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Smart and Green Packaging Made from Chitosan-based Biofilm with the Addition of Ginger Oil and Anthocyanins from Butterfly Pea Flower Extract (*Clitoria Ternatea* L)

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Abstract

A chitosan-based biofilm modified with ginger essential oil and butterfly pea flower extract has been made. This biofilm was tested for its potential as a raw material for smart and environmentally friendly packaging (smart and green packaging). The potential of biofilm as a packaging raw material is known from the characterization results, which include color changes at various pHs, mechanical properties, antioxidant properties, antibacterial properties, and morphological structure. The produced biofilms exhibit sensitivity to alterations in pH levels, manifesting distinct color transitions from pink and purple to green within the pH range of 1 to 12. This phenomenon arises due to adding butterfly pea flower extract into the biofilm, which contains anthocyanin dyes with a total concentration of 1113.3 mg/L. Adding butterfly pea flower extract and ginger oil contributes to an augmented biofilm thickness; however, this is accompanied by a reduction in both tensile strength and percent elongation. Concurrently, the addition of butterfly pea flower extract and ginger oil imparts antioxidant and antibacterial properties to the biofilm. The introduction of additional extracts enhances the antioxidant and antibacterial attributes of the biofilm. In the color and pH response assessment, the biofilm augmented with a 7.5% v/v extract exhibited a color difference value (ΔE) exceeding 5 across all assessed pH values. These findings signify the observable color variations in the biofilm due to pH fluctuations with the unaided eye. According to the outcomes of characterization and analysis, the produced biofilm holds promise as an environmentally friendly packaging solution due to its reliance on natural components and its endowed antioxidant and antibacterial properties, contributing to the prolonged preservation of packaged food items. Moreover, the biofilm demonstrates the capability to gauge the quality of food products based on their pH, which is evident through direct color alterations.

1. Introduction

Food packaging plays a crucial role in safeguarding food products from environmental elements like UV rays, oxygen, water vapor, pressure, and heat. Additionally, it contributes to enhancing food safety and prolonging shelf life by providing a barrier against chemical and microbiological contaminants [1]. However, the widespread utilization of petroleum-based materials in food packaging raises environmental concerns due to their non-sustainable origins and non-recyclable nature. As an eco-friendly alternative, the natural polymer chitosan emerges as a promising material for crafting environmentally sustainable food packaging, often called "green packaging".

As consumer awareness of food products continues to grow, producers are anticipated to carefully consider

the information displayed on food packaging. While expiry dates offer insights into the consumable period, they do not directly convey information about the freshness of the food [2]. In addition to expiration dates, it is essential to incorporate details about the freshness of food products on the packaging. This can be achieved by implementing smart labels, providing real-time information about a food product's condition. Several smart labels have been developed to provide information related to shelf life, storage temperature, freshness, pH, and water vapor permeability [3]. These labels offer insights into food status through various means, including food quality indicators, barcodes, and food analysis sensors [4]. A notable type of sensor applicable to food packaging is the colorimetric-based sensor, delivering a response in the form of a visible color change for easy visual observation.

Food products that have lost their freshness or become stale typically undergo alterations in acidity levels, which are quantitatively measured by pH. Consequently, labels equipped with pH sensors offer a means to convey information regarding the freshness status of food products. This allows consumers to distinguish between fresh and stale food merely by examining the label on the packaging, obviating the need to open it [1, 2, 3, 4].

Chitosan holds considerable potential for biomedical, cosmetic, and agricultural applications owing to its notable biodegradability and non-toxic properties [5, 6]. Additionally, chitosan can be made into an eco-friendly biofilm suitable for food packaging [7]. However, a notable drawback of chitosan biofilms lies in their suboptimal barrier and mechanical properties, which can be addressed by incorporating essential oils. The precise addition of essential oils effectively mitigates water vapor permeability and enhances the flexibility of the biofilm [8]. Importantly, a higher essential oil content corresponded to lower water absorption capacity and reduced water vapor permeability [9]. Moreover, essential oils contribute to heightened antioxidant properties in chitosan [10]. Adding ginger oil into agar and sodium alginate biofilms successfully prevents pH elevation during storage [11] and significantly inhibits microbial growth in beef, including pathogenic bacteria such as *E. coli* and *S. aureus*.

Incorporating ginger oil into carboxymethyl cellulose-polyvinyl alcohol biofilms exhibited enhanced antioxidant and effective antifungal characteristics. The utilization of this biofilm in bread packaging extends the bread's shelf life from 4 days to 30 days [12]. Nevertheless, the introduction of ginger oil induced a plasticizing impact on the film matrix, resulting in increased film flexibility.

Anthocyanins, extractable from various plants, are recognized for their capability to exhibit color variations in response to different pH conditions [13, 14, 15, 16, 17, 18, 19, 20]. The butterfly pea flower (*Clitoria ternatea* L), called the telang flower, is acknowledged for its anthocyanin content [10]. Thus, the extract from the butterfly pea flower holds promise for application as a colorimetric pH sensor. Moreover, the butterfly pea flower is recognized for its remarkable antioxidant activity, which is attributed to its rich content of diverse bioactive compounds, including phenolic acids, anthocyanins, alkaloids, steroids, tannins, and flavonoids.

Anthocyanins extracted from butterfly pea flowers exhibit a polyacrylate structure known as ternatin, specifically a polyacrylate derivative of delphinidin 3,3',5'-triglucoside, contributing to the vibrant blue coloration of the flowers [7]. These anthocyanins can be extracted using 50% ethanol as a solvent for 24 hours, and their levels can be quantified using the pH difference method [10, 15]. In addition to their antioxidant properties, butterfly pea flowers contain phytochemicals with notable attributes such as antidiabetic, antimicrobial, anti-inflammatory, and anti-cancer properties [8, 14], further enhancing their potential utility. Given these characteristics, butterfly pea flowers emerge as a promising natural source of antioxidants with potential applications in the food and pharmaceutical industries [11].

Based on the previously described information, the synthesis of chitosan-based bioplastic biofilms, incorporating ginger essential oil and butterfly pea flower extract, yields a material that can undergo color changes in response to changes in pH levels. This article discusses the fabrication process of these biofilms, exploring their potential as a raw material for smart and eco-friendly packaging (green and smart packaging). The effect of adding ginger oil and butterfly pea flower extract on the physico-chemical properties of biofilms was analyzed comprehensively to optimize their potential as raw materials for environmentally friendly smart packaging. These physicochemical properties include mechanical properties, functional groups, antioxidant properties, antibacterial properties, and color responsiveness under varying pH conditions.

2. Experimental

2.1. Tools and Materials

The tools used in this research were laboratory glassware, analytical balance (Ohaus), blender, rotary vacuum evaporator (Eleya), centrifuge (Dlab), UV-Vis spectrophotometer (FLUOstar Omega), FTIR spectrometer (Perkin Elmer UATR Spectrum Two). The materials were dried butterfly pea flowers (*Clitoria Ternatea* L) (purchased from an online shop), 50% ethanol solvent, pH 1-12 buffer solution (Merck), 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Merck), glycerol (Merck), standard solution of gallic acid and quercetin, chitosan (MW 168 kDa, DD 96.19%) (purchased from Chimultiguna), pure ginger oil and Tween 20 (bought from online supplier).

2.2. Extraction of Butterfly Pea Flowers

Dried butterfly pea flowers were cleaned from the leaf petals and stems and then crushed. Subsequently, 100 grams of finely crushed butterfly pea flower crowns were immersed in 1000 mL of 50% ethanol solvent for 24 hours. The resulting soak was filtrated, and the solvent

was evaporated using a vacuum evaporator to obtain a concentrated solution. The butterfly pea flower extract was then characterized to determine the total anthocyanin content using the pH difference method, total phenolic content (TPC) using Folin's reagent [21], flavonoid content, and antioxidant activity test.

2.3. Production of Chitosan-Ginger Oil-Butterfly Pea Flower Extract-based Biofilms

The 2% (w/v) chitosan solution was prepared by dissolving 2 grams of chitosan in 100 mL of 2% (v/v) acetic acid solution. The chitosan solution was homogenized using a magnetic stirrer, followed by adding 1 mL of each glycerol, ginger oil, and Tween 20 under stirring at 50°C until homogeneous. The resulting mixture was transferred into a petri dish with a 15 cm diameter and subsequently dried in an oven at 60°C for 24 hours [5, 6, 7]. The produced biofilms were made using the formula in Table 1.

2.4. Characterization

Biofilms were characterized for their mechanical properties, functional groups, antioxidant properties, antibacterial properties, surface morphology, and color and pH responsiveness. The mechanical properties of the biofilms were tested using a Brookfield CT 3 4500 Texture analyzer at the Integrated Laboratory of Diponegoro University, Semarang. Each test was conducted in triplicate for each sample. Notably, data analysis refrained from utilizing ANOVA or similar statistical methods; instead, it involved calculating the mean (average) of the three data points obtained from the test results.

Functional group analysis of the biofilm was performed utilizing an FTIR spectrometer (Perkin Elmer UATR Spectrum Two) within the wave number range of 400-4000 cm^{-1} at the Integrated Laboratory of Diponegoro University. Additionally, film morphology testing was conducted using the Phenom Pro.

The antioxidant activity was determined by employing the DPPH method. Biofilms were extracted using 10 mL of distilled water in a water bath set at 50°C. Subsequently, 2.8 mL of the obtained extract was incubated with the addition of 0.2 mL of 0.1 mM DPPH solution, and the mixture was left in a dark condition for 30 minutes. Then, the absorption value was measured using a UV-Vis Spectrophotometer (FLUOstar Omega) at a wavelength of 517 nm [22].

The antibacterial test was performed by utilizing the disc diffusion method. Disc-shaped biofilm sheets were created and embedded onto the surface of nutrient agar previously inoculated with *E. coli*. Subsequently, the samples were invertedly incubated for 24 hours. The bacterial inhibition was assessed by measuring the clear zones that had formed. The identical procedure was replicated using *S. aureus* [23].

The biofilm was precisely cut into 1 × 1 cm rectangles for color testing. These films were then dripped with solutions with varying pH levels (1 to 12). Then, the biofilms were left at room temperature for 15 seconds before undergoing analysis using an Android-based colorimeter. This analysis yielded values for L*, a*, and b*. The total color difference (ΔE) in the biofilm was subsequently calculated employing Equation (1), which was established by Yan *et al.* [23].

$$\Delta E = \sqrt{\Delta L^2 + a^2 + b^2} \quad (1)$$

The ΔE obtained data is standard L* 99.34; a* standard -0.26; and standard b* 1.42 [24]. The ΔL*, Δa*, and Δb* were calculated using Equation (2)-(4).

$$\Delta L^* = L^* \text{ sample} - L^* \text{ standard} \quad (2)$$

$$\Delta a^* = a^* \text{ sample} - a^* \text{ standard} \quad (3)$$

$$\Delta b^* = b^* \text{ sample} - b^* \text{ standard} \quad (4)$$

3. Results and Discussion

The dye extraction from butterfly pea flowers was conducted through maceration with ethanol solvent, resulting in a yield of 55%. Multiple studies have demonstrated that this extract contains anthocyanins [24, 25, 26]. pH difference method was employed to determine the total anthocyanin content in the extract [9]. The analysis revealed a total anthocyanin level of 1113.3 mg/L. In a study conducted by Koshy *et al.* [27], the extraction of butterfly pea flower dye yielded anthocyanin cyanidin 3-glucoside levels of 20.66 mg/L.

Additionally, another method for extracting butterfly pea flower dye involves using hot water and a 50% ethanol solvent with 1% citric acid. This method yielded total anthocyanin levels ranging between 6146.90-7925.29 mg/L, depending on the specific extraction technique employed [25]. The total anthocyanin levels obtained from butterfly pea flower dye extraction using conventional and ultrasonic-assisted extraction methods with 60% ethanol solvent were 3010.9 and 3530.2 mg/L, respectively [21].

Table 1. Composition of chitosan-ginger oil-butterfly pea flower extract

Sample code	Chitosan (mL)	Ginger oil (mL)	Glycerol (mL)	Tween 20 (mL)	Anthocyanin (mL)
KM0	100	1	1	1	0
KM5	100	1	1	1	5
KM7.5	100	1	1	1	7.5
KM10	100	1	1	1	10
KM12.5	100	1	1	1	12.5
KM15	100	1	1	1	15

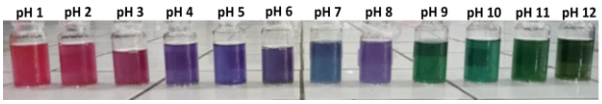

Figure 1. Color of butterfly pea flower extracts at different pH conditions

The change in the extract's color in reaction to pH variations was assessed by adjusting the pH of the extract from 1 to 12. The color changes are visually represented in Figure 1. The extract exhibited a red color at pH 1, transitioning gradually to purple and blue as the pH was raised to 7. As the pH surpassed 7 (alkaline condition), the extract's color shifted to green, with its intensity deepening with increasing pH levels. These findings underscore the pH-dependency of the extract's coloration.

The color analysis of the extract under various pH conditions was conducted using a colorimeter, and the results were expressed through L*, a*, and b* values based on the CIELab system, as detailed in Table 2. The L* value represents brightness, with lower values indicating darker colors. The red/green color component (a*) denotes red when positive, while the blue/yellow color component (b*) indicates blue when negative. Across pH 1-5, both L* and a* values exhibited a decrease.

The outcomes of the calculations conducted using the CIELab system validate the observed colors of the extract, as depicted in Figure 1. These calculations performed with the colorimeter offer quantitative insights into the color variations exhibited by the extract in response to changes in pH. Such quantitative data holds significant utility across diverse applications reliant on colorimetry or necessitating sensitivity to color changes.

Once confirmed that the extract obtained from butterfly pea flowers can produce different colors depending on various pH levels, its potential as a pH indicator becomes apparent. In this study, butterfly pea flower extract was utilized to produce chitosan biofilm. The resulting butterfly pea flower extract-chitosan biofilm was then assessed for color changes in response to pH to evaluate its potential biofilms as smart labels specifically for food packaging, capable of displaying distinct colors corresponding to different food conditions resulting from changes in acidity (pH). The color responsiveness of biofilms containing various concentrations of anthocyanins was examined across a range of pH conditions. The test results are presented in Figure 2.

As shown in Figure 2, samples KM5 and KM10 exhibit no discernible color changes across varying pH conditions. Conversely, samples KM7.5, KM12.5, and KM15 demonstrate notable color alterations. A reddish-purple purple appears in acidic solutions (pH < 3) due to a combination of blue and red pigments produced by flavylium cations, a form of anthocyanin oxonium [28]. As the pH rises (3 < pH < 7), anthocyanins show a deep blue color attributed to their hemiketal or quinoidal anhydrous base form. Meanwhile, in alkaline conditions (pH > 7), the hydroxyl groups facilitate the cleavage of the central pyrrole in the anthocyanin ring, resulting in the formation of a yellow-green chalcone compound [27, 28, 29].

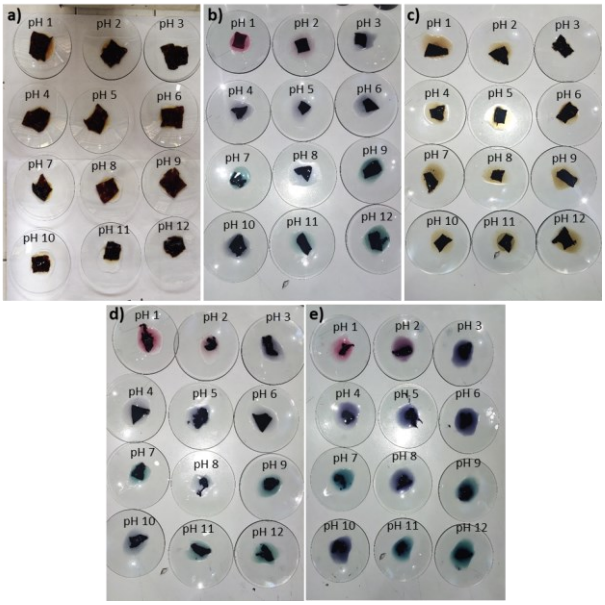

Figure 2. Color response test results of (a) KM5, (b) KM7.5, (c) KM10, (d) KM12.5, and (e) KM15, each treated with a pH buffer ranging from 1 to 12

Table 2. Results of color analysis of butterfly pea flower extract using the CIELab system

pH	Color	L*	a*	b*
1		42.909	45.941	12.535
2		40.629	40.221	2.705
3		32.025	33.715	-0.961
4		29.445	18.585	-24.632
5		26.284	14.288	-26.665
6		29.054	15.070	-24.412
7		21.234	-2.742	-10.764
8		29.504	11.954	-20.934
9		22.748	-21.274	12.569
10		25.076	-6.441	-11.658
11		35.957	-15.308	17.388
12		31.022	-7.987	23.579

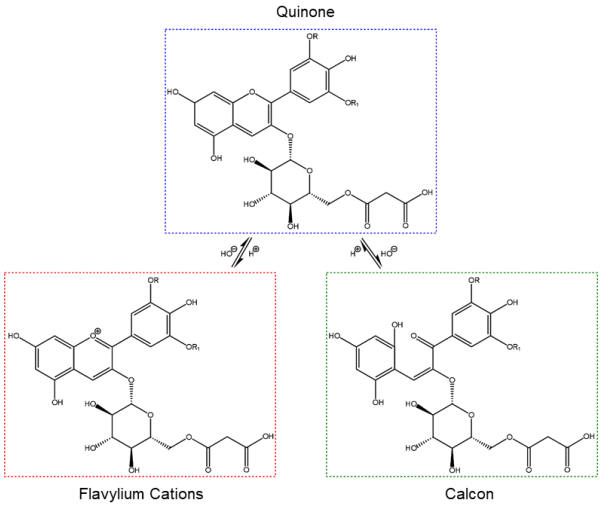

Figure 3. Structural changes of cyanidin-3-glucoside in response to different pH [28]

Under acidic conditions, flavylium cations (AH^+) predominate in the anthocyanin structure, imparting a red color. This species is fully protonated, bearing a positive charge that spreads across the chromophore. Upon deprotonation, the flavylium ion converts into a neutral quinodal base (A), resulting in a purple color. As pH increases, the quinoidal base loses a proton, forming the quinoidal base anion (A^-) with a negatively delocalized charge, leading to a blue color. Additionally, a rise in pH induces a green-yellow color due to the hemiketal structure's instability, resulting in the formation of chalcone [28]. The results underscore the significant influence of pH on altering the color of butterfly pea flower extract, known for its anthocyanin content. Consequently, the anthocyanin present in butterfly pea flower extract can serve as a color indicator, effectively demonstrating the pH value of a solution, as depicted in Figure 3.

The outcomes of the biofilm color response test conducted at different pH levels were analyzed using a colorimeter. The results were then quantified and expressed by L*, a*, and b* values, along with △E values, as detailed in Tables 4 to 6. The data in Tables 4, 5, and 6 demonstrate that increasing the anthocyanin concentration in the chitosan film results in noticeable changes in color parameters (a* and b*) and △E value. Notably, the KM7.5 sample displays the highest sensitivity to pH variations, as indicated by an △E value exceeding 5. This suggests that the KM7.5 sample holds considerable promise as an effective smart label capable of detecting pH changes in food products. Furthermore, the substantial △E value indicates that the resultant color changes can be easily perceived with the unaided eye [4]. Subsequently, the suitability of the biofilm as a material for food packaging was evaluated based on its mechanical properties. The results of this assessment are shown in Table 7.

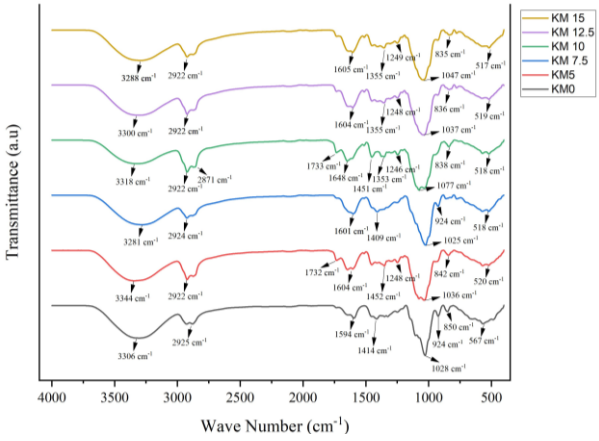

Figure 4. FTIR spectra of biofilms

The mechanical properties of the biofilm depend on the interaction between the polymer matrix of chitosan, anthocyanin and ginger essential oil components. An increase in sample thickness occurred along with an increase in the amount of butterfly pea flower extract added. However, this does not significantly increase the tensile strength and elongation percentage. The only significant difference observed between the two was that the control sample (without anthocyanin, KM0) versus the sample containing anthocyanin, KM0, showed the best mechanics. The addition of 5% extract and beyond reduces the mechanical properties of the film formed.

The addition of ginger oil is thought to have a negative effect on mechanical properties, notably by substantially diminishing tensile strength and elongation at break. According to Singh *et al.* [20], essential oils significantly decreased the tensile strength and elongation at break, indicating a reduction in intermolecular interactions between polymer chains. Similarly, adding cinnamon oil has decreased tensile strength and elongation at break in chitosan films combined with gelatin [20].This study's results, presented in Table 7, indicate that the incorporation of butterfly pea flower extract results in a reduction in elongation at break, thereby weakening the film network. Consequently, the formed film matrix exhibits brittleness, leading to diminished elongation at break. The data concerning the mechanical properties of the KM15 biofilm were unavailable due to the sticky nature of the biofilm, rendering testing unfeasible.

Intermolecular interactions between chitosan, ginger oil and butterfly pea flower extract were analyzed using FTIR analysis, as shown in Figure 4. The anthocyanin-rich butterfly pea flower extract exhibited characteristic peaks at 3254 cm^{-1} as -OH stretching, 2870 cm^{-1} representing CH stretching, 1709 cm^{-1} and 1575 cm^{-1} indicating CC and C=O stretching vibrations, 1474 cm^{-1} associated with stretching CC, and 747 cm^{-1} arising from out-of-plane CH bending vibrations of anthocyanin.

Table 4. Results of color analysis of KM7.5 samples using the CIELab system at different pH conditions

Sample	L*	a*	b*	△E
KM7.5	18.383	2.181	2.183	0
KM7.5 + pH 1 buffer	141.864	12.725	4.064	12.596
KM7.5 + pH 2 buffer	10.729	2.937	2.793	11.469
KM7.5 + pH 3 buffer	134.167	7.943	11.017	12.374
KM7.5 + pH 4 buffer	172.675	9.559	4.270	13.657
KM7.5 + pH 5 buffer	195.447	9.014	4.314	14.449
KM7.5 + pH 6 buffer	216.423	13.706	3.844	15.296
KM7.5 + pH 7 buffer	159.797	10.890	4.147	13.222
KM7.5 + pH 8 buffer	142.767	4.7548	4.764	12.340
KM7.5 + pH 9 buffer	77.338	4.7849	4.760	9.3211
KM7.5 + pH 10 buffer	22.347	7.777	0.718	5.553
KM7.5 + pH 11 buffer	59.482	4.755	4.764	8.307
KM7.5 + pH 12 buffer	77.919	11.198	4.155	9.658

Table 5. Results of color analysis of KM12.5 samples using the CIELab system at different pH conditions

Sample	L*	a*	b*	△E
KM12.5	7.386	0.361	1.434	0
KM12.5 + pH 1 buffer	5.479	4.599	2.226	3.508
KM12.5 + pH 2 buffer	1.981	0.072	2.543	2.144
KM12.5 + pH 3 buffer	8.439	3.827	6.188	4.296
KM12.5 + pH 4 buffer	11.137	1.307	1.778	3.771
KM12.5 + pH 5 buffer	7.607	0.910	1.844	3.219
KM12.5 + pH 6 buffer	0.214	2.060	1.686	1.9901
KM12.5 + pH 7 buffer	0.003	0.109	2.345	1.568
KM12.5 + pH 8 buffer	2.784	1.021	1.827	2.373
KM12.5 + pH 9 buffer	3.353	0.151	2.047	2.356
KM12.5 + pH 10 buffer	0.039	1.467	2.421	1.982
KM12.5 + pH 11 buffer	1.105	0.209	1.540	1.689
KM12.5 + pH 12 buffer	5.568	4.057	0.003	3.103

Table 6. Results of color analysis of KM15 samples using the CIELab system at different pH conditions

Sample	L*	a*	b*	△E
KM15	9.626	1.725	2.605	0
KM15 + pH 1 buffer	40.137	2.,669	6.588	7.028
KM15 + pH 2 buffer	11.669	1.343	14.232	5.219
KM15 + pH 3 buffer	2.707	1.769	14.755	4.385
KM15 + pH 4 buffer	13.003	2.977	6.786	4.771
KM15 + pH 5 buffer	0.017	12.703	1.171	3.727
KM15 + pH 6 buffer	0.065	10.516	1.792	3.517
KM15 + pH 7 buffer	8.530	2.977	6.786	4.277
KM15 + pH 8 buffer	0.045	8.339	4.718	3.619
KM15 + pH 9 buffer	0.122	7.564	1.401	3.014
KM15 + pH 10 buffer	0.170	5.127	0.066	2.316
KM15 + pH 11 buffer	15.677	1.281	7.079	4.903
KM15 + pH 12 buffer	6.761	5.326	2.107	3.767

Table 7. Measurement results of biofilm's mechanical properties

Sample	Chitosan: ginger oil: extract	Mechanical test		
		Thickness (mm)	Tensile strength (MPa)	% Elongation
KM0	100: 1: 0	0.15	3.97	64.2
KM5	100: 1: 5	0.44	0.46	203
KM7.5	100: 1: 7.5	0.45	0.08	168
KM10	100: 1: 10	0.48	0.41	130
KM12.5	100: 1: 12.5	0.52	0.04	136
KM15	100: 1: 15	-	-	-

The spectrum of chitosan bioplastic exhibits characteristic bands corresponding to chitosan and ginger oil at 3278 cm^{-1} (OH stretching), 2833 cm^{-1} (CH bending vibration), 1638 cm^{-1} (amide-I, CO stretching), 1418–1324 cm^{-1} (bending vibration CH_2), 1189 cm^{-1} (frame vibration of chitosan pyranose structure), and 740 cm^{-1} (bending vibration CH). After the addition of anthocyanin-rich butterfly pea flower extract to the polymer matrix, discernible differences in band positions and peak intensities are observed in the KM0 biofilm. Specifically, the band at 3278 cm^{-1} (OH stretching) undergoes slight broadening and shifts to 3300 cm^{-1}. The CH stretching shifts from 2833 to 2894/2896 cm^{-1}, indicating hydrogen bond formation among chitosan, ginger oil, and anthocyanin. Furthermore, the C=O stretching shifts from 1638 cm^{-1} to 1651/1653 cm^{-1}, accompanied by increased band intensity. These shifts in the amide-II and amide-III bands are attributed to the formation of electrostatic interactions.

Antioxidant activity in secondary metabolites is usually obtained from the content of phenolic and flavonoid compounds. Based on the analysis results, butterfly pea flower extract has total phenolic and flavonoid levels of 372.75 ppm and 40.79 ppm, respectively. According to Jeyaraj *et al.* [30], the glycosylated B-ring structure of anthocyanins contributes to their high antioxidant activity, whereas ortho hydroxylation and methoxylation significantly increase the antioxidant activity. In this study, antioxidant activity was analyzed using the DPPH method. The analysis results show that the IC_{50} value of butterfly pea flower extract is 1.77 ppm, which is included in the very strong antioxidant properties category. Meanwhile, the biofilm antioxidant test results are presented in Table 8.

Essential oils are also known as strong antioxidants due to their rich chemical composition, including terpenoids and phenolic acids. Ginger oil contains many bioactive compounds, such as terpenes (sesquiterpenes, hydrocarbons) and phenolic compounds (gingerol, shogaol) [31]. In addition, other research suggests that the antioxidant activity in ginger oil is related to the synergistic effect between two or several components [32]. A study by Mi *et al.* [33] reported that ginger oil has a high free radical scavenging capacit. This attribute is attributed to the presence of phenolic compounds like gingerol, parasol, and shogaol in ginger oil. These components function as donors of hydrogen atoms or electrons, thereby interrupting the oxidation chain reaction initiated by free radicals.

The ability to capture free radicals through antioxidant activity is crucial for producing high-quality food packaging. Incorporating antioxidants into packaging materials serves to prolong the shelf life of food by delaying spoilage and oxidative deterioration caused by free radicals. Data presented in Table 8 indicates that all tested samples exhibit strong DPPH radical scavenging activity. Furthermore, the scavenging activity of DPPH radicals in the samples increases proportionally with the addition of anthocyanins. This increase in the antioxidant capacity of the biofilms is primarily caused by the presence of polyphenols found in anthocyanins and ginger oil. A similar increase is also observed in chitosan when combined with extracts from mangosteen peel and purple tomatoes [2, 4, 34, 35].

Table 8. Antioxidant test results

Sample code	IC_{50} concentration (mg/L)	Category
KM5	8.636	Very strong
KM7.5	12.446	Very strong
KM10	6.706	Very strong
KM12.5	13.151	Very strong
KM15	12.397	Very strong

Table 9. Results of measurement of inhibition zone and inhibitory strength of samples

Bacteria	Sample	Inhibition zone diameter (mm)	Inhibitory effect effectiveness (%)	Category
S. aureus	KM0	0.575	4.315	Weak
	KM5	1.625	12.195	Weak
	KM7.5	6.875	51.595	Moderate
	KM10	3.375	25.328	Weak
	KM12.5	8.9	66.792	Moderate
	KM15	12.875	96.622	Strong
	Ampicillin (Control +)	13.325	100	Strong
	Sterile distilled water (Control -)	0	0	Weak
E. coli	KM0	0.2	1.223	Weak
	KM5	1.1	6.728	Weak
	KM7.5	1.7	10.398	Weak
	KM10	2.45	14.984	Weak
	KM12.5	3.425	20.948	Weak
	KM15	5.325	32.569	Moderate
	Ampicillin (Control +)	16.35	100	Strong
	Sterile distilled water (Control -)	0	0	Weak

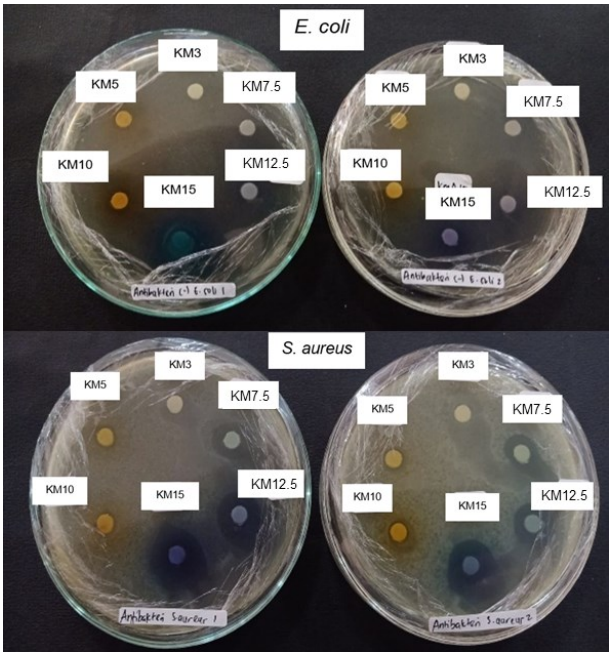

Figure 5. Images of the biofilm inhibition zone against *E. coli* and *S. aureus* bacteria

Evaluation of the antimicrobial properties of biofilms is crucial for gauging their effectiveness in mitigating pathogenic microbial contamination when employed in food packaging. The findings of the antibacterial assessment of chitosan-ginger oil-butterfly pea flower extract biofilms are presented in Table 9 and Figure 5. The antibacterial assay conducted against both *S. aureus* and *E. coli* bacteria indicates varying degrees of inhibitory efficacy ranging from weak to strong within the biofilms.

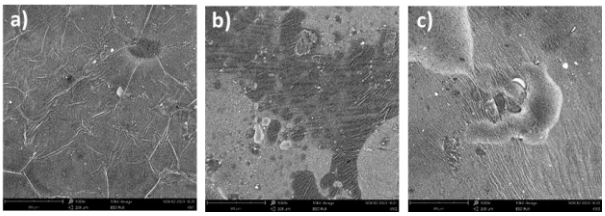

Figure 6. SEM images of (a) KM7.5, (b) KM10, and (c) KM12.5 biofilms

Table 9 illustrates that all biofilms exhibit antibacterial activity against *S. aureus*, with inhibitory strength varying from weak to strong. Notably, the addition of butterfly pea flower extract correlates with enhanced antibacterial activity. Conversely, all samples demonstrate antibacterial activity against *E. coli*, ranging from weak to moderate inhibition. Generally, the antibacterial activity against *S. aureus* surpasses that against *E. coli*. Among the samples, KM15 biofilm displays the most strong antibacterial activity. The antimicrobial mechanism of phenolic compounds is attributed to their hydrophilic properties, facilitating interaction with the bacterial cytoplasmic membrane and lipopoly-saccharides.

This interaction induces instability and eventual cell death in bacterial cells. The antimicrobial activity of anthocyanins is closely linked to the formation of pores within bacterial cytoplasmic membranes. This phenomenon arises from the interaction between flavylium cations and negatively charged phospholipid groups. Such interactions facilitate the release of intracellular contents, induce physiological alterations,

and ultimately lead to microbial inactivation [2, 35]. Biofilms possessing antimicrobial properties hold promise as potential raw materials for food packaging, offering the capability to prolong the shelf life of food products.

Figure 6 presents the results of SEM analysis regarding the morphology of the biofilm surface. The images show numerous voids evident on the film surface, resulting from the evaporation of ginger essential oil during the production process. Notably, the surfaces of films containing 10% (KM10) and 12.5% (KM12.5) butterfly pea flower extract exhibit reduced smoothness and increased void formation (Figures 6b and 6c). This observation suggests that the addition of 10% extract diminishes the compatibility between chitosan and ginger essential oil. The presence of anthocyanins, characterized by phenolic groups, allows for effective dispersion within the polymer matrix.

This dispersion is facilitated by electrostatic interactions and hydrogen bonding between ginger oil, Tween 20, glycerol, anthocyanin, and the polymer matrix. However, mechanical testing results, particularly the tensile strength of the KM10 film, demonstrate significantly higher values than the KM7.5 and KM12.5 films (Table 7). Despite having the lowest thickness, the KM7.5 film exhibits the highest elongation percentage, indicating greater elasticity. Previous studies reported comparable findings concerning the fabrication of chitosan films incorporating anthocyanins derived from jambolan fruit extract and purple eggplant extract [34, 36].

4. Conclusion

Biofilms composed of chitosan, butterfly pea flower extract, and ginger oil have been successfully synthesized. Increasing the quantity of extract in the biofilm formulation resulted in thicker products yet led to a reduction in both tensile strength and percent elongation. Conversely, increasing the concentration of ginger extract and oil enhanced the antioxidant and antibacterial properties of the biofilm. In the color and pH response test, the biofilm with the addition of 7.5% v/v extract showed an ΔE value of more than 5 at all pH values evaluated, indicating discernible color changes perceptible to the unaided eye. The research outcomes underscore the potential of the developed biofilm as environmentally friendly packaging, owing to its natural materials and inherent antioxidant and antibacterial attributes conducive to prolonging the shelf life of packaged food items. Moreover, the biofilms demonstrate the capability to assess food product quality based on pH, which is evident through observable color shifts.

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