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Effect of Sterilization on the Degree of Esterification, FTIR Analysis, and Antibacterial Activity of Durian-Rind Pectin

(Kesan Pensterilan terhadap Tahap Pengesteran, Analisis FTIR dan Aktiviti Antibakteria Pektin Kulit Durian)

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ABSTRACT

Pectin is a common food ingredient used as a rheology modifier and recently recognized as an emerging bioactive compound. Degree of esterification (DE) and molecular weight (MW) are important determinants of its bioactivity. This study evaluated the effect of moist heat sterilization (121 °C, 15 min) on pectin from Indonesian durian rind as an alternative method to modify pectin. Sterilized pectin was compared in terms of DE, Fourier transform infrared (FTIR) analysis, gel-forming ability, and antibacterial activity to non-sterilized pectin and standard citrus-peel pectin. Durian-rind pectin was identified as a low-methoxyl pectin with DE of 26.50% and weak antibacterial activity. After sterilization, the DE and pH decreased. It lost the ability to form a gel which indicated pectin was degraded to lower molecules. Loss of bands at 1760-1745 cm^{-1} indicated that pectin underwent ester hydrolysis and generated free carboxyl groups. On the other hand, the sterilized durian-rind pectin showed strong antibacterial activity towards *Staphylococcus aureus* and *Escherichia coli*, with a reduction of 5 log cycles and 3 log cycles, respectively (with the initial bacterial level of 5 log cfu/mL). These results indicated that depolymerization and deesterification of pectin by heat sterilization was able to improve the antibacterial activity of durian-rind pectin.

Keywords: Antibacterial activity; durian rind; FTIR analysis; pectin; sterilization

ABSTRAK

Pektin ialah bahan makanan biasa yang digunakan sebagai pengubah reologi dan baru-baru ini diiktiraf sebagai sebatian bioaktif yang baru. Tahap pengesteran (DE) dan berat molekul (MW) adalah penentu penting bioaktivitinya. Kajian ini menilai kesan pensterilan haba lembap (121 °C, 15 min) ke atas pektin daripada kulit durian Indonesia sebagai kaedah alternatif untuk mengubah suai pektin. Pektin tersteril dibandingkan daripada segi DE, analisis transformasi Fourier inframerah (FTIR), keupayaan membentuk gel dan aktiviti antibakteria kepada pektin tidak disterilkan dan pektin kulit sitrus piawai. Pektin kulit durian dikenal pasti sebagai pektin metoksil rendah dengan DE sebanyak 26.50% dan aktiviti antibakteria yang lemah. Selepas pensterilan, DE dan pH menurun. Ia kehilangan keupayaan untuk membentuk gel yang menunjukkan pektin telah terdegradasi kepada molekul yang lebih rendah. Kehilangan jalur pada 1760-1745 cm^{-1} menunjukkan bahawa pektin mengalami hidrolisis ester dan menghasilkan kumpulan karboksil bebas. Sebaliknya, pektin kulit durian yang disterilkan menunjukkan aktiviti antibakteria yang kuat terhadap *Staphylococcus aureus* dan *Escherichia coli* masing-masing dengan pengurangan 5 kitaran log dan 3 kitaran log (dengan tahap bakteria awal 5 log cfu/mL). Keputusan ini menunjukkan bahawa penyahpolimeran dan penyahsterilan pektin melalui pensterilan haba dapat meningkatkan aktiviti antibakteria pektin kulit durian.

Kata kunci: Aktiviti antibakteria; analisis FTIR; kulit durian; pektin; pensterilan

INTRODUCTION

Pectin is a group of complex anionic heteropolysaccharides found in the cell wall of higher plants. Pectin has diverse and complex chemical structures with the main structure consists of partially methylated galacturonic acid chains. The degree of esterification (DE) describes the ratio

of esterified galacturonic acid to the total amount of galacturonic acid units. The DE value classifies pectin into two categories: high-methoxyl pectin (HMP) with DE value above 50% and low-methoxyl pectin (LMP) with DE value below 50%. The diversity of pectin structure is determined by the plant source, especially the species and physiological stage, and the extraction condition (Minzanova et al. 2018; Müller-Maatsch et al. 2016). The chemical structure of pectin is highly correlated to its functional and biological properties and affects its application in food.

Pectin is a common ingredient in foods, mainly applied as a gelling agent, stabilizer, or thickening agent (Voragen et al. 2009). Pectin researchers have begun to explore pectin as a health product due to its abundance in nature and possible biological activities. Pectin has been studied to exert various beneficial biological activities, such as potential immunoregulatory, anti-inflammatory, hypoglycemic, antibacterial, antioxidant, and antitumor (Minzanova et al. 2018). Commercial pectin is generally extracted from citrus peel and apple pomace. However, other food wastes have also gained interest as possible sources of pectin (Müller-Maatsch et al. 2016). The most common extraction method employed is by heating the plant materials in acidic water followed by alcohol precipitation. Phenolic compounds are often found in the recovered pectin and contributed to the brown hue of pectin as the alcohol precipitation is not able to completely remove those compounds (Schieber et al. 2003; Wikiera et al. 2021).

Durian (*Durio zibethinus*) is a popular tropical fruit in Southeast Asia, especially in Thailand, Malaysia, Indonesia, and The Philippines. This large fruit generates a substantial amount of waste, particularly its rind, accounting for more than 50% of the fruit weight (Cheok et al. 2018). The high amount of waste leads to environmental problems. Thus, the transformation of durian rind into valuable products has been explored by various researchers. Durian rind waste still contains some valuable bioactive compounds which can be incorporated into food as functional ingredients. The important biological activity of durian rind is often attributed to phenolic compounds, triterpenoids, and glycosides (Feng et al. 2018, 2016). However, those components are present in limited quantities in the rind. Wanlapa et al. (2015) noted that durian rind is significantly lacking in total phenolic and flavonoid content compared to other tropical fruit wastes. The major composition of durian rind consists of non-starch polysaccharides and lignin; therefore, durian rind is a suitable source to extract bioactive dietary fiber, such as pectin and cellulose (Wanlapa et al. 2015). The water-soluble polysaccharide fractions were reported to inhibit some Gram-positive and negative bacteria (Lipipun, Nantawanit & Pongsamart 2002; Pholdaeng & Pongsamart 2010; Pongsamart et al. 2005; Thunyakipisal et al. 2010). Pectin was identified as the main component of the fraction (Hokputsa et al. 2004). These findings offer an opportunity to utilize durian rind as potential source of pectin with antimicrobial activity.

Pectin as an antibacterial agent is presently understudied to date. A study by Wu et al. (2014) showed that DE value and molecular weight (MW) are important structure-activity determinants for the antibacterial activity of citrus pectin. The definite value of DE and MW needed to inhibit bacterial growth are not currently known. In general, lower DE and lower MW were shown to have better antibacterial capabilities (Li et al. 2016; Wu et al. 2014). As a result, reduction of DE and MW through depolymerization and deesterification process appeared to be a promising step to increase the antibacterial activity of pectin. Heat treatment, such as moist heat sterilization by autoclaving (121 °C for 15 min) is often regarded as being detrimental to pectin structure and functionality (Munarin et al. 2013). However, this treatment presents the practicability to simultaneous decrease the DE and MW of pectin (Chen et al. 2015) while at the same time sterilize it. During extraction of pectin, aseptic techniques are not implemented which can result in microbial contamination of the obtained extract. In this case, sterilization is sometimes needed to prevent the reduction of the antimicrobial activity and ensure its microbiological safety (Harjanti, Wahyono & Ciptaningtyas 2020). Moist heat sterilization is also easier to perform for pectin, because pectin generates viscous solution which makes it difficult and ineffective to be sterilized using 0.22 μm membrane filters.

The impact of moist heat sterilization on important physical and chemical properties of pectin has been previously studied by Munarin et al. (2013) but no correlation to its possible change of antibacterial activity was reported. During moist heat sterilization, pectin underwent β-elimination reaction and acid hydrolysis which can generates a number of pectin degradation products, including unsaturated uronides and oligogalacturonides. Hence, this study was subjected to evaluate the effect of sterilization on the antibacterial activity of durian-rind pectin. The properties of pectin and its heat-treated products were characterized by DE value, Fourier Transform Infrared (FTIR) analysis, gel strength, and antibacterial activity against two most

common food-related bacteria, *Staphylococcus aureus* and *Escherichia coli* as representative of Gram positive and Gram negative bacteria, respectively.

Materials and Methods

Sample preparation

The durian rind was obtained from a local durian farm in Bogor, West Java, Indonesia. The ripe durian fruit was harvested 4 months after flowers appeared during the peak harvest between August and December. To avoid variation, all batches were mixed into one composite sample. The part of rind used to extract pectin only consisted of the white inner husk. The rind was cut into small pieces and dried in an oven at 60 °C. The dried rind was then ground and sieved through 60-micron mesh to obtain powder. The powder was stored in polyethylene plastic at refrigerator temperature (4 °C) until extraction. The powdered durian rind was subjected to proximate analysis according to the official method (AOAC 2012).

Pectin was extracted by a conventional hot water-acid extraction adopted from a similar study by Hokputsa et al. (2004). Durian-rind pectin was extracted in hot water (90 - 100 °C) set at pH 4.5 (with citric acid) for 20 min. The extracted pectin was precipitated by adding acidified ethanol. After the precipitation step, the pectin gel was dried and ground. The yield of extraction was calculated as the weight of dried pectin gel obtained from 100 g of dried durian-rind powder. Pectin from citrus peel (Sigma-Aldrich, USA) was used as standard pectin. The two pectin samples were further divided into two groups: untreated pectin and sterilized pectin. The untreated pectin was used as it was, while the sterilized pectin was subjected to moist-heat sterilization using autoclave at 121 °C and 0.16 MPa for 15 min. Untreated pectin and sterilized pectin were stored in a dark sealed container at refrigerator temperature (4 °C) before further analysis.

Degree of esterification (DE) analysis

The DE value was determined using an acid-base titrimetric method, as described by Wai, Alkarkhi and Mat Easa (2010).

Gel strength analysis

The gel strength test was conducted by measuring the strength needed to break the gel formed by the tested pectin. The pectin gel was prepared according to the method used by Takamine et al. (2007) with citrate buffer used in the current preparation instead of lactate buffer. The hot viscous solution obtained was immediately poured into a cylindrical polyvinyl chloride container (31 mm in height and 27 mm in inner diameter). Each preparation was enough to fill three containers. The container was sealed with HDPE plastic film and allowed to gel at 4 °C for 20 h. Before measurement was taken, each gel was allowed to stand until it reached room temperature (25 °C). The plastic cover was removed just before the analysis was conducted.

The measurement was conducted using a Texture Analyzer (Stable Micro Systems TA-XT2i, UK). A cylindrical probe (1/2" Cyl. Delrin, 12.7 mm in diameter) was used as the test probe. The speed was set to be at 1.0 mm/s pre-test speed, 1.0 mm/s test speed, and 5.0 mm/s post-test speed. The distance penetrated was set to be 10 mm with force applied to be 205 g. Due to the poor gel formed, the measurement was taken with the gel remaining inside the cylindrical container. Gel strength was measured as the force (expressed in g) which was necessary to break the gel formed by the pectin solution at the set conditions.

Analysis of functional groups by FTIR

Durian-rind pectin was analyzed using an FTIR instrument to compare the infrared spectra of durian-rind pectin and standard citrus-peel pectin, before and after sterilization. The analysis was conducted on a Bruker Tensor 37 FTIR spectrophotometer (Bruker Optik GmbH, Germany) with DTGS (deuterated triglycine sulfate) detector. FTIR spectra were obtained by transmittance measurement using KBr (Sigma-Aldrich, USA) pellets technique. The range was set from wavenumber 4000 to 400 cm^{-1}.

Preparation for test bacteria

Staphylococcus aureus (ATCC 25923) and *Escherichia coli* (ATCC 25922) were selected as model bacteria. The bacteria were maintained in TSA (Tryptone Soya Agar from Oxoid, England) slants at 4 °C for the bacterial test. Before each analysis, the bacteria were grown in fresh liquid TSB (Tryptone Soya Broth from Oxoid, England) and incubated overnight at 37 °C. The bacterial suspension was diluted in phosphate buffer saline (Oxoid, England) solution by 10-fold serial dilution until the final concentration of 1×10^3 and 1×10^5 colony forming units (cfu)/mL was obtained.

ANTIBACTERIAL ASSAY

This assay was carried out by counting the surviving bacteria after exposure to pectin at each designed time and concentration in accordance with the method used by Thunyakipisal et al. (2010). Pectin was dissolved in sterile distilled water to achieve the concentration of 50 and 100 mg/mL. The pH of each pectin solution used in the antibacterial analysis was measured to observe possible pH change. The pectin solution (5 mL) was then exposed with a 5 mL suspension of the two tested bacteria strains (10^3 and 10^5 cfu/mL) for four different exposure times: 1, 2, 3, and 6 h. After each exposure, the solution was diluted and the number of surviving bacteria was measured using the pour plate method on TSA media. Pectin might flocculate test bacteria but not inactivated them; therefore, vigorous shaking before sampling was carried out to prevent the possibility. The Petri dish was incubated for 18 h at 37 °C.

STATISTICAL ANALYSIS

All analyses were carried out in three replications in duplicate, except for gel strength that was done in triplicate. The obtained data were processed statistically using two-way ANOVA with a 95% confidence level. P-values lower than 0.05 ($p < 0.05$) were considered significant. The computer application used for data processing was Microsoft Excel, while the application for statistical evaluation was Minitab 18 (Minitab Pty Ltd., Australia). Results were expressed as mean values ± standard deviation.

RESULTS AND DISCUSSION

YIELD AND CHARACTERISTICS OF DURIAN RIND PECTIN

The powdered durian-rind, as the source of pectin in this study, had a relatively low protein-, ash- and lipid-content (Table 1). The hot water-acid extraction yielded in 3.99% of pectin, lower than that found by Hokputsa et al. (2004). Hokputsa et al. (2004) used the Monthong durian rind and achieved an extraction yield of 10%. Both extraction yields were still within the range reported by Wai, Alkarkhi and Mat Easa (2010), i.e., ranging from 2.13 to 10.25%. The difference in extracted pectin yield was likely due to the difference in durian varieties and physiological state.

TABLE 1. Chemical composition of dried ripe durian rind powder

	Composition (%)
Moisture (in wet basis)	3.32 ± 0.05
Ash	6.22 ± 0.16
Crude lipid	1.02 ± 0.28
Crude protein	6.09 ± 0.18
Carbohydrate (*by difference*)	86.67 ± 0.52
Crude fiber	32.43 ± 0.67

The durian-rind pectin and standard citrus-peel pectin showed DE values lower than 50% (Table 2), and were therefore classified as low-methoxyl pectin (LMP). The DE value was apparently affected by the sterilization treatment. The DE value of durian-rind pectin decreased while citrus-peel pectin tended to increase after sterilization. Munarin et al. (2013) also reported the decrease of the DE value after sterilizing of pectin with moist heat (121 °C, 2 atm, 15 min, saturated steam). Different results were reported by Wang, Chen and Lü, (2014), where the DE value of apple-pomace pectin tended to increase after heat treatment, while the citrus-peel pectin fluctuated depending on the temperature applied.

TABLE 2. Characterization of durian rind pectin and citrus peel pectin

Parameters	Before sterilization		After sterilization	
	Durian rind pectin	Citrus peel pectin	Durian rind pectin	Citrus peel pectin
Degree of esterification (%)	26.50 ± 1.89[a]	23.31 ± 0.39[a]	19.60 ± 0.59[b]	25.34 ± 0.68[b]
Gel strength* (g)	15.5 ± 1.97[a]	9.71 ± 0.57[b]	-	-
pH of solution				
— 100 mg/mL	3.20 ± 0.04[a]	3.38 ± 0.01[a]	1.98 ± 0.03[b]	2.71 ± 0.01[b]
— 50 mg/mL	4.04 ± 0.10[a]	4.09 ± 0.01[a]	2.18 ± 0.02[b]	2.92 ± 0.01[b]

*The force expressed in g necessary to penetrate 10 mm of the gel with a standard 0.5 inch cylinder probe
Notes: Different superscript in the same row showed statistically difference ($p < 0.05$)
(-): no gelling effect was observed

The ability of pectin to form gel is apparently also influenced by the heat treatment. Untreated durian-rind pectin showed a firmer gel strength compared to citrus-peel pectin. However, both pectin lost their ability to form a gel after sterilization, and turned to a viscous solution. This result is consistent with the finding reported by Munarin et al. (2013) that also observed a decrease in pectin viscosity after treatment with moist-heat sterilization (121 °C, 2 atm, 15 min). Heat treatment has been known to cause severe effect on the functional properties of pectin and induce pectin depolymerization. Breakdown of the pectin chain was responsible for the decrease of the pectin's rheological properties (Munarin et al. 2013). Gel strength analysis proved that depolymerization occurred during moist heat sterilization, generating low molecular weight pectin which was expected to have better antibacterial activity that the untreated pectin. Although moist-heat sterilized pectin could not be used as a gelling agent, the pectin could still be useful for other applications such as food thickener.

The decrease of pH was observed in both durian-rind pectin and citrus-peel pectin after sterilization. A previous study by Munarin et al. (2013) found that the pH of 6% pectin after moist heat treatment ranged from 2.7 to 3.4. Our current finding was still within the pH range observed by Munarin et al. (2013). Breakdown of the pectin chain into its galacturonic acid monomer might be responsible for the decrease of pH.

FTIR ANALYSIS

Spectroscopic analysis was performed to confirm structural change after moist-heat sterilization process. All of the important bands to characterize pectin, which include bands appearing at 3600 - 3000 (OH), 1743 (C=O), 1640 (C-O), 1146 (C-O glycosidic), 1100 - 1103 (C-O), and 1017 - 1019 (C-O glycosidic) cm^{-1} (Bichara et al. 2016; Szymanska-Chargot & Zdunek 2013) were detected on the untreated pectin (Table 3). Sterilized pectin did not show bands at 2930 - 2950, 1745 - 1740, 960, and 833 cm^{-1}. The same pattern was detected on both durian-rind pectin and citrus-peel pectin (Figure 1), demonstrating that the same reaction occurred during sterilization regardless of the pectin source.

Additionally, some new bands were observed in the region lower than 600 cm^{-1} which were not observed on the pectin before sterilization. Most of the changes appeared at the fingerprint region (1400 - 400 cm^{-1}). Therefore, it was difficult to assign the peaks to a specific functional group due to spectrum overlap (Gnanasambandam & Proctor 2000). FTIR spectra of durian-rind pectin showed a similar profile as standard citrus peel pectin before and after sterilization treatment.

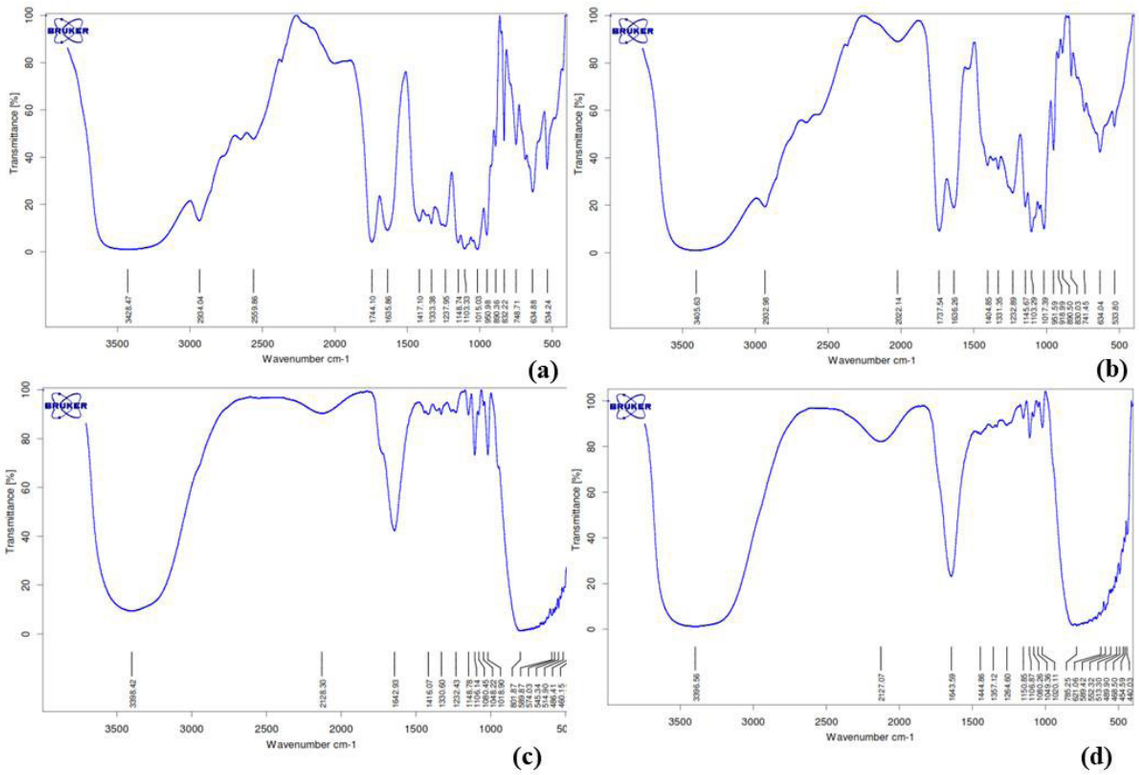

FIGURE 1. FTIR spectra of citrus-peel pectin (a, c) and durian-rind pectin (b, d) before and after sterilization process, respectively

Loss of band at approximately 2950 cm^{-1} (C-H) could point to depolymerization during thermal treatment (Muñoz-Almagro et al. 2017). Absorption bands at 2950 cm^{-1} is assigned to C-H linkages, including –CH, $–CH_2$, and $–CH_3$. Loss of this absorption band at sterilized pectin suggested that breakage of carbon backbone had taken place. Ester hydrolysis and formation of free carboxylate group were indicated by the loss of bands appearing at 1760 - 1745 cm^{-1} (Gnanasambandam & Proctor 2000), thus, lowering the DE value of treated pectin. This interpretation agreed with conventional titrimetric analysis, where the DE value was lower for sterilized durian-rind pectin. It could be concluded that sterilization-treated pectin underwent depolymerization and de-esterification, which formed low molecular pectin with low DE.

The general term of pectin refers to the polymer consisted mainly of glycosidc-linked α-1,4-D-galacturonic acids which are partially methyl esterified at C-6. The observed loss of several absorption bands in sterilized pectin indicated that depolymerization and de-esterification occurred simultaneously during sterilization process. Pectin is known to be susceptible of thermal process (Munarin et al. 2013; Shpigelman et al. 2014). During sterilization process, pectin is subjected to both high temperature and high pressure which promote cleavage of glycosidic bonds and release of methyl esters from the galacturonic acid residues. Thermal process induces degradation through various mechanisms, including de-esterification and backbone hydrolysis via β-elimination and acidic hydrolysis reaction (Diaz et al 2007; Fraeye et al. 2007). Breakdown of pectin backbone resulted in shorter chains of α-1,4-galacturonic acids, while release of methyl esters exposed the carboxyl groups attached to C6 and lowered the initial DE value. These changes in pectin structure are expected to increase the antibacterial activity of pectin according to previous study (Li et al. 2016; Wu et al. 2014).

TABLE 3. Assigned functional groups of pectin

Observed wavenumber (cm^{-1})				Wavenumber of references (cm^{-1})*	Assigned functional groups
Durian rind pectin		Citrus peel pectin			
Before sterilization	After sterilization	Before sterilization	After sterilization		
3405.63	3396.56	3428.47	3398.42	3600-3000	O-H *stretching*
2932.28	-	2934.04	-	2950	C-H *absorption*
1737.54	-	1744.10	-	1745-1740	C=O *stretching vibration of alkyl ester*
1636.26	1643.59	1635.86	1642.93	1630-1600	COO^- *antisymmetric stretching*
1404.85	1444.86	1417.10	1416.07	1400	COO^- *symmetric stretching*
1331.35	1357.12	1333.38	1330.60	1320	*Ring vibration*
1232.89	1264.60	1237.95	1232.43	1243	C-O *stretching*
1145.67	1150.85	1148.74	1148.78	1146	O-C-O *asymmetric stretching*
1103.29	1106.87	1103.38	1106.14	1100	C-O *stretching*, C-C *stretching*
1017.39	1020.11	1015.03	1080.45	1019	C-O *stretching*, C-C *stretching*
951.59	-	950.98	-	960	CO *bending*
830.03	-	832.22	-	833	*Ring vibration*
741.45	785.25	748.71	801.87	-	-
634.04	621.06	634.88	589.87	-	-
533.80	589.42	534.24	574.03	-	-
-	552.32	-	545.34	-	-
-	513.30	-	514.90	-	-
-	489.90	-	486.41	-	-
-	468.50	-	460.15	-	-
-	454.59	-	444.25	-	-
-	440.03	-	415.18	-	-

assigned according to Szymanska-Chargot and Zdunek (2013) comparison to distinguish pectin from other cell wall materials of pumpkin, celery, carrot, tomato, and potato obtained using hot alcohol insoluble solids (CMW residue) and fractioned with trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA residue)

ANTIBACTERIAL ACTIVITY OF DURIAN RIND PECTIN

Antibacterial activity of plant extracts is often attributed to the presence of phenolic compounds. However, bioactive plant polysaccharides might also play a role. The untreated durian-rind pectin and citrus-peel pectin showed a weak antibacterial activity when they were exposed to initial bacterial load of 3 log cfu/mL (Table 4) as well as to 5 log cfu/mL (Table 5). However, after sterilization process, the durian-rind pectin showed a strong antibacterial activity, which can reduce the number of *S. aureus* up to 5 log cycles and *E. coli* up to 3 log cycles after 6 h of exposures (Table 5). Hence, sterilization treatment greatly increased the antibacterial activity of pectin (p-value < 0.05).

TABLE 4. Antibacterial activity of pectin exposed to 3 log cfu/mL bacteria

Bacteria	Initial bacteria (log cfu/mL)	Time (hours)	Surviving bacteria (log cfu/mL) after exposure to			
			Durian-rind pectin		Citrus-peel pectin	
			100 mg/mL	50 mg/mL	100 mg/mL	50 mg/mL
Before sterilization						
S. aureus	3.19 ± 0.07	1	2.53 ± 0.01	2.63 ± 0.06	2.52 ± 0.04	2.62 ± 0.02
		2	2.61 ± 0.06	2.70 ± 0.08	2.54 ± 0.05	2.66 ± 0.03
		3	2.63 ± 0.04	2.70 ± 0.09	2.60 ± 0.05	2.67 ± 0.01
		6	3.53 ± 0.02	3.62 ± 0.08	2.66 ± 0.05	2.71 ± 0.02
E. coli	3.40 ± 0.25	1	2.42 ± 0.17	2.65 ± 0.01	3.36 ± 0.16	3.51 ± 0.20
		2	2.58 ± 0.07	2.62 ± 0.10	3.38 ± 0.12	3.51 ± 0.20
		3	2.58 ± 0.10	2.61 ± 0.11	3.45 ± 0.17	3.55 ± 0.14
		6	3.53 ± 0.01	3.47 ± 0.24	3.48 ± 0.21	3.73 ± 0.01
After sterilization						
S. aureus	3.35 ± 0.34	1	< 1.00	< 1.00	< 1.00	< 1.00
		2	< 1.00	< 1.00	< 1.00	< 1.00
		3	< 1.00	< 1.00	< 1.00	< 1.00
		6	< 1.00	< 1.00	< 1.00	< 1.00
E. coli	3.68 ± 0.36	1	2.61 ± 0.23	3.11 ± 0.27	< 1.00	< 1.00
		2	1.49 ± 0.85	2.86 ± 0.33	< 1.00	< 1.00
		3	< 1.00	1.99 ± 0.86	< 1.00	< 1.00
		6	< 1.00	< 1.00	< 1.00	< 1.00

TABLE 5. Antibacterial activity of pectin exposed to 5 log cfu/mL bacteria

Bacteria	Initial bacteria (log cfu/mL)	Time (hours)	Surviving bacteria (log cfu/mL) after exposure to			
			Durian-rind pectin		Citrus-peel pectin	
			100 mg/mL	50 mg/mL	100 mg/mL	50 mg/mL
Before sterilization						
S. aureus	5.24 ± 0.14	1	4.06 ± 0.02	4.10 ± 0.03	3.55 ± 0.03	3.61 ± 0.02
		2	4.10 ± 0.04	4.13 ± 0.02	3.60 ± 0.02	3.68 ± 0.03
		3	4.17 ± 0.04	4.23 ± 0.04	3.65 ± 0.06	3.74 ± 0.06
		6	4.25 ± 0.02	4.30 ± 0.02	3.71 ± 0.08	3.80 ± 0.03
E. coli	5.41 ± 0.33	1	4.41 ± 0.16	4.51 ± 0.23	4.15 ± 0.26	4.17 ± 0.19
		2	4.46 ± 0.17	4.58 ± 0.23	4.17 ± 0.21	4.20 ± 0.22
		3	4.54 ± 0.14	4.65 ± 0.16	4.21 ± 0.09	4.30 ± 0.15
		6	4.61 ± 0.18	4.72 ± 0.23	5.00 ± 0.04	5.05 ± 0.07
After sterilization						
S. aureus	5.11 ± 0.14	1	< 1.00	< 1.00	< 1.00	< 1.00
		2	< 1.00	< 1.00	< 1.00	< 1.00
		3	< 1.00	< 1.00	< 1.00	< 1.00
		6	< 1.00	< 1.00	< 1.00	< 1.00
E. coli	5.35 ± 0.32	1	5.27 ± 0.25	5.37 ± 0.34	4.76 ± 0.19	4.99 ± 0.42
		2	4.95 ± 0.70	5.00 ± 0.69	4.72 ± 0.19	4.96 ± 0.43
		3	3.71 ± 0.30	4.53 ± 0.41	4.57 ± 0.04	4.92 ± 0.45
		6	2.00 ± 1.73	2.02 ± 1.77	4.36 ± 0.28	4.80 ± 0.32

There was no statistically different found between the antibacterial activity of durian-rind pectin and citrus-peel pectin (p-value = 0.691). The antibacterial activity was also not significantly affected by the pectin concentration of 50 mg/mL and 100 mg/mL (p-value = 0.343). Furthermore, *S. aureus* appeared to be more susceptible to the exposure to pectin in comparison to *E. coli*. No growth of *S. aureus* was observed since the first hour after exposure, at moderate- as well as at high initial load. For *E. coli*, a longer time is needed to significantly reduce the surviving bacteria, especially at a high initial load (5 log cfu/mL). This finding is consistent with previous research using untreated pectin (Lipipun, Nantawanit & Pongsamart 2002; Pongsamart et al. 2005). Moreover, a previous study by Lipipun, Nantawanit and Pongsamart (2002) also showed that *S. aureus* was more susceptible to the addition of pectin compared to *E. coli*. Thunyakipisal et al.

(2010) showed that the survivability of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* against untreated durian-rind pectin depended on the exposure time and concentration. The Gram negative *A. actinomycetemcomitams* had better resistance against pectin compared to the Gram positive *S. mutans*. This observation showed that Gram negative bacteria most likely tolerated pectin better than Gram positive bacteria.

Our present study suggests that moist heat sterilization results in a better antimicrobial activity of durian-rind pectin. The reduced pH stimulated by sterilization seemed to be an important factor for decreasing the viability of the tested bacteria. Heat-treated durian-rind pectin had a lower DE value than untreated pectin (Table 2), which indicated a higher presence of the free carboxyl group. Introduction of carboxyl groups via oxidation reaction has been employed to synthesis oxidized polymers capable of inhibiting various Gram-negative and Gram-positive bacteria (Köllnberger, Schrader & Briehn 2020; Spangler et al. 2003; Zhu et al. 2017; Zi et al. 2018). Furthermore, the low pH conditions limited the capabilities of bacteria to grow or caused direct damage to the bacterial cell wall. The mechanisms to inhibit bacterial growth by pH effect are unspecific. Therefore, it is unlikely that bacteria will develop resistance (Köllnberger, Schrader & Briehn 2020; Spangler et al. 2003).

Uronic acids, such as galacturonic acid, are a group of sugars which, in addition to carbonyl groups, also have carboxylic acid as functional groups. Stalheim et al. (2009) proposed the pH lowering effect to be the general mechanism for the observed antibacterial activity of uronic acid-containing carbohydrates. Antibacterial activity was also reported from other uronic-acid containing and depolymerized polysaccharides, such as alginate and fucoidan (Ashayerizadeh, Dastar & Pourashouri 2020; Hu et al. 2005; Liu et al. 2017; Saravana et al. 2018). Liu et al. (2017) observed that the most active fucoidan fractions against bacteria also had the highest uronic acid content than the other factions. The presence of uronic acid might contribute to the antibacterial activity of certain polysaccharides, including pectin.

It could not be denied that the presence of phenolic compounds could also contribute to the observed antibacterial activity, especially for the untreated pectin. Phenolic compounds are often found bound tightly to pectin that they were often co-extracted during the extraction of pectin (Schieber et al. 2003; Wikiera et al. 2021). The presence of oxidized phenolic gives the pectin a brown hue. Wikiera et al. (2021) showed that extracted pectin could retain up from 27 to 43% of the phenolic compounds. The content of recovered phenolic compounds is lower with harsher extraction condition. Acid-assisted conventional extraction resulted in lower phenolic content compared to enzymatic-assisted extraction method. Identification of phenolic compounds of durian rind showed the presence of derivatives from several different groups, including coumarin, benzoic acid, and cinnamic acid (Feng et al. 2016). Several studies stated that these groups of phenolic have antibacterial activity (Smyth, Ramachandran & Smyth 2009; Tonari, Mitsui & Yonemoto 2002). However, another study by Vodnar et al. (2017) observed elimination of antibacterial activity of carrot wastes after heat processing (80 °C for 10 min) which was attributed to the reduction of cinnamic acid due to thermal degradation. Depending on the structure, phenolic compounds have different stability to heat treatment. It is likely that the phenolic compounds retained in the sterilized pectin underwent thermal degradation and no longer possessed antibacterial capabilities after repeated cycles of heating (drying, solvent evaporation during pectin extraction, and moist heat treatment). Therefore, the improved antibacterial activity of sterilized pectin is attributed to pectin with lower DE and MW.

Conclusion

This study evaluated the possible application of commercial sterilization (121 °C for 15 min) to enhance the antibacterial potential of durian-rind pectin. The moist heat sterilization condition was able to decrease the DE and MW of pectin, generating pectin degradation products with improved ability to inhibit the growth of *S. aureus* and *E. coli*, compared to untreated pectin. Reduction of pH was observed and FTIR analysis suggested that free carboxylate was generated after sterilization. These results indicated that the sterilized pectin had a higher presence of carboxylate which lowered the pH of media and created an environment that limit the growth of bacteria. However, the harsh thermal treatment diminished durian-rind pectin's ability to form a gel. Therefore, the sterilized pectin could no longer be applied as gelling agent. This current research reports that the sterilized durian-rind pectin showed potency as an antibacterial agent. Further study is needed to evaluate its application in the food system.

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