closely with the equivalent weight of the pectin [18]. The equivalent weight of the pectin was determined by Ranganna's method [19]. In so doing, 0.5 g of the pectin sample was introduced into a 250 ml conical flask, and 5 ml of ethanol was added to it, followed by 1 g of sodium chloride, 100 ml of distilled water, and 6 drops of phenol red. The mixture was titrated against 0.1 N NaOH until pink color was obtained, and the equivalent weight was calculated using the following equation:

$$\text{Equivalent weight} = \frac{\text{weight of sample} \times 1000}{\text{ml of alkali} \times \text{normality of alkali}}. \quad (6)$$

The methoxyl content was determined using Ranganna's method [19]. A neutral solution was collected during the determination of the equivalent weight, and 25 ml of sodium hydroxide (0.25 N) was added to it. The mixture was stirred thoroughly and kept at room temperature for 30 min, followed by the addition of 0.25 N hydrochloric acids, and the mixture was titrated against 0.1 N NaOH. Methoxyl content was calculated using the following equation:

$$\text{Methoxyl content (\%)} = \frac{\text{ml of alkali} \times \text{normality of alkali} \times 3.1}{\text{weight of sample}}. \quad (7)$$

2.4.3. Physicochemical Properties

(1) *The Solubility of Dry Pectin in Cold and Hot Water.* This was done according to the method described in [22], in which 0.25% of the pectin samples were separately placed in a conical flask containing 10 ml of 95% ethanol and 50 ml of distilled water. The mixture was shaken

vigorously to form a suspension which was then heated at 85–95°C for 15 min.

(2) *The Solubility of Pectin in Cold and Hot Alkali (NaOH).* This was done according to the method described by Kanmani et al. [20], in which 1 ml of 0.1 N NaOH was added to 5 ml pectin solution and then heated at 85–90°C for 15 min.

2.4.4. Functional Properties

(1) *The Degree of Esterification (DegE)*. The degree of esterification (DegE) of the pectin was determined by the direct titrimetric method as described by Pinheiro et al. [23]. The initial weight of 200 mg of pectin in 50 ml conical flasks was moistened using ethanol and then dissolved with 20 ml of deionized water at 40°C for 2 hours. After complete dissolution, one drop of phenolphthalein was added to the sample and it was titrated against 0.1 M sodium hydroxide to obtain a result that was recorded as V1. This was followed by the addition of 10 ml of 0.1 M sodium hydroxide in the conical flasks, which were covered with a glass stopple. The solution was stirred at room temperature for two hours before the addition of another 10 ml of 0.1 M hydrochloric acid with continuous stirring until the pink color disappeared. The solution was then titrated against 0.1 M sodium hydroxide again, and the final result was recorded as V2. The DegE was calculated using the following equation:

$$\text{DegE}\% = \frac{V2}{V1 + V2} \times 100. \quad (8)$$

2.4.5. *FTIR Spectroscopy of Pectin*. The extracted pectin was characterized according to its spectra from the Fourier transform infrared spectrometer (FTIR) (Bruker Make, Model: ALPHA-P) in the IR region 400 cm⁻¹–4000 cm⁻¹, with 4 cm⁻¹ resolutions as described by Griffiths and De Haseth [24]. Pectin (2 mg) was mixed with 300 mg of dry KBr crystals and printed using a rotary vacuum pump. The pellet of KBr was scanned using FTIR equipment.

2.5. *Statistical Analysis*. The data obtained were inputted into Microsoft Excel version 19, and data statistical analysis was performed by using Statgraphics Centurion XVII. One-way analysis of variance (ANOVA) and means were compared using Fisher's least significant difference (LSD). Sigma plot was used to derive the response surface plots. All significances were determined at a 5% confidence level.

3. Results and Discussion

3.1. *Evaluation of the Effect of Microwave and Acid Extraction on the Physical, Chemical, and Functional Properties of Pectin from Waste Biomass Fruit Peels of A. angustifolium*. The comparison of the two extraction methods on the physical, chemical, and functional properties of the pectin from *A. angustifolium* is shown in Table 3.

3.1.1. Physical Properties of the Pectin Extracted by Acid Extraction and Microwave-Assisted Extraction Methods

(1) *The Percentage Yield of Pectin*. The percentage yield recorded for the microwave-assisted extraction method (4.74 ± 0.10) was significantly greater than that for acid extraction method (3.09 ± 0.03). The yield of pectin and its degree of esterification tends to vary with variations in fruit

peels, change in parameters, and nature of extraction carried out. The results obtained was similar to the work carried out by Kute et al. [25] on orange peel powder and recorded values of 15.79% and 8.78% for microwave-assisted extraction and acid extraction, respectively. This could be attributed to the fact that during the microwave extraction process, the microwave disrupted the water-containing cells through the rapid dielectric heating process, which resulted in a better extractability of the biomolecules.

(2) *Color*. The pectin extracted from both acid extraction and microwave-assisted methods when matched to the color chart was found to be light brown, as indicated in Figure 4. Kute et al. [25] also recorded a brownish-orange color for acid extraction and a yellowish color for microwave-assisted extraction. This coloration can be associated with the existence of carotenoids in extracted pectin from *A. angustifolium*.

The obtained result is similar to that obtained by the authors in [26], who worked with five noncitrus agrofood wastes. The pectin that was extracted from these samples had a brown color. However, IPPA (IPPA, 2016) reported that standard pectins are usually light colored because light colors represent quality gel. The same obviations had been reported by many authors working on some citrus peels [27]. They further suggested that factors such as the surface contamination, environmental factors types of agricultural material used, and human error may have contributed to the discrepancy in the pectin color.

3.1.2. Chemical Properties of the Pectin Extracted

(1) *Moisture Content*. The percentage moisture content for both extraction methods was 6.5 ± 0.14 and 6.9 ± 0.42 for microwave-assisted extraction and acid extraction, respectively. According to IPPA, the maximum moisture content requirement for dry pectin is not more than 12%. The extracted pectin water content meets this requirement. Low moisture content is necessary for pectin for safe storage as well as to inhibit the growth of microorganisms. Results from this study are less than those reported by Yadav et al. [18], with a moisture content of 8.1–10% for microwave-assisted extraction and acid extraction pectin moisture content values ranging from 9.4% to 11.3%. However, they are similar to those obtained by Attri and Maini [28] and by Kalapathy and Proctor [29] from soy hull and galgal (*Citrus pseudolimon* Tan.) peels which were 6–7% and 6–8%, respectively. The extracted pectin is very hygroscopic and therefore needs to be preserved in a closed dry atmosphere [30].

(2) *Ash and Alkalinity of the Ash and Mineral Content*. The percentage of ash obtained from the complete incineration of the extracted pectin sample had no significant difference from both extraction methods with values of 1.56 ± 0.34 and 1.56 ± 0.54 for MAE and acid extraction, respectively. This was within the IPPA [31] standards <11%. They also recorded alkalinity of 2.55 ± 0.07 and 2.45 ± 0.07, respectively.

TABLE 3: Physical, chemical, physicochemical, and functional properties of pectin extracted from *A. angustifolium*.

Characteristics	Properties	Microwave extraction	Acid extraction	
Physical	Yield (%)	4.74 ± 0.10 ^a	3.09 ± 0.03 ^b	
	Color	Light brown	Light brown	
Chemical	Moisture (%)	6.5 ± 0.14 ^a	6.9 ± 0.42 ^a	
	Mineral ion (mg/kg)	Ca	5440 ± 1.41 ^a	5440.5 ± 0.71 ^a
		Mg	3303.55 ± 0.35 ^a	3304.65 ± 0.21 ^a
		K	6741.66 ± 0.07 ^a	6740.76 ± 0.06 ^a
		Na	131.01 ± 0.65 ^a	131.46 ± 0.02 ^a
	Alkalinity	2.55 ± 0.07 ^a	2.45 ± 0.07 ^a	
	Ash (%)	1.56 ± 0.34 ^a	1.56 ± 0.54 ^a	
Equivalent weight (mg/ml)	852.49 ± 16.59 ^b	882.1 ± 9.04 ^a		
Methoxyl content (%)	33.06 ± 1.65 ^a	31.85 ± 0.36 ^a		
Physicochemical	Solubility in hot water	Soluble		
	Solubility in cold water	Partly dissolves after vigorous shaking	Partly dissolves after vigorous shaking	
	Pectin suspension soluble in cold alkali (0.1 N NaOH)	Pectin suspension forms yellow precipitate	Pectin suspension forms yellow precipitate	
	Pectin suspension soluble in hot alkali (0.1 N NaOH)	Pectin suspension dissolves and turns milky	Pectin suspension dissolves and turns milky	
	Degree of esterification (% DegE)	67.96 ± 1.18	66.61 ± 0.46	

Scores with different letters on superscripts (a and b) are significantly different at $p < 0.05$.



FIGURE 4: Sample of extracted pectin with light brown color.

Further analysis revealed the concentrations of the major mineral cations present in the samples are shown in Figure 5 and are not significantly different for both extraction methods. The ash had a high level of potassium ion (6741.67 ± 0.07 and 6740.76 ± 0.06 mg/kg) and a low concentration of sodium (131.01 ± 0.65 and 131.46 ± 0.02 mg/kg) for both MAE and acid extraction, respectively.

(3) *The Equivalent Weight and the Methoxyl Content of the Extracted Pectin.* The equivalent weight of the pectin extracted via different methods was found to be significantly different ($p > 0.05$), as presented in Figure 6. Acid-extracted pectin had a higher equivalent weight value (882.1 ± 9.04) as

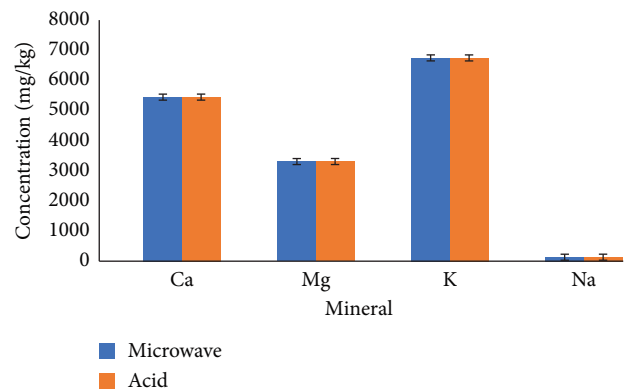


FIGURE 5: Mineral content in the extracted pectin.

compared to that of microwave-assisted extraction (852.49 ± 16.59). Low equivalent weight could be a result of a higher partial degradation of the pectin due to high temperatures or microwaves shun through the sample [32]. The equivalent weight, which is the total galacturonic acid content, depends on the pH and the extraction solvent used in the extraction process [33]. The equivalent weight of pectin obtained from this study was found to be higher than the one reported by Muthukumaran et al. [34] who obtained pectin of equivalent weight of 384.5g/mol while working on pectin extracted from *Cucumis melo* (muskmelon) peels.

The methoxyl value of pectin represents the distribution capacity of the pectin in water. According to Mugampoza et al. [35], the gel capacity of high methoxyl pectin may suggest the existence of strong cohesive and adhesive forces which might infer the increase of firmness of the food commodity. The methoxyl content of both the acid and the

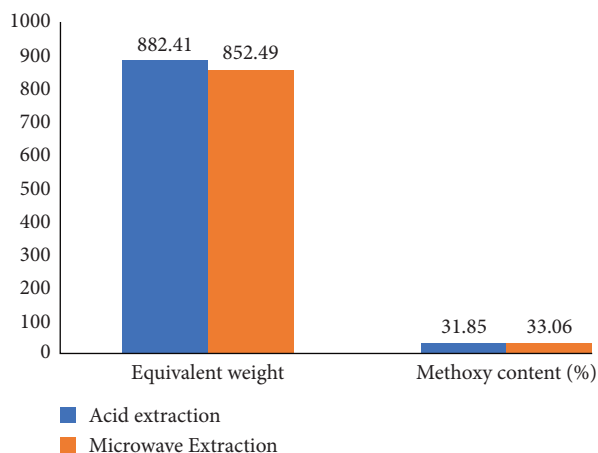


FIGURE 6: Equivalent weight (mg/ml) and % methoxy content in the pectin sample.

microwave pectin extracted from *Aframomum angustifolium* was not significantly different from each other ($31.85 \pm 0.36\%$ and $33.06 \pm 1.65\%$, respectively) but was significantly higher than the methoxyl content of standard pectin which ranges from 2.5 to 7.8, as reported in the International Pectin Producers Association [31]. This is in contrast with the finding of Liew et al. [33], who reported a higher value of 54.78% methoxyl content in passion fruit peel. The methoxyl content of pectin helps to determine its gel strength, the setting time, and the sensitivity of the pectin metal ions. This value has been reported to vary based on the source of the pectin and the extraction conditions. The degree of methylation (DM) is also used in the classification of pectin into two major groups [36]: the high-methoxyl (HM) pectin's and the low-methoxyl (LM) pectin's. Pectins with a degree of methylation ranging from 60 to 70% are classified as high-methoxyl pectins and can form higher sugar gels with a rapid setting time of 20–70 seconds while those having a lower degree of methylation (lesser than 50%) fall under low methoxyl pectins, which also indicate a lower concentration of sugar gels, having a slow set time of 180–250 seconds [37].

3.1.3. Physicochemical Properties of Pectin Extracted

(1) *Solubility of the Pectin in Cold and Hot Water and the Solubility of Suspension in Cold and Hot Alkali (0.1N NaOH)*. The extracted pectin powders obtained through both methods were insoluble in cold water, whereas they were soluble in hot water. When placed in hot water, the pectin cells imbibe water and swell. The solubility in hot water can be attributed to the distributions of hydrophilic and hydrophobic groups in the pectin molecule. During hot water treatment, the hydrophobic bonds might be weakened, paving the way for the hydrophilic groups to be attached to water, which might have led to solubility. Partial demethoxylation of pectin lowers its solubility in water [25].

This was also the case observed with its solubility in cold and hot alkali as a yellow precipitate was formed and suspended in cold alkali, whereas in hot alkali, it dissolved to form a uniform milky solution.

3.1.4. *Functional Properties of the Pectin Extracted (Degree of Esterification)*. The degree of esterification of the extracted pectin was not significantly different between the two extraction methods, as shown in Figure 7. The pectin extracted using the acid extraction method had a slightly higher degree of esterification value of $67.96 \pm 1.18\%$ as compared to that extracted using the microwave extraction method, with a value of $66.61 \pm 0.46\%$. Pectins can be classified as a high methoxyl (HM) pectin when the DegE $>50\%$ (commercially available food-grade high methoxyl pectin) or a low methoxyl (LM) pectin when the DegE $<50\%$ [38]. High methoxyl (HM) pectins require a relatively high concentration of soluble solids and a low pH for gel formation while the LM pectins can form rigid gels by the action of calcium or multivalent cations, which cross-link the galacturonic acid chains [39].

3.1.5. *Pectin FTIR*. The preliminary qualitative analysis of the major functional groups of pectin using characteristic bands of functional groups was done with the help of FTIR spectroscopy [40, 41]. The Fourier transform infrared spectrum analysis revealed four main groups of absorption bands, namely, those between 3000 cm^{-1} and 3500 cm^{-1} ; $3000\text{--}2500$, $2000\text{--}1500$, and $1000\text{--}500\text{ cm}^{-1}$, as presented in Figure 8. The broad and intense bands between 3000 cm^{-1} and 3500 cm^{-1} can be associated with the elongation vibration of the -OH group, associated with inter- and intramolecular H-bonds [42, 43]. The major absorptions at 3287.75 cm^{-1} were associated with the stretching of the hydroxyl group. This is similar to a report by Kozioł et al. [43], who observed major absorption at spectra range of $3296\text{--}3339\text{ cm}^{-1}$ for apple pectin and citrus pectin using FTIR. The bands between 2500 cm^{-1} and 3000 cm^{-1} indicate the presence of the C-H elongation bond [44, 45]. The absorption band between 1700 cm^{-1} and 1500 cm^{-1} is a characteristic of the methyl-esterified carboxyl group (COO-R) and the ionic carboxyl groups (COO⁻) vibration [42], antisymmetric stretch vibrations, and polygalacturonic acid while those between 1300 cm^{-1} and 1400 cm^{-1} (1328.11 cm^{-1}) are associated to ring stretching vibrations. The strong band at 1634.73 cm^{-1} corresponds to the C=O ester stretch vibration of the C=O.

Following the FTIR analysis, the spectral range between 1100 cm^{-1} and 930 cm^{-1} indicates the noticeable structural features ascending with particular conformations around the glycosidic bonds of pectin. The absorbance at $1077.73\text{ cm}^{-1}\text{--}1148.53\text{ cm}^{-1}$ is associated with the ring vibration coupled with C-OH bending vibrations of alcoholic groups and carboxylic acids [42, 45, 46], while the $931.22\text{ cm}^{-1}\text{--}1077.730\text{ cm}^{-1}$ (997.92 cm^{-1}) absorption band corresponds to the C-C bond. The medium intensity bands below 931.22 cm^{-1} are mainly attributed to the vibration of the C-O-C bridges typical of polysaccharides [46–49]. These different values lead us to believe that the pectin sample presented in this work is largely functionalized, as represented in Figure 7.

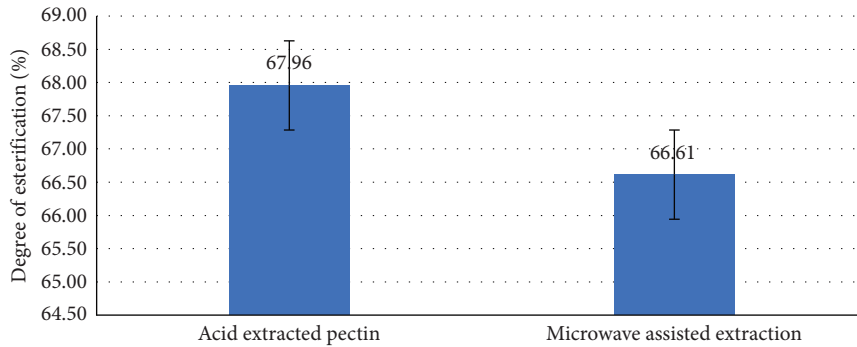


FIGURE 7: Degree of esterification of the pectin extracted via the two methods.

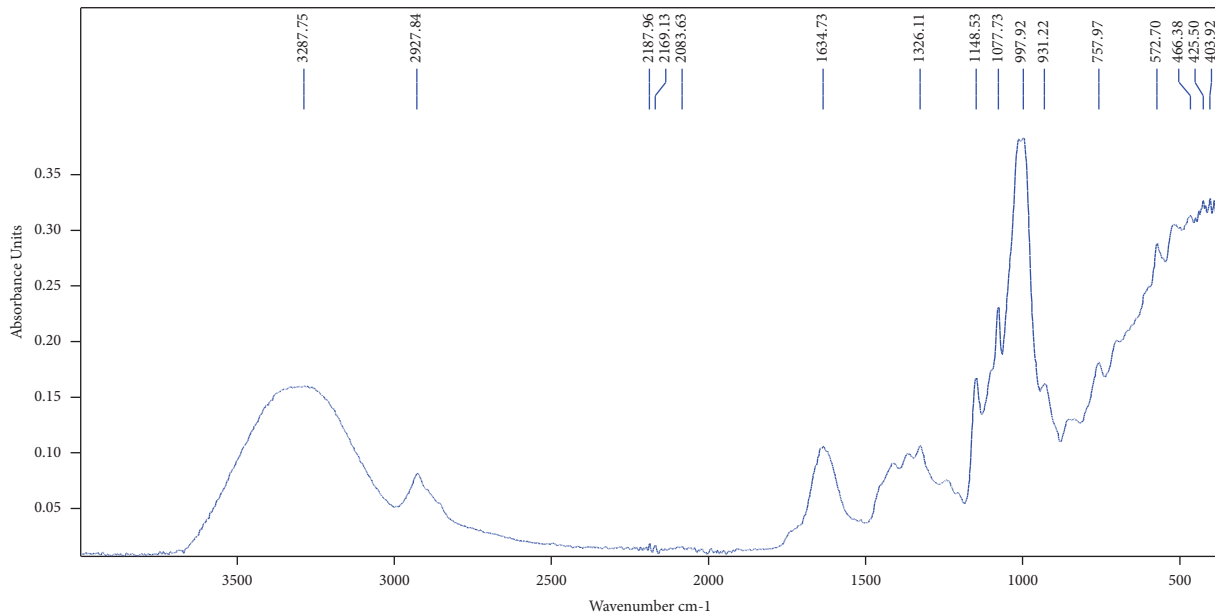


FIGURE 8: FTIR spectrum of *A. angustifolium* pectin.

3.2. Evaluating the Effects of Processing Parameters on Pectin from *A. angustifolium*

3.2.1. Effects of Processing Parameters for Microwave-Assisted Extraction. To study the effect of processing factors of the microwave extraction process of *Aframomum angustifolium* fruit peel, a central composite rotatable design with three variables (X_1 : power, X_2 : pH of acid, and X_3 : time) with response percent yield and degree of esterification (DegE) were used. Table 4 presents the result of the effect of the different process factors on the percentage yield of pectin and the % DegE from *Aframomum angustifolium* fruit peel at each central composite rotatable design point.

(1) *The Effect of Extraction Power, pH, and Time on the Percentage Yield of Pectin from A. angustifolium Fruit Peel.* The pectin yield ranged from 1.43 to 7.00%, which was out of the typical values reported of 10.07 and 8.83% for dried lemon peels and apple pomace, as presorted by Zarei et al. [50]. The results indicate that the *A. angustifolium* fruit peel pectin can be classified as high methoxyl pectin [51] due to

its DegE which is higher than the reference values of 50% [52], as indicated in Table 4.

The Pareto graph of the results was used to determine the effects of power (X_1), pH of acid (X_2), and extraction time (X_3) on each of the responses of *A. angustifolium* fruit peel pectin. From the Pareto graph in Figure 9, the time (X_3) and time-squared (X_3^2) significantly affect pectin yield at $p < 0.05$, whereas the power, pH, and other interactions had no significant effect on the pectin yield. Time (X_3) has a positive significant effect ($p < 0.05$), while time-squared (X_3^2) has a negative significant effect ($p < 0.05$) on pectin yield. However, power (X_1), pH (X_2), pH-squared (X_2^2), power-squared (X_1^2), and interaction pH-time (X_2X_3) have a negative significant ($p > 0.05$) effect on pectin yield. Also, power (X_1) and the interaction power-time (X_1X_3) and power-pH have a positive significant effect ($p > 0.05$) on the pectin yield (Figure 9).

The increase in the pectin yield as the time (X_3) increases could be because there is a longer interaction time for increased penetration of the solvent into the solid matrix, thus causing the increase of the polysaccharides mass going out from the solid particles into the solution [53]. Power (X_1)

TABLE 4: Central composite rotatable design and response results for microwave-assisted extraction.

Experimental runs	Experimental factors			Responses	
	X_1 Power (W)	X_2 pH of acid	X_3 Time (min)	Y_1 % yield	Y_2 % DegE
1	550	2	30	6.60	60.6
2	550	2	30	7.00	61.3
3	500	1	40	6.67	55.1
4	500	3	40	5.67	65.7
5	600	1	20	4.33	61.8
6	600	3	40	5.60	68.1
7	600	1	40	7.43	58.1
8	550	2	13.2	1.43	60.1
9	634.1	2	30	6.00	57.2
10	550	3.68	30	5.13	62.4
11	500	1	20	5.00	52.9
12	550	2	46.8	6.53	60.8
13	500	3	20	4.87	57.6
14	600	3	20	5.07	58
15	550	0.32	30	6.60	50.7
16	465.9	2	30	4.67	60.4

X_1 = power (W), X_2 = pH, X_3 = time (min), Y_1 = pectin yield (%), and Y_2 = degree of esterification (%).

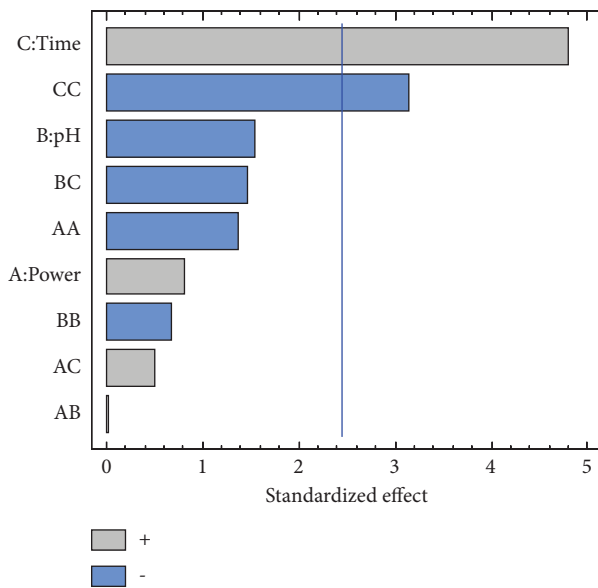


FIGURE 9: Effects of different process factors and their interactions on pectin yield from *A. angustifolium* fruit peel during microwave-assisted extraction.

also increased pectin yield because power increase in power can lead to the swelling and loosening effects during the extraction process, which will improve the solubility of pectin increasing the extraction yield [54]. This positive effect on the pectin yield was also observed with the interaction effects of power-time (X_1X_3) and power-pH (X_1X_2).

Significant decrease in pectin yield with the time-squared effect (X_3^2) was also noticed with an increase in pH (X_2), pH-squared (X_2^2), power-squared effect, and combined effects of pH-time (X_2X_3).

The model equation obtained from the regression coefficient for pectin yield was;

$$\begin{aligned}
 \text{Pectin Yield (Y)} = & -48.903 + 0.158X_1 + 1.6308X_2 \\
 & + 0.5447X_3 - 0.00015X_1^2 + 0.001X_{1,2} \\
 & + 0.00029X_{1,3} - 0.1848X_2^2 \\
 & - 0.43X_{2,3} - 0.0085X_3^2.
 \end{aligned} \tag{9}$$

The model is considered valid as the R^2 value for pectin yield is 86.63% and adjusted $R^2 = 66.58\%$ with a standard error of Est. = 0.83 (<10%). The optimized conditions for the maximum pectin yield of 7.82% are power: 572.19 W; pH: 0.32; time: 40.94 min. Generally, the pectin yield (4.33–7.43%) was similar to those reported by Kute et al. [25] and Lu et al. [55], who had pectin yields of 7.25–22.75% and 15.79%, respectively.

(2) *The Effect of the Extraction Power, pH, and Time on the Degree of Esterification (DegE) of Pectin from A. angustifolium Fruit Peel.* The effect of the extraction power, pH, and time on the degree of esterification (DegE) of the extracted pectin from *A. angustifolium* fruit peel is presented in Figure 10. From the Pareto graph in Figure 10, the pH (X_2) had a significant effect on the degree of esterification at $p < 0.05$, whereas power, time, and other interactions did not show any significant effect on the degree of esterification. The pH (X_2) had a positive significant effect ($p < 0.05$) on the degree of esterification, whereas time (X_3), power (X_1), time-squared (X_3^2), and the interaction (pH-time (X_2X_3)) have a positive significant effect at $p > 0.05$. However, power-squared (X_1^2), pH-squared (X_2^2), and the interaction terms (power-pH (X_1X_2) and power-time (X_1X_3)) have a negative insignificant effect ($p < 0.05$) on the degree of esterification.

Therefore, there will be a significant increase in the degree of esterification with an increase in pH (X_2). This increase in the degree of esterification was also noticed with an increase in power (X_1), time (X_3), time-squared (X_3^2), and

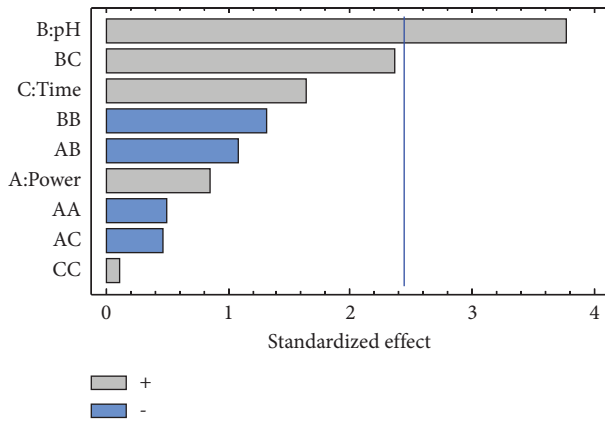


FIGURE 10: Effects of different factors and their interactions on % degree of esterification during microwave-assisted extraction.

the combined effect of pH-time (X_2X_3). Also, the Pareto chart shows that there will be a decrease in the DE with an increase in pH-squared (X_2^2), power-squared (X_1^2), and the interactions' power-pH (X_1X_2) and power-time (X_1X_3) effect on the DegE.

The model equation obtained from the regression coefficient for DegE was as follows;

$$\begin{aligned} \text{DegE} (Y_2) = & -45.142 + 0.299346X_1 + 13.2398X_2 \\ & + 0.112295X_3 - 0.00191772X_1^2 - 0.02275X_{1,2} \\ & - 0.000975X_{1,3} - 1.27493X_2^2 + 0.24625X_{2,3} \\ & + 0.00103931X_3^2. \end{aligned} \quad (10)$$

The model is considered valid as the R^2 value for pectin yield is 81.79% and adjusted R -squared = 54.48% with a standard error of Est. = 2.95 (<10%). The optimized conditions for the maximum DegE of 73.63% are power: 484.10 W; pH: 3.68; time: 46.982 min. The DegE (50.7–68.1%) was similar to that reported by Wong and Alkarkhi [56], who recorded a value greater than 50 for DegE.

High methoxyl pectin (DegE >50%) has been obtained from pomelo peel [41], pomelo albedo [57], and watermelon rind [58], indicating that our findings are in agreement with these previous works. It was noticed that the DegE increased for more extensive extraction pH.

3.2.2. Effects of Processing Parameters for Acid Extraction. To study the effect of processing factors on the acid extraction process of the pectin from *A. angustifolium* fruit peel, a central composite rotatable design with three variables (temperature: X_1 , pH of acid: X_2 ; time: X_3) was used to study the effect on the percentage yield (Y_1) and the degree of esterification (Y_2). Table 5 presents the results of the effect of different process factors on the percentage yield and the degree of esterification of the pectin extracted from *A. angustifolium* fruit peel.

(1) *The Effect of the Extraction Temperature, pH, and Time on the Pectin Yield from A. angustifolium Fruit Peel.* From the Pareto graph on Figure 11, the time (X_3) and pH (X_2) significantly affected pectin yield at $p < 0.05$, whereas the power and all other interactions had no significant effect on the pectin yield. Time (X_3) has a positive significant effect ($p < 0.05$), whereas pH (X_2) has a negative significant effect ($p < 0.05$) on the pectin yield. However, temperature (X_1) and the interaction pH-time (X_2X_3) have a positive significant effect ($p > 0.05$) on the pectin yield, whereas all the squared effects and the interactions between the temperature-time (X_1X_3) and the temperature-pH (X_1X_2) had a negative significant effect ($p > 0.05$) on the pectin yield (Figure 11).

The model equation obtained from the regression coefficient for pectin yield was;

$$\begin{aligned} \text{Pectin Yield} (Y) = & -48.903 + 0.158X_1 + 1.6308X_2 + 0.5447X_3 \\ & - 0.00015X_1^2 + 0.001X_{1,2} + 0.00029X_{1,3} \\ & - 0.1848X_2^2 - 0.43X_{2,3} - 0.0085X_3^2 \end{aligned} \quad (11)$$

The R^2 value for the equation of the pectin yield was obtained to be 84.97% and best fitted the second-order model, while the standard error was estimated to be 0.469, which was less than 10%. The model was therefore considered as valid.

The percentage of the pectin yield was observed to increase with an increase in time (X_3) and temperature (X_1). This could be attributed to the fact that high temperatures favour swelling and loosening effects during the extraction process, thus improving the solubility of the pectin which leads to an increase in the extraction yield [55]. A negative effect on the pectin yield can be observed with an increase in pH (X_2). The optimized conditions for the maximum pectin yield of 6.28% are temperature: 86.82°C; pH: 0.32; time: 118.86 min.

(2) *The Effect of Extraction Temperature, pH, and Time on the DegE of Pectin from A. angustifolium Fruit Peel.* From the analysis of variance, the pH (X_2) and pH-squared (X_2^2) significantly affected the DegE at $p < 0.05$, whereas power, time, and all other interactions did not show any significant effect on the DE. The pH (X_2) had a positive significant effect ($p < 0.05$), whereas pH-squared (X_2^2) had a negative significant effect ($p < 0.05$) on DegE. However, time (X_1), time-squared (X_3^2), and the interactions (pH-time (X_2X_3) and temperature-pH (X_1X_2)) had a positive significant effect ($p > 0.05$), whereas the squared effects of temperature (X_1^2) and the interaction temperature-time (X_1X_3) (X_1X_2) had a negative significant effect ($p > 0.05$) (Figure 12).

The DegE was observed to increase with an increase in pH (X_2) and a decrease in the pH-squared (X_1) effect. This could be due to an increase in deesterification of the polygalacturonic chains which increased the DegE [55]. A negative effect on DegE can be observed with an increase in temperature (X_1).

The model equation is obtained from the regression coefficient for DegE was as follows;

TABLE 5: Central composite design and response results for acid extraction.

Experimental runs	Experimental factors			Responses	
	X_1 Temperature (°C)	X_2 pH of acid	X_3 Time (min)	Y_1 % yield	Y_2 % DegE
1	70	3.68	85	4.33	48.7
2	53.18	2	85	4.00	60.3
3	70	2	85	5.23	63.5
4	80	3	120	4.83	68.5
5	70	2	143.9	5.50	68.8
6	60	3	50	4.03	57.3
7	86.82	2	85	5.43	56.3
8	70	2	26	3.07	56.9
9	70	0.32	85	5.37	48.4
10	80	3	50	4.00	59.3
11	60	1	50	4.93	53.1
12	80	1	50	5.17	50.5
13	80	1	120	5.93	46.1
14	60	1	120	5.67	51.3
15	70	2	85	5.20	61.3
16	60	3	120	4.93	66.7

X_1 = temperature (°C), X_2 = pH, X_3 = time (min), Y_1 = pectin yield (%), and Y_2 = degree of esterification (% DegE).

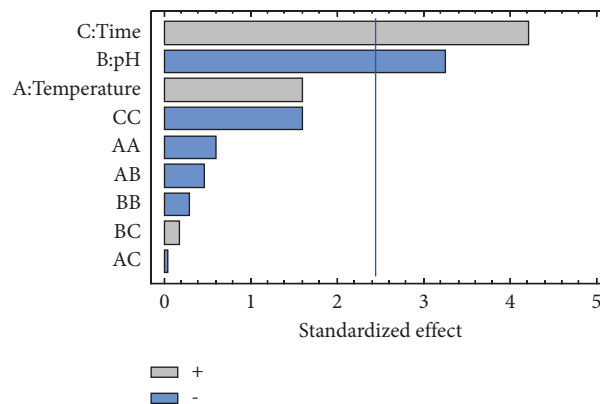


FIGURE 11: Effects of different factors and their interactions on pectin yield from *Aframomum angustifolium* fruit peel during acid extraction.

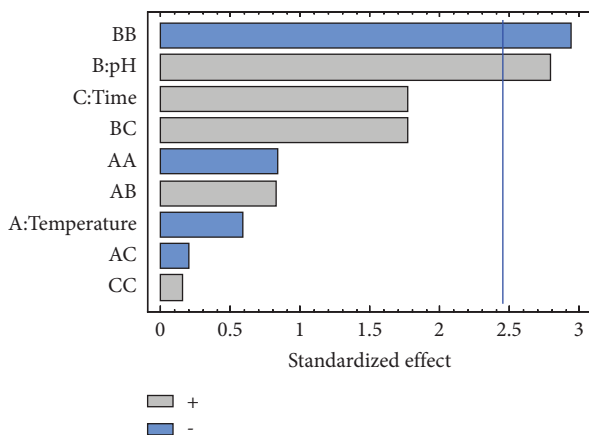


FIGURE 12: Effects of different factors and their interactions on % DegE of pectin from *Aframomum angustifolium* fruit peel during acid extraction.

$$\begin{aligned}
 \% \text{ DegE } (Y_2) = & -0.374202 + 1.61957X_1 + 5.30417X_2 \\
 & - 0.0739277X_3 - 0.0135937X_1^2 + 0.145X_{1,2} \\
 & - 0.001X_{1,3} - 4.80652X_2^2 + 0.0885714X_{2,3} \\
 & + 0.000203509X_3^2.
 \end{aligned} \tag{12}$$

The R^2 value for the equation of the % DegE was obtained to be 81.32% and best fitted the second-order model, while the standard error was estimated to be 4.96%, which was less than 10%. The model was therefore considered as valid. The optimized conditions for the maximum % DegE of 73.63% are temperature: 69.82°C; pH: 2.93; time: 143.86 min.

3.3. Optimizing Microwave and Acid Extraction of Pectin from *A. angustifolium*. Table 6 depicts the model constants, p values, and R^2 values for the second-order polynomial

TABLE 6: Model constants for the second-order polynomial equation used to model the microwave and acid extraction of pectin.

Constant	Microwave extraction				Acid extraction			
	Y1		Y2		Y1		Y2	
	Const.	p value	Const.	p value	Const.	p value	Const.	p value
β_0	-48.903		-45.142		-4.0181		-0.3742	
β_1	0.1585	0.452	0.299346	0.4255	0.169	0.161	1.61957	0.5804
β_2	1.6308	0.1755	13.2398	0.0092	0.2516	0.0172	5.30417	0.0314
β_3	0.5447	0.003	0.112295	0.1522	0.049	0.0056	-0.07393	0.128
β_{12}	0.0001	0.9869	-0.02275	0.3173	-0.00788	0.6521	0.145	0.4408
β_{13}	0.00029	0.6375	-0.00098	0.6567	0.000018	0.9712	-0.001	0.8487
β_{23}	-0.043*	0.1917	0.24625	0.0562	0.0008	0.8682	0.088571	0.1281
β_{11}	-0.00015	0.2196	-0.00019	0.6384	-0.0009	0.5652	-0.01359	0.437
β_{22}	-0.1848	0.5216	-1.2749	0.2364	-0.0461	0.775	-4.80652	0.0258
β_{33}	0.0085	0.0202	0.00103	0.9181	-0.0002	0.1621	0.000204	0.8836
R2	86.6321		81.7928		84.972		81.3177	

Y1 = pectin yield and Y2 = % degree of esterification.

equation used in modeling the microwave and acid extraction of pectins from *A. angustifolium*.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1 x_1 + \beta_{22} x_2 x_2 + \beta_{33} x_3 x_3 + E. \quad (13)$$

Conclusively, results of multiple response optimization analyses revealed optimized conditions for microwave extraction resulted in power, pH, and time of 555.18 W, 2.79, and 40.69 min, respectively, with optimum desirability of 0.829. Also, acid extraction resulted in optimized conditions of temperature of 72.95°C and pH and time of 2.31, and 142.55 min, respectively, with overall desirability of 0.88.

4. Conclusion

The aim of this research was the extraction and assessment of pectin's physicochemical and functional properties from waste biomass peels of *Aframomum angustifolium* as an alternate source using acid (AAE) and microwave extraction (MAE) methods. Pectin as successfully extracted from the peels of *Aframomum angustifolium* using both acid (AAE) and microwave extraction (MAE) methods. Though MAE had a significantly greater percentage yield of pectin than the acid extraction method, the results indicated that MAE and acid extraction had similar attributes concerning their physical, chemical, physicochemical, and functional properties. Concerning MAE, the extraction time and the pH had significant effects on the percentage yield of pectin and the degree of esterification, respectively, whereas for acid extraction, the temperature and the pH showed significant effects on the percentage yield of pectin and the degree of esterification. The optimal conditions for MAE were seen to be power 555.18 W, pH 2.79, and time of 40.69 min with optimum desirability of 0.829. Also, acid extraction resulted in optimized conditions of temperature (°C), pH, and time of 72.95°C, 2.31, and 142.55 min, respectively, with overall desirability of 0.88.

Data Availability

The quantitative and qualitative data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Authors' Contributions

Pride Ndasi Ngwasiri and Mbaku Akuro Blaise conceptualized the study and contributed to methodology; Pride Ndasi Ngwasiri and Mbaku Akuro Blaise were responsible for software; Wilson Agwanande Ambindei and Ngwa Martin Ngwabie validated the data; Mbaku Akuro Blaise performed the formal analysis; Pride Ndasi Ngwasiri and Mbaku Akuro Blaise investigated the data; Ngwasiri Pride Ndasi, Mbaku Akuro Blaise, and Ngwabie Martin Ngwa were responsible for resources; Pride Ndasi Ngwasiri and Mbaku Akuro Blaise performed data curation; Pride Ndasi Ngwasiri and Mbaku Akuro Blaise prepared the original draft; Martin Benoit Ngassoum reviewed and edited the manuscript; Pride Ndasi Ngwasiri was involved in study supervision. All authors have read and agreed to the published version of the manuscript.

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