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Physical, Mechanical, Barrier, Antibacterial Properties, and Functional Group of Carrageenan-based Edible Film as Influenced by Pectin from *Dillenia serrata* Fruit Peel and Curcumin

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Abstract

The use of carrageenan-based edible film has increased since it can be functionalized with other biopolymers and active ingredients. *Dillenia serrata* peel pectin and curcumin were mixed at various proportions to form a carrageenan-based edible film by casting method. In this work, the main objectives are to utilize the peel of *Dillenia serrata* fruit as a source of pectin combined with curcumin for carrageenan-based edible film production and to assess the effect of various concentrations of pectin and curcumin on the physical, mechanical, barrier, antibacterial properties, and functional group of films. Nine carrageenan-based edible films produced by the casting method were designed on the basis of a complete factorial design with three concentrations of pectin and curcumin. Tensile strength, thickness, WVTR, swelling, colour, antibacterial activity, and FTIR analysis were measured. The results revealed that the concentration of pectin significantly influenced the thickness, WVTR, and swelling, while the addition of curcumin presents significantly influenced the WVTR and colour of films. The edible film containing high pectin and curcumin gave the lowest thickness and WVTR. All films showed a lower inhibitory zone for *Escherichia coli* than *Staphylococcus aureus* when higher curcumin was incorporated into the biopolymer matrix. FTIR analysis revealed that curcumin can be used along with *Dillenia serrata* pectin to form a good-quality carrageenan-based edible film. These findings suggested that carrageenan-based edible film with addition of pectin and curcumin improved overall performance. This approach can be a good

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strategy to encourage sustainable utilization of endemic fruit wastes (*Dillenia serrata* fruit peel) for development of edible packaging film.

Introduction

The success of edible film manufacturing as packaging is determined by the proper biopolymer used, the type and concentration of plasticizer added, and the solvent used.[1] Much emphasis has been given to the production of edible film based on fruit by-products such as peel. Fruit peel contains pectin, a polysaccharide that contains D-galacturonic acid through the α-1,4-glycosidic bond,[2] has the ability to form edible film and possesses active compound carrier properties. Fruit peel has been utilized for decades due to its composition's rich nutrients, making it a possible source for edible packaging in various food products. It is estimated that 25-30% of total fruit and vegetable production is food waste, including peels, pomace, rind, and seeds.[3-4] Many industrial products derived from peel waste include microbiological media, fortified probiotics, green nanoparticles, carbon quantum dots, and edible film/coating.[3]

One of the fruit peels that can be used as material for edible film fabrication is *Dillenia serrata* fruit. This fruit is produced by the endemic *Dillenia serrata* Thunb. plant, which is common in the Sulawesi region, Indonesia. The residents of Sulawesi, particularly those in South and Central Sulawesi, are familiar with this species. People in South Sulawesi refer to this fruit as Dengen. *Dillenia serrata* fruit is similar to orange fruit but has a very sour taste; hence, locals do not cultivate it, but this fruit has antioxidant activity that can be used to inhibit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans* bacteria.[5] In addition, *Dillenia serrata* contains koetjapic, betulinic acids, and 3-oxoolean-12-en-30-oic that can inhibit the production of prostaglandin E2 (PGE2).[6]

Pectin is commonly found in plant main cell walls, particularly in the gap between cellulose and hemicellulose. Pectin is used as a raw material for edible film because it has some advantages, such as good biodegradation performance, biocompatibility and non-toxicity.[7] The most commonly used biopolymer in the production of edible films is pectin, which is extracted from a variety of sources including orange peel,[1] pomelo peel,[8] pumpkin waste,[9] and mulberry leaves.[10]

The disadvantage of edible films manufactured from hydrocolloids is that they deteriorate quickly due to microbial decomposition. Packaging containing antimicrobial chemicals is required to prolong the shelf life of packaged product.[11] Curcumin is one of the few naturally occurring antibacterial compounds. Curcumin is an antibacterial and antiviral agent that can inhibit a broad spectrum of gram-negative and gram-positive bacteria.[12] Curcumin is a phenolic molecule that can limit bacterial growth by denaturing and destroying cell membranes, hence disrupting metabolic activities. Furthermore, the addition of curcumin to edible film reveals antioxidant characteristics as well as free radical scavenging and reduction capability,[13] while the addition of curcumin to edible coating prevents oxidation of cheese during storage.[14] Moreover, the inclusion of curcumin can boost the tensile strength and thermal stability of the resultant film.[15] Some authors reported that emulsion-based systems and the type of carrier oil employed in emulsion formulation affect curcumin's stability, bioavailability and bioaccessibility.[16] Curcumin is highly effective at inhibiting or killing pathogenic/rotting bacteria that cause food contamination. It can minimize the production of unpleasant flavours due to the direct inclusion of active components in food.[12]

The characterization of carrageenan-based edible film influenced by polysaccharide and bioactive compounds has been known to affect the quality of the film and coating. To the best of our knowledge, no investigations have been conducted on the physical, mechanical, barrier, antibacterial activity, and functional group of carrageenan-based edible film as influenced by pectin from *Dillenia serrata* fruit peel subjected to the addition of curcumin. In addition, there is a growing demand for the utilization of fruit peels as materials for edible packaging. Thus, the characterization of the physical, mechanical, barrier, antibacterial activity, and functional group of carrageenan-based edible film as influenced by pectin from *Dillenia serrata* fruit peel and curcumin

was conducted in this study. The aims of this study are: (a) to utilize the peel of *Dillenia serrata* fruit as a source of pectin loaded with curcumin for carrageenan-based edible film production, (b) to assess the effect of various concentrations of pectin and curcumin on the physical, mechanical, barrier, and antibacterial activity of films as well as their functional group. To reach this goal, nine carrageenan-based edible films, corresponding to a complete factorial design based on three concentrations of pectin, were produced and loaded with three levels of curcumin concentration.

Materials and Methods

Materials

Dillenia serrata fruits were obtained from Soroako, a small mining town located in East Luwu Regency, South Sulawesi province. Kappa-carrageenan, glycerol, and curcumin used in this work were of food-grade quality. The chemical reagents used were analytical reagent grade.

Preparation of Sample

The fruits of *Dillenia serrata* were properly rinsed with tap water. The fruits were then peeled and the peels were rinsed under running water. The rinsed peels were dried for ten hours at 55°C. Finally, the dried peels were pulverized into powder with a blender and sieved through a 100-mesh sieve to facilitate the extraction of the pectin. The obtained peel powder was stored until used for pectin extraction.

Extraction of Pectin from *Dillenia Serrata* Fruit Peel

Pectin was extracted according to previous method with slight modification.[17] 10 g of fruit peel was prepared and added with 150 ml of distilled water. 2 M HCl was added into the mixture to obtain a pH of 2.0 and mixed, then heated at 70°C for 60 min until it was homogenous. The filtrate was filtered, and then 150 mL of ethanol was added and allowed in a temperature room for 60 min. The precipitated pectin was immediately washed with ethanol (1:2) and then centrifugated at 1500 rpm for 30 min. The obtained pectin was heated at 50°C for 24 hours. Finally, the dried pectin was crushed and then passed through a ten-mesh filter.

Preparation of Edible Film

For this study, nine edible films with various pectin and curcumin concentrations were created using a complete factorial design based on two factors: the pectin concentration level (P0=0%; P1=1%; P2=2%) and the curcumin concentration level (C0=0%; C1=0.5%; C2=1%). Pectin, kappa carrageenan, curcumin, and glycerol were utilized to make the edible film. These materials are all of food-grade quality.

To make carrageenan edible films, pectin was dissolved in warm water (60°C) for 5 min with a hot plate stirrer, followed by the addition of carrageenan. Curcumin and glycerol were effectively combined and mixed for 5 min at 60°C on high speed. Finally, the solution was homogenized for 3 min at a speed of 24.000 rpm using an ultra-turrax. Following that, 20 ml of film-forming solution was placed into a petri dish and dried at 60°C oven for 24 hours. After drying, the films were settled in a desiccator to keep the temperature and humidity consistent. Then, the physical, mechanical and barrier properties of the edible films were measured, including tensile strength, thickness, water vapour transmission rate (WVTR), swelling, colour, antibacterial activity, and FTIR analysis.

Film Characterization

Tensile Strength

Determination of film tensile strength was obtained according to previous method with slight modification.[18] Characterization of films was obtained by using a Testometric Materials Testing Machine. The edible film was cut into square pieces with 20 x 50 mm dimensions. The testing equipment clamps the sample. The tool pulls the samples at a speed of 100 mm/min until the samples break. The film tensile strength was calculated by the following equation, where τ indicated tensile strength (MPa), Fmax was voltage (N), and A showed broad cross-section transverse (mm^2).

$$\tau = Fmax/(A)$$

Film Thickness

The film thickness was carried out with a digital calliper to the nearest 0.01 mm at five random positions. The thickness values are the means of five measurements chosen at random from each evaluated sample.[19]

Water Vapor Transmision Rate (WVTR)

The WVTR of film was calculated according to previous method.[20] A test cup was filled with 10 mL of distilled water. With a 3 cm diameter and without touching the water, the film was cut into a circle and placed on the test cup's mouth. The system's weight (test cup+ water+ film) was measured every 24 hours for six days while it was kept in a desiccator containing silica gel. The WVTR was expressed using the following equation, where ΔW is the amount of water absorbed by silica gel as a function of the time, A is the area of the film (m^2), being the slope (ΔW/Δt) of each line determined by linear regression (R^2> 0.99).

$$WVTR=\Delta W/(\Delta t.A)$$

Swelling

The swelling of films was obtained according to the previous method with slight modification.[21] A film with 20 mm × 20 mm was weighed as dry weight (Wi) and it was afterwards submerged in distilled water for 10 seconds. The film was taken off, and any residual water was wiped off with filter paper before being weighed once again as wet weight (Wf). The following equation was used to determine the degree of swelling:

$$Swelling\ (\%)= (wf-wi)/(wi)\times 100$$

Colour

A Minolta CR 300-chromameter was employed to examine the colour of films. Then, the colour reader was turned on using the L, a, b system. The testing was carried out by locating sensors on the surface of films and firing rays at two different par. For each section, measurements were taken three times. The collected data were then averaged.

Antibacterial Activity

The antibacterial activity of edible film was tested to identify the concentration of curcumin that could inhibit the growth of spoilage bacteria (*Escherichia coli* and *Staphylococcus aureus*) in the product. The diameter of the inhibition zone created by the disk diffusion method was measured during the test. The cut film fragments were placed on MHA (Müller-Hinton agar) media that had been inoculated with 0.1 ml of bacterial suspension containing 106 CFU/ml. Petri dishes were then incubated at 35±2°C for 24 hours. After the incubation period, the inhibition zone will appear, and the diameter of the inhibition zone will be measured with a calliper. The inhibitory zone's diameter was determined as the diameter of the produced clear zone (including the diameter of the edible film). This measurements was done in duplicate.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis (QATR-S, Shimadzu, Japan) was used to characterize the films. The scan range was 4000 cm^{-1} to 400 cm^{-1}, and 32 scans with a resolution of 2 cm^{-1} were established. The edible films were located in crystal cells and mounted on the FTIR analyzer.

Data Analysis

Data obtained in this study were analyses using R software (release 4.1.0, 2021). For the physical, mechanical, barrier characteristics, and antibacterial activity of films, ANOVA was done using the stats package's lmtest function. The effects and differences were considered significant for all data analyses for all data analyses when $p < 0.05$. In addition, principal component analysis (PCA) of function of the FactoMineR package (version 2.4) was also carried out to evaluate the similarity and variation of physical, mechanical, barrier properties, and antibacterial activity between samples.

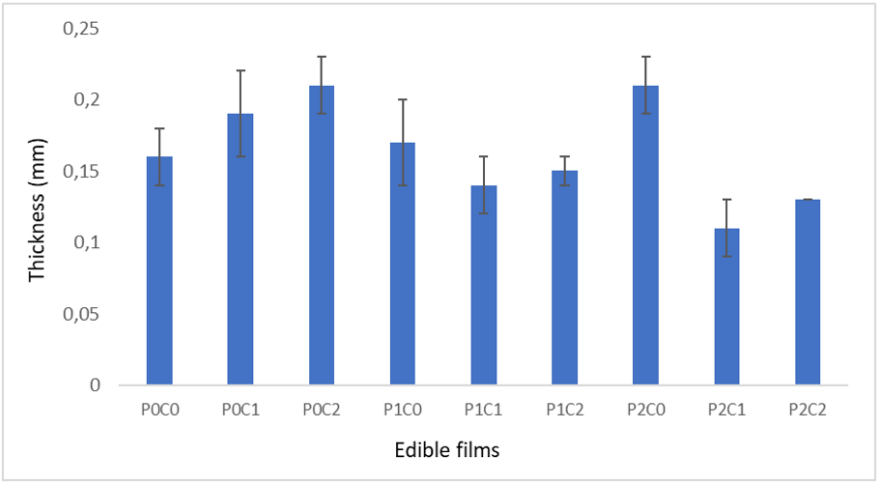
Results and discussion

Physical, Mechanical, and Barrier Properties

A series of ANOVAs were carried out on each parameter with the pectin and curcumin as the fixed factors in order to further study the impact of composition elements on the physical, mechanical, barrier characteristics, and antibacterial activity of edible films.

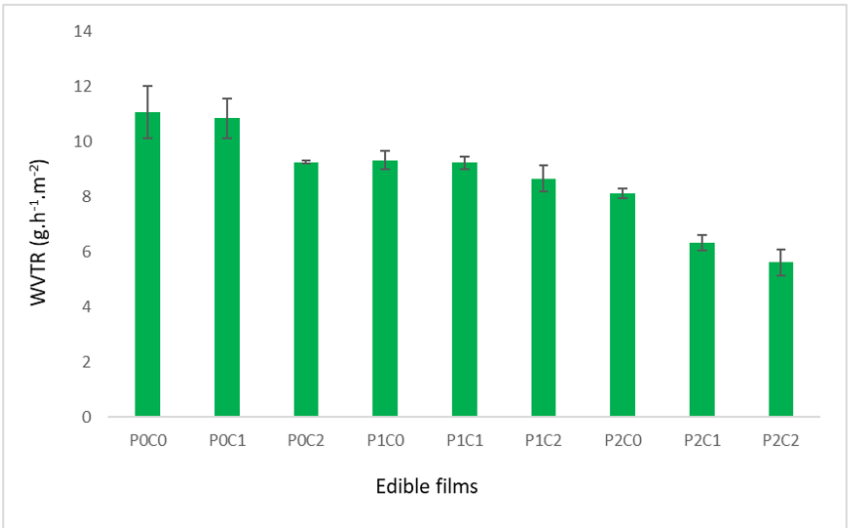
The mean values of thickness of the films were between 0.11 to 0.21 mm showing edible films without pectin and higher curcumin (P0C2) and films with pectin and without curcumin (P2C0) were found to be thicker, as seen in Fig. 1. A two-way ANOVA (pectin, curcumin) on thickness revealed a significant effect of pectin ($F(1;23)=6.36$, $p=0.019$) and interaction between pectin and curcumin ($F(1;23)=16.13$, $p<0.0001$). However, curcumin was not significant in thickness ($P>0.05$), as shown in Table 2. This phenomenon occurs because a higher amount of added pectin can enhance the polymer

content forming the film matrix. Consequently, the total solids content in the edible film increases, thus affecting its thickness. This aligns with the statement by some authors who suggest that increasing the concentration of the constituent polymer up to a certain limit can enhance both the thickness and stability of the edible film.[22] Curcumin can interact with pectin, which can change the structure or texture of the film. This interaction may cause a change in film thickness due to the absorption of curcumin into the film matrix. When curcumin interacts with components of pectin, certain chemical reactions may occur. These reactions can change the properties of the film base material and affect the final film thickness.[15]



P0C0 (EF without pectin and curcumin)
P0C1 (EF without pectin : curcumin 0.5%)
P0C2 (EF without pectin : curcumin 1%)
P1C0 (EF with pectin 1% : without curcumin)
P1C1 (EF with pectin 1% : curcumin 0.5%)
P1C2 (EF with pectin 1% : curcumin 1%)
P2C0 (EF with pectin 2% : without curcumin)
P2C1 (EF with pectin 2% : curcumin 0.5%)
P2C2 (EF with pectin 2% : curcumin 1%)

Fig. 1. Thickness of edible films with various of pectin and curcumin concentration



P0C0 (EF without pectin and curcumin)
P0C1 (EF without pectin : curcumin 0.5%)
P0C2 (EF without pectin : curcumin 1%)
P1C0 (EF with pectin 1% : without curcumin)
P1C1 (EF with pectin 1% : curcumin 0.5%)
P1C2 (EF with pectin 1% : curcumin 1%)
P2C0 (EF with pectin 2% : without curcumin)
P2C1 (EF with pectin 2% : curcumin 0.5%)
P2C2 (EF with pectin 2% : curcumin 1%)

Fig. 2. WVTR values of edible films with various pectin and curcumin concentration

The mean of the WVTR value on each edible film at varied pectin and curcumin concentrations can be seen in Fig. 2. The WVTR value range between 5.61 g.h-1.m-2 to 11.07 g.h-1.m-2. The analysis of variance revealed a significant effect of pectin concentration ($F(1;23)=143.30$, $p<0.0001$) and curcumin concentration ($F(1;23)=28.97$, $p<0.0001$) on the WVTR. However, it was not observed in their interaction ($p>0.05$), as seen in Table 2. As pectin and curcumin concentrations were added, there was a trend for the WVTR of edible film to drop. These findings suggest that pectin and curcumin tend to decrease the rate of water vapour transport. This result was comparable to curcumin and sulfur nanoparticles added to pectin films[23] and kappa carrageenan incorporated with curcumin.[15] It could be due to the presence of long carbon chain and hydrophobic benzene ring of curcumin, which can lower the film's affinity for water vapour by blocking the internal network of pectin.[23] The other reason was that the hydrophobicity of curcumin promotes poor dispersion, which agglomerates it and affects the compact structure of the film.[24] This could be due to hydrophobic interactions and the creation of hydrogen bonds, which limit the number of free OH groups that can interact with the water in the edible film.[25] Moreover, the addition of pectin into the film-forming solution improves intermolecular contact between polymers, resulting in the production of a denser film matrix, presenting lower water vapour permeability because it is difficult to penetrate the formed film matrix.[8]

Table 1: Tensile strength, swelling, and colour properties of films

Edible films	Tensile Strength (N/mm^2)	Swelling (%)	L	a*	b*
P0C0	0.14±0.03	51.40±22.87	51.78±3.97	-7.50±0.65	3.38±0.91
P0C1	0.53±0.08	38.78±2.10	50.69±0.59	-5.80±1.07	14.97±4.17
P0C2	0.03±0.00	21.78±5.40	45.38±3.64	-5.17±1.27	15.56±5.14
P1C0	0.06±0.01	65.99±14.87	55.35±0.32	-7.52±0.29	2.86±0.15
P1C1	0.04±0.01	46.24±19.10	49.36±0.85	-5.14±0.32	17.33±0.83
P1C2	0.04±0.02	67.18±5.68	50.09±1.47	-4.89±0.46	17.72±1.39
P2C0	0.08±0.04	71.42±10.86	54.00±3.03	-7.62±0.47	3.50±0.69
P2C1	0.04±0.00	67.43±35.84	51.83±1.76	-6.66±0.14	9.05±1.10
P2C2	0.03±0.00	66.91±10.58	51.57±0.94	-5.96±0.33	12.64±4.02

The data are displayed with the mean ± standard deviation.
L for lightness, a* and b* for the color-opponent dimensions of redness–greenness and blueness–yellowness.

P0C0 (EF without pectin and curcumin) P1C0 (EF with pectin 1% : without curcumin) P2C0 (EF with pectin 2% : without curcumin)
P0C1 (EF without pectin : curcumin 0.5%) P1C1 (EF with pectin 1% : curcumin 0.5%) P2C1 (EF with pectin 2% : curcumin 0.5%)
P0C2 (EF without pectin : curcumin 1%) P1C2 (EF with pectin 1% : curcumin 1%) P2C2 (EF with pectin 2% : curcumin 1%)

The tensile strength of edible films depends on the type of polymer matrix and additive compounds employed[26] and reveals whether a film can withstand external pressure and maintain its integrity without breaking.[27] In this study, all film samples had tensile strength in the range of 0.03-0.53 N/mm^2 as shown in Table 1. A two-way ANOVA (pectin, curcumin) on thickness revealed no significant difference between pectin, curcumin and their interaction ($P>0.05$), as shown in Table 2. Notably, the tensile strength was lower, regardless of the concentration of pectin and curcumin used (with the exception of edible film without pectin and 0.5% of curcumin, P0C2). The low tensile strength observed might possibly be influenced by the poor compatibility between pectin and curcumin due to the differing polar characteristics of these two compounds. Pectin, being a polysaccharide, tends to be polar (containing polar carboxyl groups), while curcumin is a nonpolar compound. Due to this difference in polarity, curcumin tends to exhibit a lower affinity towards pectin in solution. Pectin

comprises branched polysaccharide chains, and these branches contribute to the susceptibility of inter-chain bonds within the polysaccharide to break. The amorphous nature of pectin results in significant void spaces, limiting the overall mass density of the fruit peel's pectin.[2] Consequently, this leads to an insufficiently compact structure in the resulting edible film, ultimately causing the low tensile strength observed in the films.

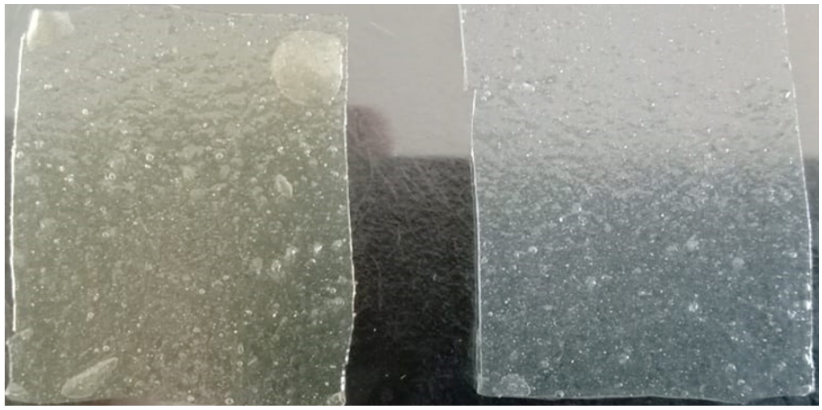
Table 2: ANOVA results for carrageenan-based edible film as influenced by pectin and curcumin. Light grey cells denote the absence of significant effects (p>0.05), while blue cells imply significance at p<0.05

		Thickness strength	Tensile	WVTR	Swelling	L	a*	b*
Pectin level	F(1;23)	6.36	1.77	143.30	14.91	2.88	2.65	2.45
	p-value	0.019	0.196	<.0001	<.0001	0.103	0.117	0.131
Curcumin level	F(1;23)	0.57	0.184	28.97	1.84	9.47	37.92	42.24
	p-value	0.456	0.672	<.0001	0.188	0.005	<.0001	<.0001
Pectin*Curcumin	F(1;23)	16.13	0.32	0.84	1.60	0.00	0.58	0.45
	p-value	<.0001	0.86	0.37	0.218	0.990	0.455	

The swelling of films with different concentrations of pectin and curcumin is shown in Table 1. To assess the influence of pectin and curcumin on swelling capacity, we also carried out a two-way ANOVA. The results showed a significant effect of pectin concentration ($F(1;23)=14.91$, $p<0.0001$). However, curcumin and interaction between pectin and curcumin were not significant ($p>0.05$), as shown in Table 2. The higher the concentration of pectin applied, the more swelling is created. The swelling of edible film without pectin and curcumin (P0C0) was 51.40% and increased in the edible film prepared with the addition of 2% pectin without curcumin (P2C0), which was 71.42%. This is because pectin can easily bind water molecules and the presence of hydroxyl groups in its structure. The hydration ability of pectin allows it to absorb water, increasing the edible film's swelling capacity. Pectin is amorphous in nature, meaning it does not have a regular crystalline arrangement. This amorphous structure creates a lot of space in the pectin matrix, allowing water to penetrate and be absorbed by the film more easily.[28] Due to the more amorphous nature of pectin, there are numerous voids, resulting in a relatively low bulk density of pectin in fruit peels, which allows for significant water absorption, thus leading to low water resistance. However, the addition of curcumin can increase the bulk density of the edible film, reducing the amount of absorbed water.[1] Without pectin, the edible film did not promote swelling. The pectin-based film matrix can be altered by adding curcumin in particle form. There will be more water absorption sites in the film if the curcumin particles are evenly distributed, increasing the water absorption capacity. Curcumin, a flavonoid molecule, is insoluble in water; as a result, the curcumin particles in the resultant film matrix have a harder time absorbing water, which lowers the edible film's ability to absorb water.[29]

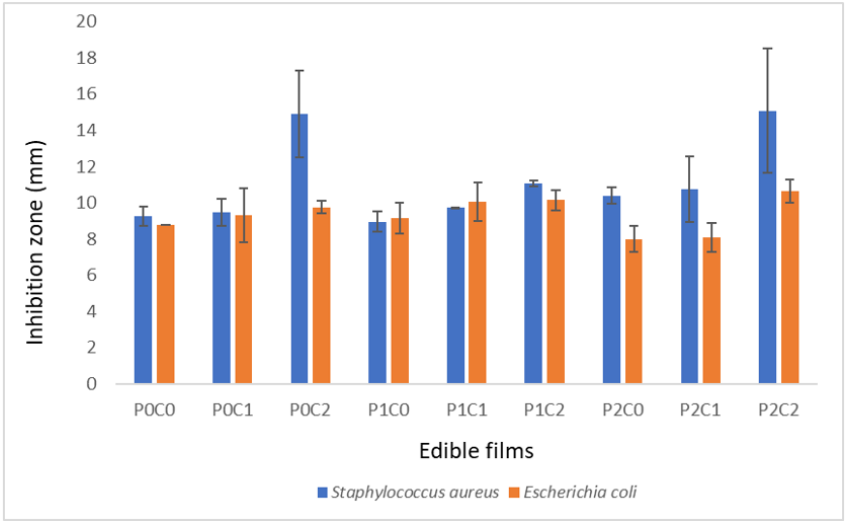
The colour parameters of *Dillenia serrata* pectin films incorporated with curcumin are presented in Table 1. Incorporation of curcumin had significant effect on L values ($F(1;23)=9.47$, $p=0.005$), a values ($F(1;23)=37.92$, $p<0.0001$), and b values ($F(1;23)=42.24$, $p<0.0001$). However, pectin and interaction between pectin and curcumin had no significant ($p>0.05$) on colour parameters, as shown in Table 2. The L* value (lightness/darkness) of edible films decreases with increasing curcumin concentration from 55.35-45.38, respectively. It means that films with higher curcumin addition (P0C2) were darker compared to films without curcumin (P1C0). Higher curcumin concentrations will typically result in a more intense hue, which could make the edible film appear darker.[30] The same results were also shown by a* value (redness/greenness). The a* values of films decreased with the addition of curcumin from -4.89 to -7.62, indicating that films without curcumin (P2C0) tended to be green with low intensity compared to films with higher curcumin addition (P1C2). The addition of pectin with varied solubilities, on the other

hand, can affect the interaction between curcumin and other elements in the film.[31] In contrast, b* values (yellowness/blueness) significantly increased with increasing concentration of curcumin from 3.38-17.72, respectively. Films containing higher curcumin (P1C2) were more yellow than those without curcumin (P1C0). A substance called curcumin has a noticeable yellow-to-orange colour. Curcumin can give the film matrix a yellow or orange when added to edible film components, as seen in Fig. 3. The L* and b* values of this present study were similar to edible zein/shellac composite film loaded with curcumin [32] and kappa carrageenan added with plant essential oil.[33] The darker films produced in this work have certain advantages, such as protecting packaged foods like fresh meat from light exposure.[34] Also, the red and yellow films obtained in this study can enhance the appearance of particular foods, such as baked products.[35]



(A) (B)

Fig. 3. Edible film with curcumin (A) and edible film without curcumin (B)



P0C0 (EF without pectin and curcumin)
P0C1 (EF without pectin : curcumin 0.5%)
P0C2 (EF without pectin : curcumin 1%)
P1C0 (EF with pectin 1% : without curcumin)
P1C1 (EF with pectin 1% : curcumin 0.5%)
P1C2 (EF with pectin 1% : curcumin 1%)
P2C0 (EF with pectin 2% : without curcumin)
P2C1 (EF with pectin 2% : curcumin 0.5%)
P2C2 (EF with pectin 2% : curcumin 1%)

Fig. 4. Inhibition zone (mm) of carrageenan-based edible films containing Dillenia serrata peel pectin and curcumin against different bacteria. The data are displayed with the mean ± standard deviation

Antibacterial Activity

The antibacterial activity of nine edible films tested against bacteria, *Staphylococcus aureus* (gram +) and *Escherichia coli* (gram -), is shown in Fig. 4. As shown, all films presented antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*. As expected, curcumin concentration significantly affected the inhibitory zone of the edible film against *Staphylococcus aureus* (F(1;14)=9.38, P=0.0085) and *Escherichia coli* bacteria (F(1;14)=5.943, P= 0.03). However, the interaction of pectin and curcumin did not significantly affect both *Staphylococcus aureus* and *Escherichia coli* bacteria (P>0.05), as seen in Table 3. The inhibitory zone formed increases with curcumin concentration. The curcumin-loaded *Dillenia serrata* pectin films inhibited the growth of both bacteria, indicating that the diameter of the inhibitory zone formed against *Staphylococcus aureus* bacteria is bigger than that produced against *Escherichia coli* bacteria. The best treatment for *Staphylococcus aureus* bacteria is P2C2, which has an inhibition zone diameter value of 15.08 mm, while the treatment with the lowest clear zone, P0C0, has a value of 9.25 mm, showing that curcumin has an antibacterial function. Some authors reported that curcumin has a higher sensitivity to Gram-positive than Gram-negative bacteria[36 15] This result was contrary to [23] who found that pectin added with curcumin was more effective against the Gram-negative (*E. coli*) than the Gram-positive (L. monocytogenes) bacteria. This is because gram-positive and gram-negative bacteria have different wall structures and composition. Peptidoglycan layers that form a thick and stiff structure make up the majority of the cell walls of gram-positive bacteria like *S. aureus* and *Streptococcus sp*.[37] However, Gram-positive bacteria's cells are surrounded by a thick peptidoglycan layer containing an additional class of lipoteichoic acids (LTAs), but they lack an outer membrane (OM) [36 38] so the bacterial cell wall can be damaged by curcumin attaching to the peptidoglycan, leading to bacterial cell lysis.[39]

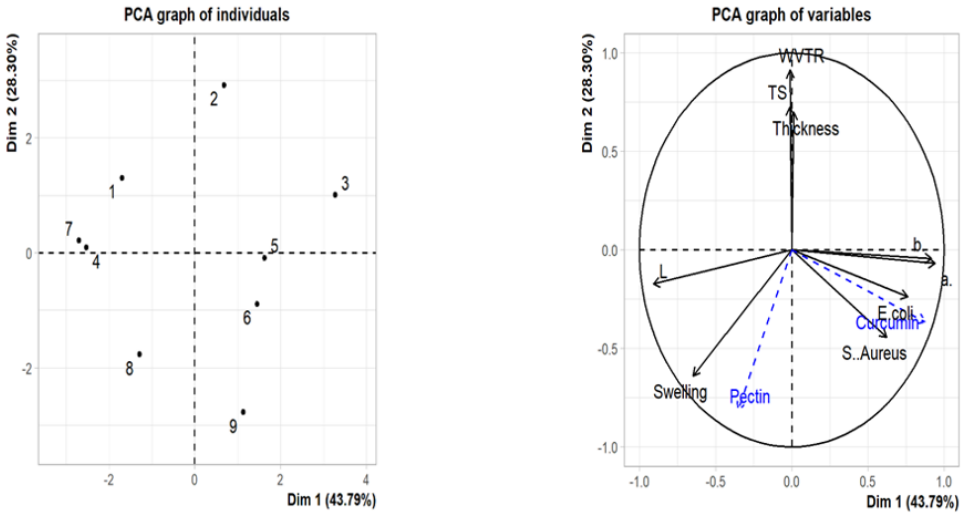
Table 3. ANOVA results for carrageenan-based edible film as influenced by pectin and curcumin. Light grey cells denote the absence of significant effects (p>0.05), while blue cells imply significance at p<0.05

		Staphylococcus aureus	*Escherichia coli*
Pectin level	F(1;14)	0.411	0.340
	p-value	0.53	0.5692
Curcumin level	F(1;14)	9.38	5.943
	p-value	0.0085	0.03
Pectin*Curcumin	F(1;14)	0.087	1.218
	p-value	0.773	0.288

Principal Component Analysis

A Principal Component Analysis (PCA) was done on the physical, mechanical, barrier characteristics, and antibacterial activity, with information for both samples and variables represented for the two first dimensions. Fig. 5 depicts the value of two main components, the first (PC1) and second (PC2) accounting for, respectively, 43.8% and 28.3% of the overall variation in the data. The ability to visualize the films based on the PC1 and PC2 is provided by Figure 2A (individuals). For example, edible film with more pectin and lower curcumin (P2C1) was spread along PC1 and PC2 at negative values, whereas edible film without pectin with lower curcumin (P0C1) and higher curcumin (P0C2) was spread along PC1 and PC2 at positive values. Two edible films with lower and higher pectin without curcumin (P1C0 and P2C0) located on PC2 and control film (P0C0) have been distinguished by opposite PC1 values. The edible film with higher pectin and lower curcumin and higher curcumin (P1C2 and P2C2) were characterized by high value of PC1 and opposite of PC2. Figure 2B (variables) presents the distribution of each variable of samples. The most positive effects on PC1 were colour parameters (a* and b* values) and antibacterial activity (*Staphylococcus aureus* and *Escherichia coli*), which were influenced by curcumin along PC1 and the most negative effect were L value and swelling, which were influenced by pectin along PC2. Whereas edible film without pectin

and addition curcumin (P0C0, P0C1, and P0C2) have higher WVTR. Thus, edible films composed of higher pectin and curcumin (P2C2) have the lowest WVTR with higher bacterial growth inhibition on *Staphylococcus aureus* than *Escherichia coli*.



1=P0C0 (EF without pectin and curcumin) **4**=P1C0 (EF with pectin 1% : without curcumin) **7**=P2C0 (EF with pectin 2% : without curcumin)
2=P0C1 (EF without pectin : curcumin 0.5%) **5**=P1C1 (EF with pectin 1% : curcumin 0.5%) **8**=P2C1 (EF with pectin 2% : curcumin 0.5%)
3=P0C2 (EF without pectin : curcumin 1%) **6**=P1C2 (EF with pectin 1% : curcumin 1%) **9**=P2C2 (EF with pectin 2% : curcumin 1%)

Fig.5. Plot of PCA of the physical, mechanical, barrier and antibacterial activity of nine edible films. Individuals (A) and variables (B) of the two principal components

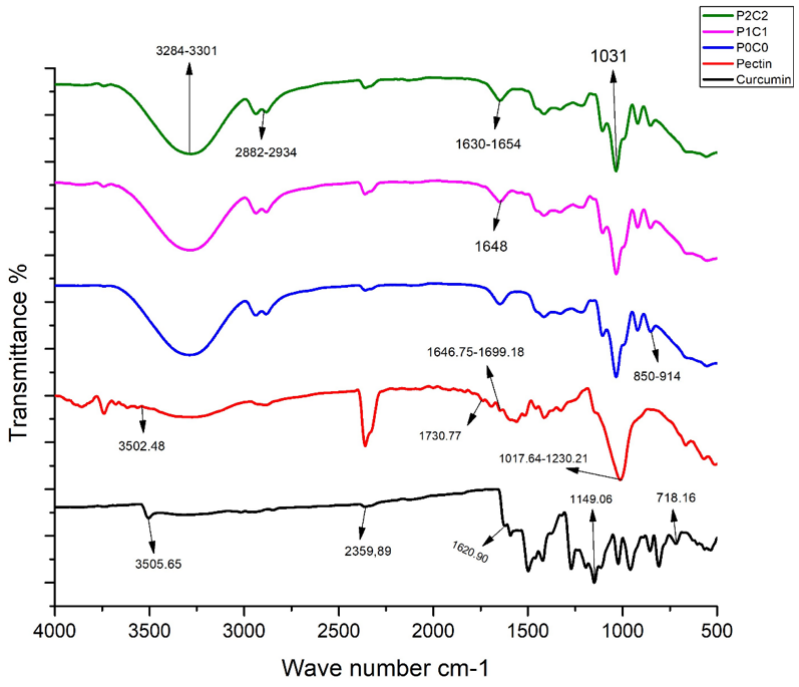


Fig. 6. FTIR spectra of curcumin, pectin, and edible films

FTIR

The effect of *Dillenia serrata* peel pectin and curcumin on the FT-IR spectra of the carrageenan-based films was studied to have information about the interactions of the chemical group involved, as illustrated in Fig. 6. In the curcumin and pectin, peaks at 3505.65 cm^{-1} and 3502.48 cm^{-1} are related to O–H stretching vibrations originating from the O-H groups, indicating the bond between oxygen and hydrogen atoms in the hydroxyl group of phenols and pectin. There is a high amount of phenolic compounds in curcumin.[40] In the control film without pectin and curcumin (P0C0), peak at 850 cm^{-1} was observed that belonged to the O-SO3 stretching vibration mode.[41-42] Overall, the FT-IR spectra of the edible films were fairly similar, however there were notable differences in the peak positions of numerous distinctive bands. For films containing lower pectin and curcumin (P1C1) and the highest pectin and curcumin (P2C2), the intensity of peaks at 3284-3301 cm^{-1} in the film's matrix was also related to stretching O-H (alcohol) vibrations. The characteristic bands for curcumin, pectin, and blend films are around 3284-3505.65 cm^{-1} due to the hydrogen bonding of hydroxyl groups of polysaccharide and hydrogen bonding of carboxylic acid.[43] New absorption peaks were observed at 2852.55 – 2921.49 cm^{-1} for the blend films, compared to the curcumin and pectin, which could be related to C-H vibration of methyl esters.[44] Peaks at 1730.77 cm^{-1} in pectin may be attributed to the -NH bending (amide II), which can create peaks between 1740 and 1725 cm^{-1} [45] while the peaks at 1620.90-1699.18 cm^{-1} in all samples could be related to aromatic moiety C=C stretching [44] which usually indicates the presence of ethyl or methyl groups. In addition, peaks at 1017.64 cm^{-1} to 1230.21 cm^{-1} in pectin correspond to the ether R-O-R and cyclic C–C ring linkages of the pectin structure[46] indicating that methoxyl groups are present in pectin (methoxyl pectin).

Conclusion

To conclude, we showed that adding pectin from the peel of *Dillenia serrata* and curcumin to carrageenan-based edible film modified their physical, mechanical, and barrier properties. The high thickness of films was conducive to the higher pectin and curcumin. WVTR showed a decrease with an increase in the pectin concentration, while the colour of the film was different due to curcumin addition. In addition, the films with higher pectin showed more hydrophilic characteristics (i.e., higher swelling value). Furthermore, a disk diffusion test revealed that films containing curcumin had a greater antibacterial impact on *Staphylococcus aureus* than on *Escherichia coli*. The results of FTIR spectra confirmed the presence pectin and curcumin within the film matrix were crosslinked by hydrogen bond. Therefore, carrageenan-based edible film containing pectin from *Dillenia serrata* fruit peel and curcumin providing potential benefits as base ingredients for development of edible packaging.

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Conflict of Interest

There was no conflict of interest associated with this study by any of the authors.

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