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Research Article

Extraction and Physicochemical Characterization of Pectin from Noni (*Morinda citrifolia*) Fruit

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Abstract

Noni (*Morinda citrifolia*) is a popular plant in Asian countries and has great potential as pectin source for thickening agents in food products. In general, ethanol has been widely used to precipitate pectin, but the proportion can affect the quality of the product. Therefore, this study aimed to characterize the physicochemical properties of pectin from noni fruit. Extraction was performed using 0.1 N HCl at pH 1.5 and heated at 80°C for 15 min, followed by precipitation with different proportions of 96% ethanol including A (1:1), B (1:2), and C (1:3) v/v, while commercial orange pectin was used as a reference. The results showed that all pectin extracted belonged to the high methoxyl category. The FTIR spectra confirmed the presence of important functional groups including OH-hydroxyl, CH_3-saturated aliphatic methyl, C=O carbonyl, and -O- cyclic ether. Furthermore, the crystalline and amorphous structures of noni pectin were confirmed by X-ray diffraction observation. The highest yield was achieved using the proportion of 1:3, followed by 1:2, and 1:1 on a wet basis. The MeO and AUA content of sample B (1:2) were relatively similar to those of the reference. Based on extraction results, noni possesses good characteristics and has the potential for commercial pectin production.

Keywords: Extraction; noni (*Morinda citrifolia*); pectin; physicochemical; precipitation

1. INTRODUCTION

Noni (*Morinda citrifolia* L) is a popular fruit-bearing plant in Asian countries valued for its versatility as a vegetable and medicine. The plant can reach a height of 6 m and produces fruit 10-30 cm in length, yellowish green in color, and irregular surface, while the round stem has coarse bark [1]. Exploration of noni fruit remains limited, despite the widespread distribution in most parts of Asian countries. It primarily comprises peel, seeds, and juice, which has been applied in several products, including foods and non-foods. Noni fruit chemically consists of polysaccharides, including various monosaccharides, namely glucuronic acid (GlcA), galactose (Gal), arabinose (Ara), and rhamnose (Rha). Based on GC-MS analysis, monosaccharides in noni fruit were reported to be Gal*A*p 53.6%, Gal*p* 17.19%, Ara*f* 13.6%, Rha*p* 9.5%, Glc*p* 21.13%, Xyl*p*, Man*p*, and Fuc*p* [2]. Ridley et al. (2001) also reported an appreciable quantity of pectin and galacturonic acid in noni fruit. In general, pectin is a complex and heterogeneous polysaccharide within primary cellular walls and between cells in plants. It comprises acidic polymers known as homogalacturonan (HG) and rhamnogalacturonan (RG) with neutral sugar polymers including arabinans, galactans, and arabinogalactans in the side chain [4]. Furthermore, pectin is already widely used in the food industry as a gelling agent, stabilizer, emulsifier, or thickening agent [11]. The conventional method for extraction consists of two main steps, including the hydrolysis of protopectin to pectin, using acids, followed by precipitation with ethanol [7].

The conditions of extraction affect pectin characteristics, and the physical properties depend on the chemical characteristics. High temperatures during extraction can increase pectin yield [26] while the addition of ethanol potentially reduces stability and promotes the formation of clotting due to dehydrating effects which disturb water and pectin balance. The quality obtained is shown by extraction performance and gelling ability when rehydrated. Moreover, pectin forms gel properly with high molecular weight, MeO, and polygalacturonate content [5].

Pectin in noni fruit can be isolated using acid or water as solvents, while stability is achieved using a single water layer through electrostatic bonds between negative charges in pectin and positive charges in water molecules. In this study, noni pectin was extracted using acid (HCl) followed by heating at 80ºC for 15 minutes using various proportions of 96 % ethanol. Yadav (2015) used 95% ethanol twice the volume of filtrate to precipitate orange peel pectin. Extraction using acid solvents is a commonly used method due to the low possibility of damage. Pectin in plant tissues exists as a protopectin that is insoluble in water, hence, hydrolysis into water-soluble pectin is carried out using acidic solvents during extraction. To obtain optimal extraction results, it is necessary to adjust the level of acidity (pH), temperature, and duration of hydrolysis. Prolonged extraction time leads to the hydrolysis of pectin into galacturonic acid [5].

The level of methoxyl crucially dictates the functionality of pectin, as well as its structure and texture. At a high level, pectin can form a gel with the addition of sugar and acid. Meanwhile, at a very low level, pectin is less soluble in water [6]. The quantity of galacturonic acid and charges in pectin significantly affect the functional properties, as well as the structure and texture of the gel formed. A higher content of galacturonic acid positively relates to the quality of pectin. Functional groups of pectin can be identified using FTIR (*Spectroscopy Fourier Transform Infrared*), providing insights into the wavenumber absorbance acquired from standard. In this study, FTIR analysis was performed on noni pectin to determine the functional groups formed and provide information on the structure. Santos et al., (2020) applied FTIR to identify the degree of esterification (DE) based on functional groups such as COOR, ester, COO-, and carboxylate. Furthermore, analysis using X-ray diffraction (XRD) can offer insights into the structural coordinates as well as amorphous and crystalline properties of pectin [8].

Noni has great potential as a source of pectin in line with the quality standards of the International Pectin Producers Association (IPPA). Therefore, this study aimed to characterize noni fruit pectin isolated with acid (HCl) followed by heating at 80 °C for 15 min, using various proportions of 96% ethanol.

2. RESEARCH METHODS

Materials

Fresh and ripe noni fruit was collected from Walantaka in Banten Province, Indonesia followed by sorting and washing to remove unwanted materials. The chemical composition of the fresh fruit and reference was determined, including moisture, ash, protein, fat, carbohydrate, and total energy, referring to AOAC (2012). The chemicals used were 96% ethanol, HCl, NaOH, and AgNO from Sigma-Aldrich, Singapore. The filtrate was evaporated using a rotary evaporator (OGAWA, Japan), and pectin was collected by freeze-drying (Lyovapor L-200 BUCHI, Switzerland). To characterize functional groups, FTIR analysis was carried out using Alpha II Bruker (Billerica, MA, USA).

Extraction of Pectin

Pectin extraction from noni fruit was carried out using samples with equal solid weight. Therefore, the moisture content was determined to calculate solid content. Extraction was performed using a heated procedure at 80°C for 15 min, referring to a previous method [10] with slight modifications. Noni fruit (50 g) was added with 250 mL of distilled water and 10 mL of HCl 1.0 N pH 1.5 followed by filtration. Subsequently, the filtrate was evaporated using a rotary evaporator (OGAWA, Japan) at 40°C to reach half of the volume. The viscous filtrate was left to reach a final temperature of 25 °C and added with 96% ethanol at different proportions of A (1:1), B (1:2), and C (1:3) to allow precipitation. After 16 hours, the clots were vacuum-filtered, using a filter paper Whatman No. 1, and washed with ethanol 96% to remove chloride. Complete removal was shown by the absence of white clots in the used ethanol reacted with $AgNO_3$. Pectin was collected, freeze-dried (Lyovapor L-200 BUCHI, Switzerland), and the % yield was determined. The treatments were carried out in triplicate.

FTIR Spectra Analysis of Noni Pectin

FTIR spectra were used to characterize functional groups of pectin[11] through Alpha II Bruker (Billerica, MA, USA) at a wavenumber range of 4000 cm^{-1} to 400 cm^{-1} [11]. The ratio of the KBr: sample was 100 mg :1 mg.

XRD Analysis

XRD analysis was conducted according to the protocol prescribed by [6]. The peak position at 2θ between 200-400 in the diffractogram was observed to compare the results, with higher intensity representing more desirable crystallinity. XRD Scan Axis Genio (Germany) was measured using an X-ray polyester diffractometer, at room temperature with Cu-Kα as a source of radiation (l-0.154 nm) at 2θ and an angle range of 3-40°.

Quantification Of Moisture and Ash

Quantification of moisture and ash followed a standard method AOAC (2012). To measure the moisture content, 2 g of sample was dried at 105°C for 3 hours until a constant weight was reached. Ash content was obtained by burning 2-3 g of pectin in a furnace at 550°C.

Determination Of Equivalent Weight

Determination of equivalent weight conformed to a reported method [12] with slight modifications. About 100mg of pectin was transferred into Erlenmeyer, dissolved with 1 mL of ethanol, and added with 20 mL of NaCl 1000 ppm. A total of 6 drops of red phenol reagent were added and stirred to ensure complete dissolution. The solution was titrated with NaOH 0.1 N until a yellowish-red color was formed at pH 7.5, then left for 30 sec. Equivalent weight calculation conformed to the equation:

$$\text{Equivalent Weight} = \frac{\text{Weight of Sample (mg)}}{\text{ml of alkali x Normality of alkali}} \quad (1)$$

Determination Of MeO and Anhydrouronic Acid Content (AUA)

The neutral solution from the previous stage was added with 25 mL of NaOH 0.25 N, shaken, and left for 30 min at room temperature. About 25 mL of HCl 0.25 N was added followed by titration with NaOH 0.1 N until the color of the solution changed. This procedure conformed to a previous work [12] and the determination of Methoxyl content (MeO) was observed by using the formula:

$$\text{MeO (\%)} = \frac{\text{ml of alkali} \times 31 \times \text{Normality of alkali}}{\text{Weight of Pectin (mg)}} \times 100\% \quad (2)$$

in which 31 represents the molecular weight of MeO groups.

Determination Of DE

AUA was expressed as milliequivalent (meq) of NaOH acquired from the Equivalent Weight and Content of MeO. DE was calculated as follows [11].

$$\text{AUA(\%)} = \frac{\text{meq (alkali for free acid+alkali for methoxyl)x176}}{\text{weight of pectin (mg)}} \times 100\%. \quad (3)$$

$$DE\ (\%) = \frac{MeO\ x\ 176}{AUA\ x\ 31} \times 100\%. \quad (4)$$

Determination Of Pectin Molecular Weight

Pectin molecular weight was determined using a Column TSK gel Super AW5000 (Tosoh Bioscience, Munich) with a refractive index detector (Waters, MA, USA) [13]. Noni pectin (5.0 mg/mL) and dextran as a standard (1 mg/mL) with 6.1 and 113 k Da of weight-average molecular weights were dissolved in phosphate buffer 0.02 M at pH 6.5 and run in HPLC at a flow rate of 0.4 mL/min separately. Temperature for column and detector was set at 40°C, while other conditions were set as follows: injection volume 10µL, pressure 3 mPa, and mobile phase from aqua grade 1 for all samples.

Statistical Analysis

This experiment followed a Completely Randomized Design, and a significant difference between means was verified using Duncan at $p<0.05$. Statistical test was performed in SPSS 23.0 (SPSS, Inc., Chicago, II, USA). All analyses were carried out in three replications in duplicate and results were expressed as mean values ± standard deviation.

3. RESULTS AND DISCUSSION

Proximate Composition of Fresh Noni

Noni fruit in this study, had a relatively low protein, ash, and lipid content on a wet basis (**Table 1**), while the carbohydrate content predominated with a value of 87.76% (% dry basis). Therefore, extraction of pectin as one of the carbohydrate components from noni fruit will benefit the wide usage[1]. In this study, the moisture content reached 85.52%. Different results were reported by a previous study [14,] which obtained a moisture content of 54.21% and carbohydrate of 35.14% (wet basis) [1] presumably due to the varying ripeness of noni fruit. Furthermore, the crude fiber content was consistent with the results reported by [14] which obtained a value of 4.49%. The dietary fiber contained in noni fruit has a fairly high percentage. This fiber is a complex carbohydrate in food that cannot be digested by human digestive enzymes. The presence in food has several beneficial effects relating to indigestibility in the small intestine. However, this study focused primarily on the extraction of pectin, a soluble dietary fiber.

Table 1. Proximate Composition of Noni (*Morinda citrifolia*) Whole Fruit

	Composition	
	(%wet basis)	**(%dry basis)**
Moisture	85.52±0.20	
Ash	0.65±0.01	4.49 ±0.01
Crude lipids	0.02±0.01	0.09±0.03
Crude protein	1.11±0.05	7.66±0.03
Carbohydrate (*by difference*)	12.71±0.10	87.76±0.35
Crude fiber	4.69±0.10	30.32±0.77

All data are used as the mean ± SD of three replicates

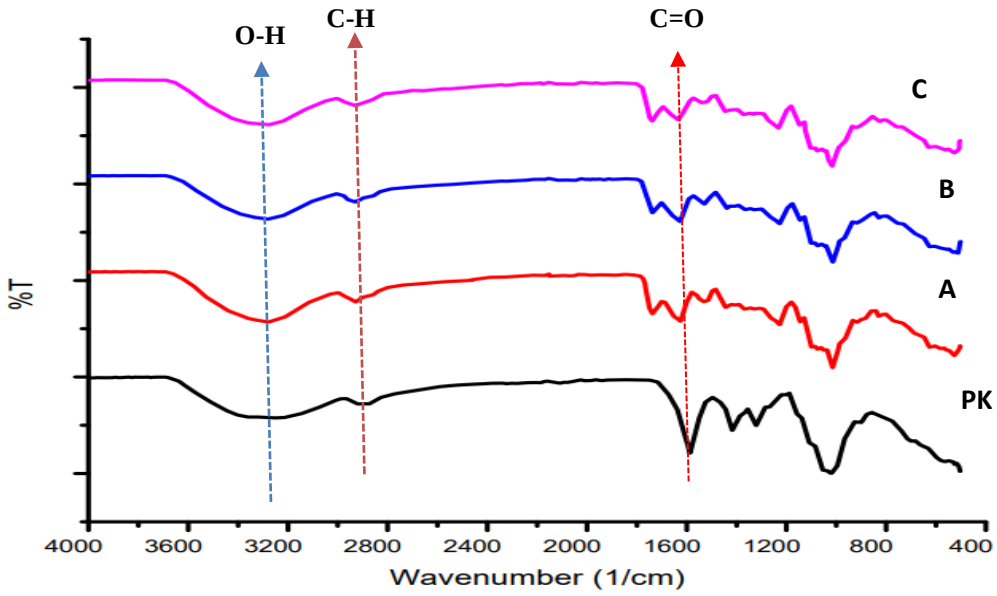

Figure 1. FTIR spectra of (PK) orange pectin from Sigma, Inc., and FTIR spectra noni pectin (A) filtrate: ethanol 96% (1:1) v/v. (B) filtrate: ethanol 96% (1:2) v/v ,(C) noni pectin filtrate : ethanol 96% (1:3) v/v

Table 2. Main Wavenumbers Observed from FTIR Spectra of Noni and Commercial Pectin (Orange Pectin)

Wavenumbers (cm^{-1})				Wavenumber of reference (cm^{-1})	Assigned functional group
A (1:1)	B (1:2)	C (1:3)	orange pectin (PK)		
3275	3277	3277	3248	-OH	Hydroxyl
2926	2928	2926	2925	-C-H (in $-CH_3$)	Saturated Aliphatic Methyl
1624	1625	1627	1584	-C=O	Carbonyl
1143	1143	1143	1204	-C-O	Cyclic Ether

(PK) orange pectin from Sigma, Inc., noni pectin obtained from extraction by (A) filtrate: ethanol 96% (1:1) v/v. (B) filtrate : ethanol 96% (1:2) v/v and (C) filtrate : ethanol 96% (1:3) v/v

FTIR Spectra of Noni Pectin

FTIR spectra provide a clear demonstration of absorbance peak showing a particular characteristic of groups specifically attributed to a compound. **Figure 1** shows FTIR spectra of noni and commercial orange pectin, presenting a cluster of functional groups. Functional groups of pectin occurred at a wavenumber between 1000-2000 cm^{-1} [11]. **Table 2** presents the wavenumber of noni and commercial pectin tested, acquired from FTIR spectra. The FTIR spectrum for noni pectin showed specific broad absorption areas at wavenumber 3275-3277 cm^{-1} for hydroxyl groups (O-H stretching) to carbon number 2. The hydroxyl group in galacturonic acid is attached to carbon atoms number 2, 3, and 6 in the monomer structure. In addition, the absorption area for aliphatic groups ($-CH_3$) occurred in the FTIR spectra for noni pectin. Carbonyl groups (-C=O) were found at a wavenumber of 1624 cm^{-1} (A), 1625 cm^{-1} (B), 1627 cm^{-1} C), and 1584 cm^{-1} (reference pectin), suggesting the samples tested were pectin. Absorption was also observed at a wavenumber of 1143 cm^{-1} (A), 1143 cm^{-1} (B), 1143 cm^{-1} (C), and 1204 cm^{-1} (reference pectin), showing the presence of cyclic ether groups (-O-). The FTIR spectra for each sample showed the fingerprint area of pectin, including 3200-3650 cm^{-1} for -OH, 2840-3500 cm^{-1} for CH_3, 1630-1850 cm^{-1} for C=O, and 1050-1260 cm^{-1} for ether -O-. The sharp peak for O-H groups was attributed to alcoholic groups. Based on the results, the peaks for O-H and C-H groups were less sharp, showing the disrupted interaction in pectin molecules [15].

XRD

XRD pattern for noni and commercial pectin standard is depicted in **Figure 2,** describing the amorphous or crystalline structure[16]. The crystalline peak observed at 2θ was found at 20.43° for reference pectin showing the shifting to higher 2θ, while the peaks for noni pectin occurred at 26.53°, 22.09°, and 22.14° for samples A, B, and C respectively. Sample C showed crystalline properties, but samples A and B had amorphous properties. XRD diffractogram for crystalline pectin showed sharp peaks, while that for amorphous indicated broad peaks. The crystallinity index may suggest the extent of hydrolyzed amorphous pectin by acid and reassociation of crystalline structure[18].

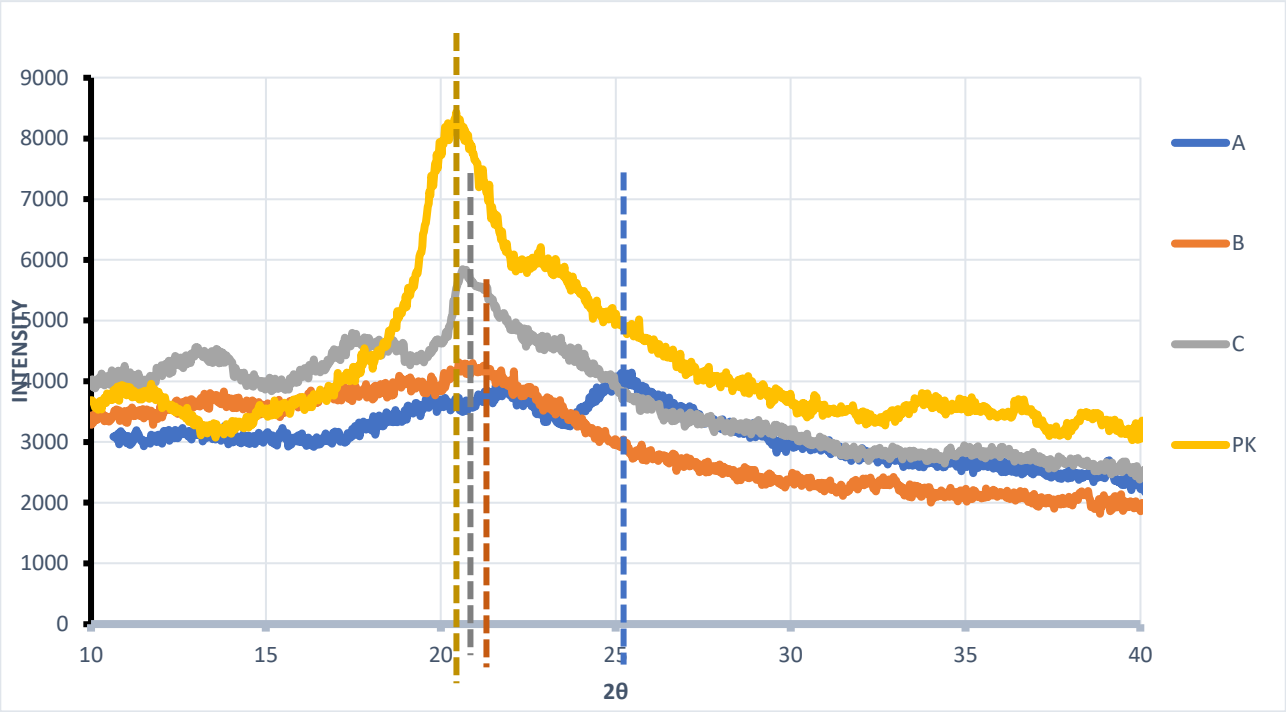

Figure 2. XRD of noni pectin (A) filtrate: ethanol 96% (1:1) v/v. (B) filtrate ethanol 96% (1:2) v/v, (C) filtrate: ethanol 96% (1:3) v/v and (PK) orange pectin from Sigma, Inc

The results were consistent with a previous study [16] which reported a range of 21.27° to 35.38°. In addition, the peak at 2θ for amorphous pectin was found at 13.7° [8]. The XRD patterns of citrus pectin after $NaHCO_3$-H_2O_2 system degradation were shown at 21.03°, indicating crystallinity, which increased after degradation due to the formation of hydrogen bonds in the hydrophilic sites of fragments [16].

XRD is a non-destructive rapid analysis technique mainly used for phase identification of crystalline materials and can provide information about the dimensions of cell units[8]. The concentration of ethanol dissolution may affect the structure of pectin, due to the impact on hydrolysis [7]. High concentrations of ethanol influence the structure to become crystalline by binding air in the pectin matrix. Therefore, ethanol potentially causes dehydration of the pectin matrix, allowing the molecules to arrange in a more regular crystalline pattern than in an amorphous state. The chemical process is important for the removal of cellulose, hemicellulose, and lignin, with these components primarily accounting for the amorphous region. This region is more susceptible to hydrolysis, leading to the enhancement of crystalline properties [19].

Yield, Moisture, and Ash Content of Pectin

Table 3 shows the yield, moisture, and ash content of freeze-dried pectin. The higher proportion of solvent caused more intensive precipitation (**Table 3**). In general, 96% ethanol is used as an extracting solvent to facilitate a dihydroxylation reaction which disturbs the equity of pectin and water, causing precipitation of pectin [13]. The presence of ethanol facilitates the dehydration of pectin, which prompts unstable colloidal solutions, leading to coagulation and easy separation. During precipitation, water molecules are exchanged with dissolved molecules. This shift enhances the interaction between chains within pectin that produces complex molecules, such as polysaccharides. Some molecules form intensive linkages through hydrogen bonds among water and trapped dissolved pectin. The rise of solution diffusion into the material's cells can increase the amount of soluble pectin [12].

In this study, HCl 0.1 N pH 1.5 was applied as a solvent, enabling the conversion of protopectin into water-soluble pectin [5]. According to a previous study, the use of acid at low pH can hydrolyze protopectin into water-soluble pectin [21]. With HCl, the conversion from protopectin to pectin occurred due to changes in Ca and Mg ions induced by H ions from chloride acid, causing the formation of calcium and magnesium chloride both precipitated under acid conditions. In addition, heat exposure during hydrolysis also intensifies the transformation of protopectin into pectin [26].

Based on the Food Chemical Codex, the moisture content of pectin powder must be below 12%. As shown in **Table 3**, the moisture content of freeze-dried pectin from this study ranged from 6.11 % - 9.38 %, while commercial pectin has a moisture content of 7.33 % according to IPPA standards. These results suggested that the moisture content of pectin extracted in this study and reference met the standard criteria. A similar value

Table 3. Extraction Yields (%Wet Basic) and Physicochemical Characteristics of Noni and Orange Pectin from Sigma, Inc

	Freeze-dried pectin (obtained from different proportions of pectin extraction) (Filtrate: Ethanol 96%) v/v			(PK) orange pectin (white powder)
	A	B	C	
Yields of pectin, %	5.83±0.65[a]	7.45±0.72[b]	9.34±0.36[c]	-
Moisture, %	8.34±2.61[a]	9.38±3.20[a]	6.11±1.31[b]	7.33±0.02[b]
Ash, %	3.32±0.64[a]	3.06±0.05[a]	3.20±0.65[a]	7.17±0.27[b]
Whiteness, %	56.38±0.08[a]	57.92±0.04[b]	56.69±0.08[a]	82.19±0.31[c]
Equivalent weight, mg	732.73±0.41[a]	749.46±1.81[a]	765.12±1.48[a]	1010.52±9.92[b]
MeO, %	9.86±1.02[a]	8.71±0.40[b]	7.65±0.34[c]	8.46±2.31[b]
AUA, %	98.97±2.06[a]	92.60±3.11[b]	88.60±1.59[c]	93.95±0.84[b]
DE, %	56.69±2.82[a]	52.16±0.14[a]	51.98±1.71[a]	63.43±1.40[b]

Note: Different superscripts in the same row showed statistical differences ($p<0.05$)
(PK) orange pectin from Sigma, Inc., noni pectin obtained from extraction by (A) filtrate: ethanol 96% (1:1) v/v. (B) filtrate : ethanol 96% (1:2) v/v and (C) filtrate : ethanol 96% (1:3) v/v

of <12% was also found in other studies[7], where the moisture content altered the shelf life of the product.

Ash content is related to the purity of pectin, representing the amount of inorganic compound residue, and varies depending on extraction and isolation methods [10]. The Standard by the IPPA [22] stipulates a maximum ash content of 10%. The ash content in the two groups of tested samples was in agreement with the IPPA standard (**Table 3)**

Equivalent Weight, DE, AUA, and MeO

As described in **Table 3**, treatments showed different significance in equivalent weight with commercial orange pectin. According to IPPA (2002), the equivalent weight ranges from 600-800 mg, suggesting that the samples tested met the criteria. The equivalent weight of pectin from *Averrhoa bilimbi* L extracted using HCl 1.0 N ranged from 577.5-650.8 mg for 8 treatments at a temperature of 60°C-100°C and maximum time of 120 min [23]. Equivalent weight can be affected by the acid used in extraction, the stronger and more concentrated the acid level, the greater the polymerization of pectin chains, leading to the elongation and reduction of free acids. Alleviation of free acids in solution caused an increase in the equivalent weight [24].

AUA content differed between samples, reaching 98.97%, 93.95%, (92.60%), and 88.60% for A, reference, B, and C respectively. The quantity of this compound crucially determines the functionality of pectin, while also affecting gel structure and texture. Therefore, the percentage of AUA represents the quality of pectin. This study also confirmed the suitability of the content compared to the IPPA standard, which stipulated a minimum of 50%. AUA can exist in materials at different levels, depending on origin, solvent, and extraction method.

MeO in noni and reference pectin ranged from 7.65% - 9.86%, classified as high (>7%). This result was consistent with Ismail *et al.* (2012) which reported MeO of 7.34% in apple pectin. MeO abundance was affected by extraction procedures responsible for the degradation of ester groups due to acid hydrolysis [25].

DE did not differ between samples tested ($p>0.05$), but the reference showed the highest value of 63.43% compared to other samples, including A (56.69%), B (52.16%), and C (51.98%). According to Aziz et al., (2018), using a high proportion of ethanol reduced DE. This parameter represents the amount of carboxyl groups in D-galacturonate acid residue esterified with ethanol [10]. In general, DE value is obtained from the MeO and galacturonate. The use of 96% ethanol could ameliorate DE value, representing the number of esterified carbonyl groups. High MeO pectin is primarily characterized by DE >50%, and in this study, noni pectin showed DE >50%. Based on the results, sample B (1:2) had a higher content of MeO, AUA, and DE than C (1:3). Although sample A had higher MeO, galacturonate, and DE than B, the % yield was smaller.

Molecular Weight

Molecular weight constitutes a crucial variable for the chemical features of a polymer. As shown in **Figure 3,** the molecular weight of noni pectin samples was 53.18 K Da (A), 54.55 K Da (B), and 59.97 K Da (C), lower than that of orange pectin standard of 129.46 K Da. Consequently, pectin molecules may have a molecular weight ranging from 50 -150 k Da. Determining the molecular weight of a sample may provide beneficial insight into the process of synthesis and polymer products. In general, polymers with high molecular weight are attributed to stronger properties.[26] The molecular weight of pectin isolated from breadnut (*Artocarpus camansi*) peels reached up to 61.17 k Da [28]. In addition, Madjaga *et al.* (2017) found molecular weight of 73.67 k Da in pectin isolated from breadfruit peels. These results suggest that different pectin source yields varying molecular weights.

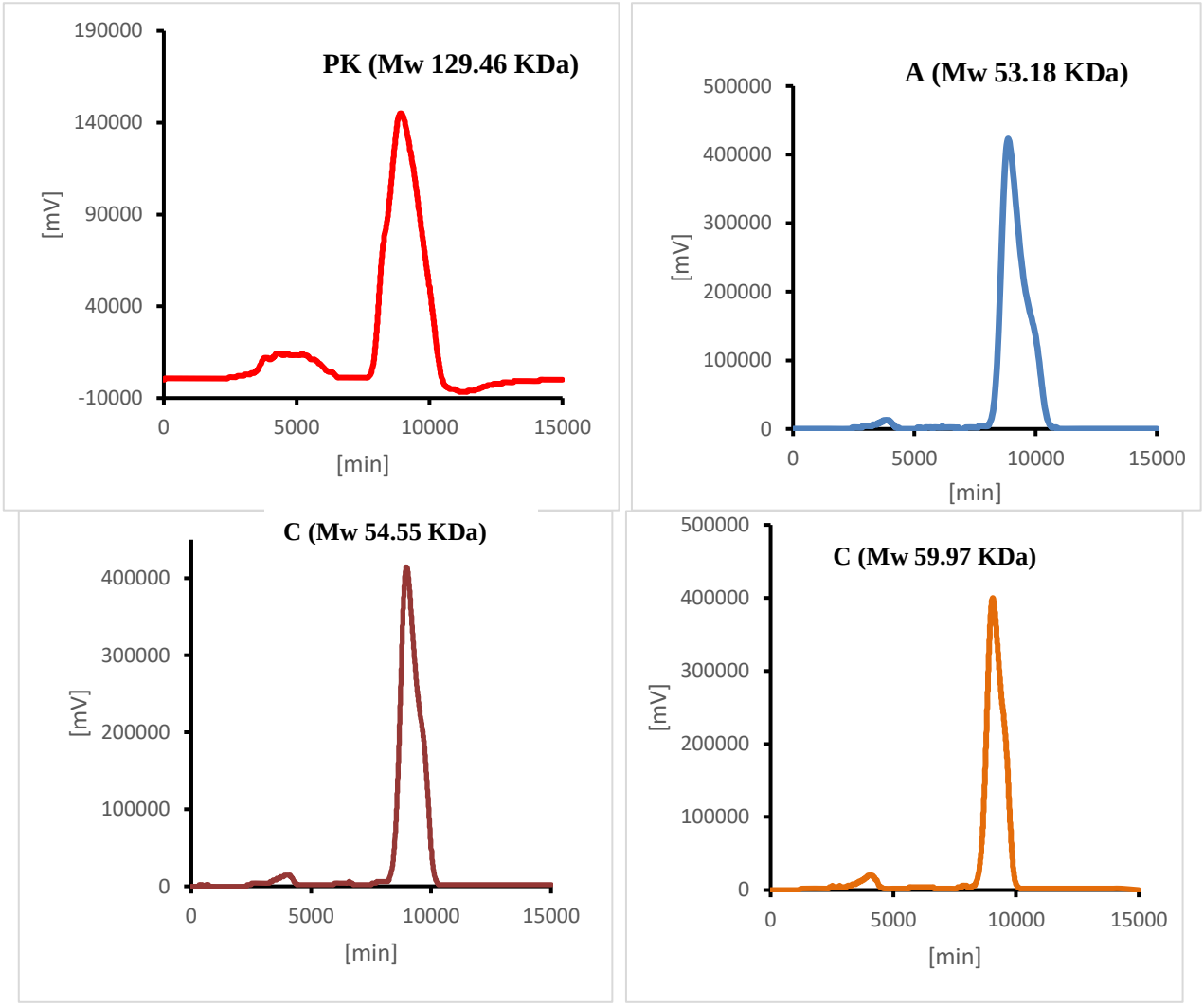

Figure 3. Molecular weights determined by HPLC using gel filtration chromatography column of (PK) orange pectin from Sigma, Inc. noni pectin obtained from (A) filtrate: ethanol 96% (1:1) v/v. (B) filtrate: ethanol 96% 1:2) v/v, (C) filtrate: ethanol 96% (1:3) v/v

Principally, pectin comprises hundreds to thousands of saccharide units within a single configuration consisting of galacturonate units attached with 1,4-α glucoside linkages. Pectin with a high molecular weight can be applied in food, drugs, and adhesives, while those with low molecular weight are applied as an antibacterial agent and prebiotic.

4. CONCLUSION

In conclusion, different extraction conditions in the form of filtrate: ethanol 96%, including A (1:1); B (1:2); and C (1:3) v/v were used to obtain pectin from noni fruit with good characteristics**.** Chemical properties, equivalent weight, MeO, and DE, all met the standard criteria. However, noni pectin prepared using a 1:2 (B) proportion had similar properties with the reference. The FTIR spectra confirmed the presence of important functional groups, including OH-hydroxyl, CH_3-saturated aliphatic methyl, C=O carbonyl, and -O- cyclic ether. In addition, noni and reference pectin samples were regarded as crystalline and amorphous, based on the XRD analysis.

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