Research Article

Physicochemical properties of Saba Banana (*Musa acuminata x balbasiana*) Alginate Starch Biofilm reinforced with Orange Peel Extracts and Its Application on Lady finger banana

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ABSTRACT

This study employed the casting method to fabricate biofilms comprising banana starch and alginate, fortified with different concentrations of orange peel extract (0, 0.5, 1, and 2 % (w/v)). The resulting films of BA, BAO 0.5%, BAO 1% and BAO 2% were subjected to analysis using Field Emission Scanning Electron Microscopy (FESEM) and Fourier Transform Infrared Spectroscopy (FTIR). Additionally, various physical attributes of the films were investigated, encompassing parameters including thickness, density, colour, porosity, moisture content, water solubility, water absorption, and water vapor permeability. The addition of orange peel extract significantly reduced the water vapor permeability of the film from 4.25±0.03 to -1.16±0.02 $gs^{-1}mPa \times 10^{-7}$. Besides, FTIR analysis unveiled the presence of diverse functional groups within the film, notably alkanes (CH), hydroxyl (-OH), carbonyl (C=O), and carboxylic acid (-COOH), which contribute to the enhancement of fruit longevity. The empirical results indicated that a film composition comprising banana starch and alginate reinforced with 1% orange peel extract exhibited favourable effects on bananas, diminishing the occurrence of black spots and retarding the ripening process. Furthermore, the antimicrobial properties of the film were elucidated, wherein a discernible barrier effect was observed in films augmented with 1% and 2% orange peel extract. The film solution shows good potential in preserving the postharvest quality of fruits.

Keywords: alginate; antimicrobial properties; banana starch; biofilm; orange peel extract

1. Introduction

Scientifically known as Musa from the Musaceae family, bananas are among the most vital fruit crops globally and are particularly popular in Malaysia's tropical climate. In the global trade hierarchy, bananas hold the fifth important position, following cereals, sugar, coffee, and cocoa (Uma, 2008). Ecuador emerged as the largest banana producer in 2018, yielding approximately 6.65 million tonnes, with India following at 2.95 million metric tons. Most of the production in both countries is consumed domestically (Food and Agriculture Organization of United Nation, 2018). In Malaysia, bananas ranked second in plantation area

and production, next to durian. Bananas are extensively cultivated as a commercial fruit crop due to their constant availability and high demand (Reginio *et al.,* 2020).

A banana consists of two main components: the peel and the pulp. The banana's peel makes up roughly 40% of its total weight, rendering it the primary by-product, while the pulp, the edible part, is rich in essential nutrients. Bananas contain approximately 60% starch (de la Torre-Gutiérrez et al., 2008), with a notable concentration of green banana starch, making it an excellent material for film packaging. About 70 to 80% of the desiccated mass of green banana pulp comprises starch. The primary factors influencing starch production are the ripeness stage and banana cultivar. The damp grinding method is suitable for isolating banana starch due to its minimal impurity levels. Several starch isolation techniques have been documented, including alkali and non-alkali extraction methods (Marta *et al*., 2022). Previously, Vonnie *et al.* (2022) employed Green Saba banana starch as the composite matrix for curcumin and aloe vera. The use of green banana starch improved the physical properties of the film in terms of its thickness, opacity, and biodegradability.

The cell walls of brown algae, specifically *Laminaria digitata*, contain sodium, calcium, and magnesium ions bound to alginic acid. These ions are subsequently extracted and employed in the production of alginates. Alginate comprises two primary constituents, namely β-D-mannuronic acid and β-L-guluronic acid. Gheorghita *et al.* (2020) identified that sodium alginate, among various types of alginate, possesses distinctive attributes, including the ability to generate a durable, lustrous, visually pleasing, odourless, pliable film that is water-soluble and resistant to oxygen and oil. Notably, alginate is designated as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration, making it suitable for use in the production of food biofilm. Sodium alginate were commonly utilized as a coating to retain the quality properties of fruits including avocado and fresh-cut strawberries (Iñiguez-Moreno *et al.,* 2021; Alharaty and Ramaswamy, 2020).

According to Othman and Fadzil (2021), orange peel is an underutilized bio-waste often deemed undesirable in product utilization. This arises from extracting orange juice utilising only approximately half of the original fruit mass. Orange peels are highly biodegradable and contain beneficial polysaccharides such as pectin, cellulose, and hemicellulose, along with important ingredients like fermentable sugars, polyphenols, flavonoids, essential oils, and other essential compounds which exhibit robust antioxidant properties and offers numerous health benefits (Wang *et al.,* 2019). Felicia *et al.* (2024) and Amjadi et al. (2021) discovered that orange peel essential oils have the potential as the active ingredient which act as antioxidant and antimicrobial agents.

Nowadays, natural macromolecules and biopolymers have gained popularity in the food and packaging industry due to consumer preference for eco-friendly products (Contini *et al.,* 2022). Exposure to oxygen and moisture in the air can accelerate their deterioration, leading to increased food waste. Inadequate handling, packaging, storage, and transportation practices contribute to these losses, negatively impacting the food sector, as Dora *et al.* (2020) highlighted. Therefore, enhancing postharvest management practices for fruits is imperative to minimize waste and extend their shelf life. This research aimed to develop a biofilm using Saba bananas starch and alginates enhanced with orange peel extracts to preserve the postharvest quality of Lady finger bananas. The biofilms were meticulously characterized for physical attributes, including thickness, density, colour, water solubility, moisture content, opacity, water vapor permeability, and antioxidant and antimicrobial analysis.

2. Materials and Methods

2.1 Materials

Green Saba banana (*Musa acuminata x balbisiana*) (D: 8-11 cm x 3-5 cm) and Lady finger banana (*Musa acuminata* 'Lady Finger') (D: 6-8 cm x 2-4 cm) at a maturity stage of 75% were purchased from the Pasar Tamu Beaufort Sabah. Orange (*Citrus sinensis*) peel was collected from households and a nearby cafe in Kota Kinabalu. Meanwhile, sodium alginate was purchased from EvaChem.

2.2 Extraction of Starch from Banana Saba (Musa acuminata x balbisiana)

Extraction banana pulp starch using the method Vonnie *et al.* (2023) banana fruits were peeled and chopped into 2 cm pieces, soaked in a 5 mins solution of 2% (w/v) of citric acid, then crushed for 2 mins to extract the starch from the pulp. The banana pulp and residual solution were centrifuged (Centrifuge 5430 R Eppendorf, Germany) at 7500 rpm for 5 mins at room temperature before discarding the supernatant. Starch particles are dried for 18 h at 55 °C in a universal oven and sieved through a 125-micron hole.

2.3 Extraction of Orange Peel

The orange peel was extracted using the method described by Aboul-Enein *et al.* (2016) with some modifications. Desiccated orange peel was dissolved in 80% acetone in a solid-to-solvent ratio of 1:10 after 24 h of shaking at ambient temperature. The mixture was filtered using Whatman No. 1 filter paper after the extraction procedure was repeated numerous times. After being concentrated in a rotary evaporator at 60 °C, the filtrate was freeze-dried for 15-16 h.

2.4 Fabrication of Film

Fabrication of film was done with slight modifications from the method done by Ramakrishnan *et al.* (2023). A 2% (w/v) concentration of banana starch and 1% (w/v) of alginate is added, dissolved, and gelatinized in distilled water for 15 mins at 80 °C using a hot plate and then cooled forming BA films. The films without orange peel extract served as a control. The film-forming solution is created by blending different concentrations of orange peel extract (0.5, 1, 2 % (w/v)) with banana starch and alginate forming BA, BAO 0.5%, BAO 1% and BAO 2%. Then, 10 mL of solution was cast onto a Petri plate and dried for 48 h at room temperature.

2.5 Physicochemical Characterization

2.5.1 Thickness and Density

The thickness of the banana starch/alginate/orange peel film was measured at five separate locations using a digital micrometre (Starrett, Athol, MA, USA) with a resolution of 0.001 mm. Next, the average is calculated. The initial mass is determined by calculating the area of a square with dimensions of (2 cm x 2 cm). The film is subjected to a drying process at a temperature of 105 °C for 24 h, then the weight of the film is determined (Vonnie *et al.,* 2023). The density is determined using the equation (1) below.

$$Density = \left(W_i - W_f\right)(A \times t) \quad (1)$$

Where, W_i is the weight of the initial film before drying (g), W_f is the weight of the last film after drying (g) A is the film area (cm^2), and t is the thickness of the film (mm).

2.5.2 Colour Measurement

The colour of the film was measured with a colourimeter (Konica Minolta, Tokyo, Japan) in the CIE Laboratory's absorption and L *, a *, b * scale mode. Equation (2) calculates the total colour difference (ΔE) after measuring the film in three replications (Vonnie *et al.,* 2022). The whiteness and yellowness indexes were calculated using Equations) and (4).

$$\Delta E = \sqrt{(L*_2 - L*_1) + (a*_2 - a*_1) + (b*_2 - b*_1)} \quad (2)$$

$$Whiteness\ Index = 100 - (100 - L)^2 + a^2 + b^2 \quad (3)$$

$$Yellowness\ Index\ =\ 142.86\ (b/L) \quad (4)$$

Where L* (94.60), a*(-0.42) and b*(3.24) were the standard colour parameters of the calibration plate.

2.5.3 Water Solubility

The water solubility of the film was determined using the Vonnie *et al. (*2023) method with some modifications. Each film sample was immersed in 50 mL of distilled water for 24 h to test its water solubility. Upon hydration, the wet film is gently coated with filter paper, dried in a universal oven at 105^{O}C for 24 h, and the final weight is determined. The film water solubility (%) is obtained using Equation (5).

$$Water\ Solubility(\%)\ =\ \frac{M_1 - M_2}{M_1} \times 100 \quad (5)$$

Where M_1 is the film weight before soaking in distilled water (g) and $M_2$is the film weight after 24 h of soaking (g).

2.5.4 Moisture Content

Moisture content was determined using the method of Rovina *et al*. (2020). The freshly made film sample was heated for 24 h at 105^{O}C in the universal oven. The film's moist and dry weights are calculated by weighing it before and after exposure to water, as defined by AOAC. Equation (6) is used to determine the film's moisture content.

$$Moisture\ content = \frac{W_1 - W_2}{W_1} \times 100 \quad (6)$$

Where W_1 is the initial weight (g), and W_2 is the final weight (g).

2.5.5 Opacity

The UV-visible spectrophotometer (PerkinElmer, London, UK) was employed to quantify film absorption at a wavelength of 600 nm. A (4 cm × 1 cm) film section was trimmed and placed within a cuvette. In triples, measurements are acquired and the film's opacity is computed using Equation (7) (Bojorges et al., 2020).

$$Opacity = \frac{-log\ T600}{X} \quad (7)$$

The film thickness (mm) is indicated by x, while T600 represents the fractional transmittance at 600 nm.

2.5.6 Water Vapor Permeability

The water vapour permeability value was evaluated using ASTM E96's "cup method" using permeability cells designed specifically for this purpose (Cazón *et al.,* 2018). Each 400 mm^2 film is laminated with aluminium foil and secured with double-sided tape containing 30 g of silica gel. Each cup is then placed in a water-filled desiccator at 25 °C, and its weight is recorded every 24 h until a fixed weight is achieved. Equation (8) determines the water vapour transmission rate, while Equation (9) determines the permeability.

$$Water\ Vapour\ Transmission\ Rate = \frac{\Delta m}{(A \times \Delta t)} \quad (8)$$

$$Water\ Vapour\ Permeability = \frac{wvtr(x)}{\Delta P} \quad (9)$$

Where, Δm is the weight gained, A is the exposed area of the film (mm^2), Δt is the time of the test (day), x is the thickness (m) of the film and ΔP the difference in water vapor pressure through the film (Pa).

2.5.7 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was performed on film using attenuated total reflection technique (ATR) and spectrophotometer from Thermo Scientific (Nicolet Continuum, Madison, USA) over 4000–650 cm^{-1} range with 4 cm^{-1} resolution (Barbosa *et al.,* 2022).

2.5.8 Morphology Characterization

FESEM images of banana starch/alginate/orange peel extract films were obtained using a JEOL JSM-7900F electron microscope (Tokyo, Japan). The film samples were coated with gold using a sputter coater (Quorum, Q150T ES PLUS, West Sussex, England) before scanning with FESEM (Vonnie *et al.,* 2023).

2.5.9 Antioxidant analysis

The film extracts were prepared according to the methods described in Tongnuanchan *et al*. (2012). A mixture of 3 mL of distilled water and 25 mg of film was stirred for 3 h. The mixture was centrifuged at 3000 rpm for 10 mins to obtain the supernatant. The supernatant was tested for its ability to scavenge DPPH radicals. 1.5 mL of the film extract solution was mixed with 0.15 mM of 2,2-diphenyl-1-picryl hydroxyl (DPPH) in 95% ethanol. The mixture was kept in the dark at room temperature for 30 mins. The absorption of the DPPH test solution at 517 was measured using a spectrophotometer. The antioxidant activity of the film was determined based on the amount of DPPH radical-trapping activity. The percentage of free radical activity of DPPH will be calculated using Equation (10).

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \frac{Abs_{DPPH} - Abs_{extract}}{Abs_{DPPH}} \times 100\% \quad (10)$$

Where, Abs_{DPPH} is the absorption value of the ethanol DPPH solution at 517 nm and $Abs_{extract}$ is the absorption value of the sample extract at the same wavelength.

2.5.10 Antimicrobial Analysis

The antimicrobial activity of the films has been evaluated according to the method of Taweechat *et al.* (2021). The Muller-Hinton broth (MHB) is used to grow *E. coli*. The broth was then incubated in a shaker incubator for 18–24 h at 37 °C. One working microorganism stock loop was fed to the Muller-Hinton agar plate (MH) and incubated at 37 °C for 18–24 h to obtain one colony. The optical density of the culture was carried to 0.5 McFarland turbidity standards. Sterile swabs were then used to inject culture on the plates of MH agar. The film sample was sliced into a circle with a diameter of 6 mm, and it was then placed on the surface of the injected Muller-Hinton (MH) agar. The strain was treated with ampicillin (30 micril/discs) as an antibiotic. Following an 18–24 h incubation at 37 °C, the plates were inspected for the inhibitory zone on the film disc.

2.6 Biofilm Solution Application

Lady finger bananas obtained from supermarkets in Kota Kinabalu were coated by soaking them in a banana starch/alginate/orange peel extract biofilm solution with concentrations of 0.5%, 1.0% and 2% for about 30 sec. Bananas were suspended to air-dry at room temperature after dipping. Then, bananas were kept at room temperature (Dwivany *et al.,* 2020). The discolouration and presence of brown spots on banana peels is recorded for 5 days.

2.7 Statistical Analysis

The data were analyzed statistically using the IBM SPSS Statistics Version 27. A one-way ANOVA followed by a post-hoc Tukey test were used to determine the significant differences between the groups ($p<0.05$). The data obtained were presented in mean ± standard deviation with n=3 (Taweechat *et al.,* 2021).

3. Results and Discussion

3.1 The Yield of Banana Starch and Orange Peel Extract

Table 1 presents an overview of the starch and orange peel extract obtained from the Saba banana and orange peel. The maceration process of orange peel yields 34.96±0.65% of orange peel extract. Meanwhile, the extraction of banana starch yielded 71.16 g of starch flour from banana pulp weighing 296.54 g, yielding 23.88±0.25%.

Table 1. The yield of banana starch and orange peel extract

Extract	Yield (%)
Orange peel extract	34.96±0.65
Banana starch	23.88±0.25

3.2 The physical parameter values of the films.

Table 2 displays film thickness, density, moisture content, water solubility, and water barrier characteristics. The thickness of each film shows no significant variation ($p > 0.05$) and ranges from 0.02±0.00 to 0.03±0.00 mm, as indicated by the results. Adding orange peel extract at concentrations of 0.5%, 1%, and 2% show no differences in thickness values. However, the density of the film increases with the increase of orange peel extract. Zhou *et al.* (2021) and Hong *et al.* (2019) found that polyphenols, cellulose and hemicellulose in orange peel extract can modify the secondary structure of biodegradable films by engaging with their components through hydrophobic and hydrogen bonding interactions as well as the formation of covalent bonds within the film matrix, thus enhancing its density through increased cross-linking. Contrarily, Go and Song (2020) found that the incorporation of rambutan (*Nephelium lappaceum*) peel extract in *Citrus junos* pomace pectin films increase the film thickness due to the number of solids present in the film matrix.

The moisture content of the films showed an increasing trend with the increase of the orange peel extract. This phenomenon is caused by the hygroscopic nature of polyphenols, carbohydrates, cellulose and hemicellulose found in orange peel extracts, which tend to absorb moisture from their surroundings (Hong *et al.,* 2019). The reduction of moisture content in BAO 1% film might be due to the restructuring of the film matrix that leads to changes in the size and distribution of pores within the film; larger pores allow more water molecules to escape, thus facilitating greater moisture loss from the film. The water solubility of BA films exhibited the highest solubility in water at 51.79±5.63%. The water solubility decreased with the addition of 0.5% and 1% orange peel extract, however, it increased with the addition of 2% orange peel extract. Zhou *et al*. (2021) attribute the observed water solubility primarily to the weak interactions (hydrogen bonds and hydrophobic interaction) between alginate and banana starch within the film matrix, which are insufficient to maintain the integrity of film when exposed to water or solvent. The introduction of a high concentration of orange peel extracts, rich in hydrophilic and water-soluble compounds like organic acids and polysaccharides, impacts the film's water solubility.

The water vapour permeability of the prepared films fluctuates as shown in Table 2. The fluctuations might be caused by the film structure and composition that disrupt or allow the permeability of moisture. Orange peel extract contains a spectrum of chemical compounds, including flavonoids and polyphenols, that enhance the barrier qualities of the film and restrict the movement of water vapour. This film effectively

limits the movement of water vapour through the material as shown in BAO 2% film (Bansode *et al.,* 2023). The intermolecular interactions lead to a more condensed structure, which decreases the available space in the film and restricts the flow of water vapour molecules. The investigation into the antioxidant properties of BA, BAO 0.5%, BAO 1% and BAO 2%, reveals significant findings, as summarized in Table 4. Notably, BAO 2% yielded an optimal radical scavenging activity, peaking at 88.37%. This enhancement in the films' antioxidant capability can be attributed to the potent phenolic compounds within orange peel extracts, which, even at a minimal concentration of 0.5%, significantly improved the films' radical neutralizing performance compared to BA films. Parallel research by Taweechat *et al.* (2021) highlighting that the integration of rosemary extracts, known for their polyphenolic content, into film formulations elevates the antioxidant potential.

Table 2. The physical parameters of BA, BAO 0.5%, BAO 1% and BAO 2% films

Parameter	BA	BAO 0.5%	BAO 1%	BAO 2%
Thickness (mm)	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.01^{ab}	0.02 ± 0.00^{ab}
Density (g/cm^3)	0.01 ± 0.00^{c}	$0.06\pm0.00b^{c}$	0.09 ± 0.02^{ab}	0.11 ± 0.31^{a}
Moisture content (%)	7.56 ± 4.21^{b}	12.56 ± 0.93^{ab}	12.21 ± 1.71^{ab}	15.98 ± 1.30^{a}
Water solubility (%)	51.79 ± 5.63^{a}	41.35 ± 5.21^{ab}	40.78 ± 2.60^{b}	49.87 ± 0.74^{ab}
Water vapour permeability ($gs^{-1}mPa$) (x 10^{-7})	4.25 ± 0.03^{a}	2.53 ± 0.11^{b}	2.76 ± 0.11^{b}	-1.16 ± 0.02^{c}
DPPH radical scavenging activity (%)	71.43 ± 0.60^{d}	75.28 ± 0.87^{c}	77.67 ± 0.19^{b}	88.37 ± 0.05^{a}

The small letters of different superscripts in the same line show a significant difference between film samples by Tukey's test ($p < 0.05$) with mean data ($n = 3$).

3.3 The Optical and Appearance of Film

Table 3 presents the physical characteristics and optical features of different films of BA, BAO 0.5%, BAO 1% and BAO 2%. A detailed examination of the L* (lightness), a* (redness) and b* (yellowness) values across different film formulations underscores the impact of orange peel extract concentration on film appearance. BAO 2% film emerges as the lightest variant with a L* value of 43.06 ± 0.02, surpassing the BA film (40.57 ± 0.04). Conversely, BAO 0.5% and BAO 1% films manifest significantly lower L* values of 37.46 ± 0.01 and 35.43 ± 0.03, respectively, denoting a darker appearance, attributed to the pigmentation properties of orange peel extract. However, better dispersion of pigments in BAO 2% resulted in a more uniform and lighter colour appearance. Initial observations for a* values indicate a notable contrast between BA and BAO 0.5% films marked by a significant shift in the a* value from -1.03±0.05 to -1.31±0.05, indicating a darker hue ($p < 0.05$). This trend, however, reverses with higher concentrations of orange peel extract. Introducing 1% and 2% extract concentrations paradoxically neutralizes the colour intensity, evidenced by a* values stabilizing around 0.00 ± 0.12 and 0.07 ± 0.12, respectively. Moreover, the infusion of orange peel extract distinctly intensified the yellow hue of the films due to the presence of carotenoids, as evidenced by the b* values. Increase in ΔE values of films underscores a significant shift in colour properties which also attributed to the intrinsic pigments present in orange peel, which impart distinctive chromatic characteristics to the films (Viñas-Ospino *et al.,* 2024). Interestingly, despite adding orange peel extract enhancing the yellowish tint, the control films exhibited the highest whiteness index at 40.51 ± 0.04. This suggests that the natural whiteness of the banana starch and alginate matrix was overshadowed by the pigmentation introduced by the orange peel extract. Adding orange peel extract to banana starch film notably impacted its optical properties, specifically its opacity. Taweechat *et al.* (2021)

suggests that the opacity of such films is intricately linked to the amylose content. This mechanism is further evidenced by the lowest opacity value of 2.71 ± 0.74 observed in BA film. Experimental results demonstrated a progressive decrease in opacity with the incorporation of orange peel extract. This trend underscores an increment in film transparency correlating with higher concentrations of orange peel extract.

Table 3. The optical and appearance of BA, BAO 0.5%, BAO 1% and BAO 2% films

Film	BA	BAO 0.5%	BAO 1%	BAO 2%
L*	40.57±0.04^{b}	37.46±0.01^{c}	35.43±0.03^{d}	43.06±0.02^{a}
a*	-1.03±0.05^{b}	1.31±0.05^{c}	0.00±0.12^{a}	0.07±0.12^{a}
b*	2.64±0.00^{d}	16.21±0.17^{c}	21.18±0.03^{b}	24.39±0.04^{a}
Colour Difference (ΔE)	49.60±7.70^{a}	46.75±10.25^{a}	48.11±11.86^{a}	51.57±3.58^{a}
Yellowness Index	9.30±0.01^{d}	61.83±0.67^{c}	85.39±0.15^{a}	80.91±0.14^{b}
Whiteness Index	40.51±0.04^{a}	35.38±0.06^{c}	32.05±0.03^{d}	38.06±0.02^{b}
Opacity	8.95±1.21^{a}	3.11±0.53^{b}	3.01±1.31^{b}	2.71±0.74^{b}
Appearance				

The small letters of different superscripts in the same line show a significant difference between film samples by the Tukey's test ($p < 0.05$) with mean data ($n = 3$).

3.4 Microscopic Morphological Analysis

Images of surface FESEM and film cross-sections at ×10,000 and ×1500, respectively, are displayed in Figure 1. BA film opening has a non-parallel and textured shape, unlike other banana starch film surfaces, homogeneous, smooth, and free of pores and cracks as inferred by Vonnie *et al.* (2023). According to Pragya *et al.* (2021), adding alginates in the film formula provides a gel that can be formed by ionic cross-linking alginates with calcium ions. An uneven distribution of calcium ions can result in an unstable film structure. BA films also demonstrate little porosity at the cross-section. Significant porosity is less likely to arise when alginate crosslinks, especially when calcium ions are present and assist in forming a cohesive, dense film structure (Wang *et al.,* 2019). However, addition of orange peel extract in BA film is diminished by the microporous structure and non-uniform dispersion of orange peel extracts in the matrix. The increase in orange peel extract showed more uneven surfaces on the film matrix due to the agglomeration of orange peel extract. BAO 1% and BAO 2% displayed an uneven, abrasive, crack-free, and small pore holes, demonstrating the presence of starch grains on the film surface. Insufficient mixing or processing of starch

grains might lead to an uneven distribution of starch in the film matrix (Abral *et al.,* 2019).

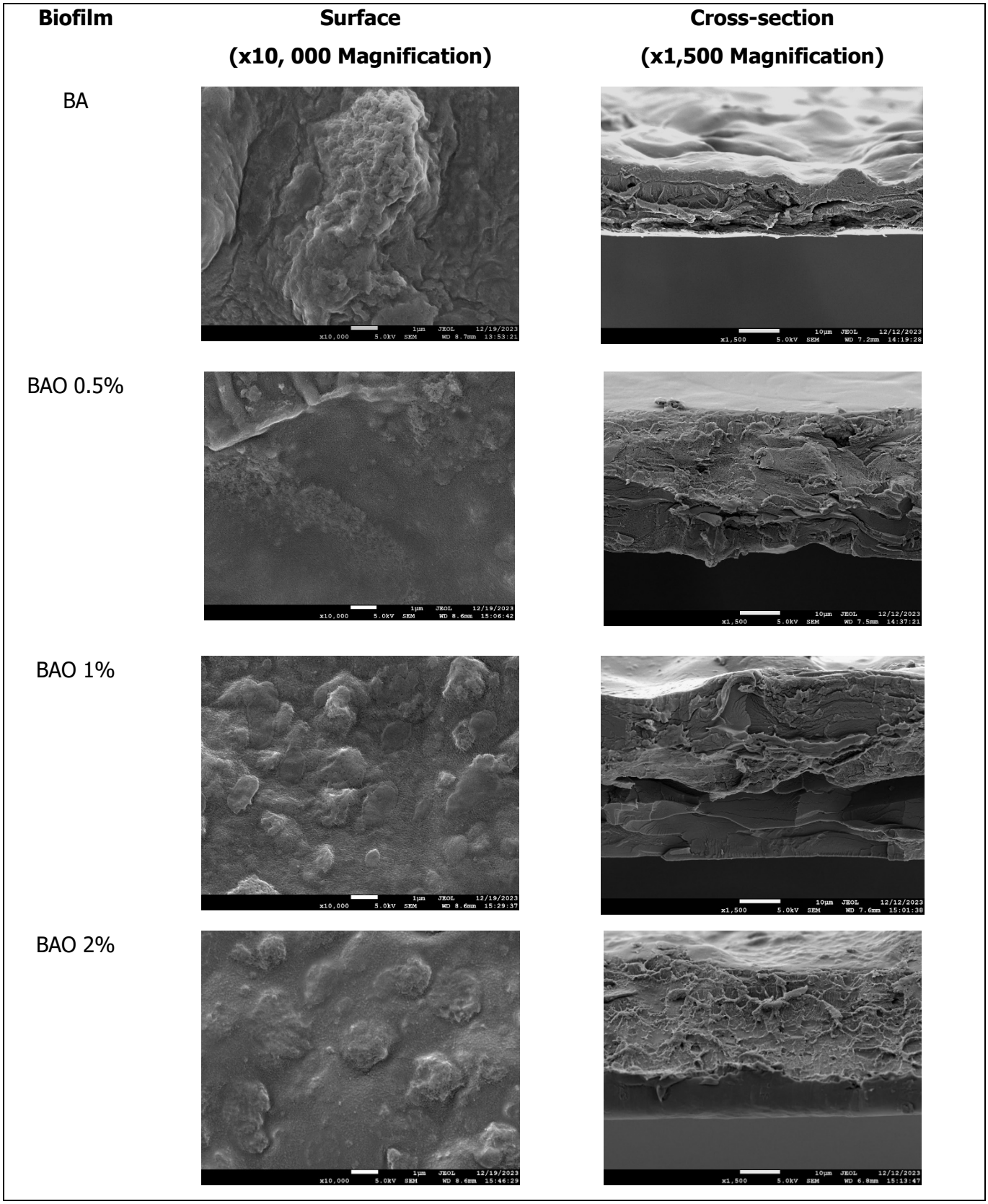

Figure 1. FESEM images of surface and cross-sectional biofilm with ×10,000 and ×1,500 magnification, respectively

3.5 Infrared Spectroscopy

The film's FTIR spectroscopic spectrum is conducted to verify the existence of many functional groups, as depicted in Figure 2. The films demonstrated that the peak band observed at 3273.28 to 3293.50 cm^{-1} is attributed to the vibrational stretch of the -OH bonding (Adeogun *et al.,* 2019). The alkane group's -CH bonding stretch is identified by a band ranging from 2926.19 to 2929.23 cm^{-1} (Alimi *et al.,* 2022). The presence of the amylose leads to the absorption of C=O stretching vibrations of the amide carbonyl group I, culminating in a peak band seen at 1634.51 to 1640.32 cm^{-1} . The amide III region corresponds to the peaks at 1340.35 and 1367.45 cm^{-1}. The bending of N-H bonds and the elongation of C-N bonds give rise to spectral bands in the amide region III. Additionally, the starch stretches of groups C-C and C-O are accountable for the bands observed within the 1148.23 to 1148.59 cm^{-1} range. The peak at 1076.43 and 1075.16 cm^{-1} was attributed to the strain in the CO bond of the carboxylic acid group (-COOH). The presence of cellulose in the orange peel extract causes the bending of COH and -CH groups at 1340.35 to 1341.01 cm^{-1} (Vonnie *et al.,* 2023).

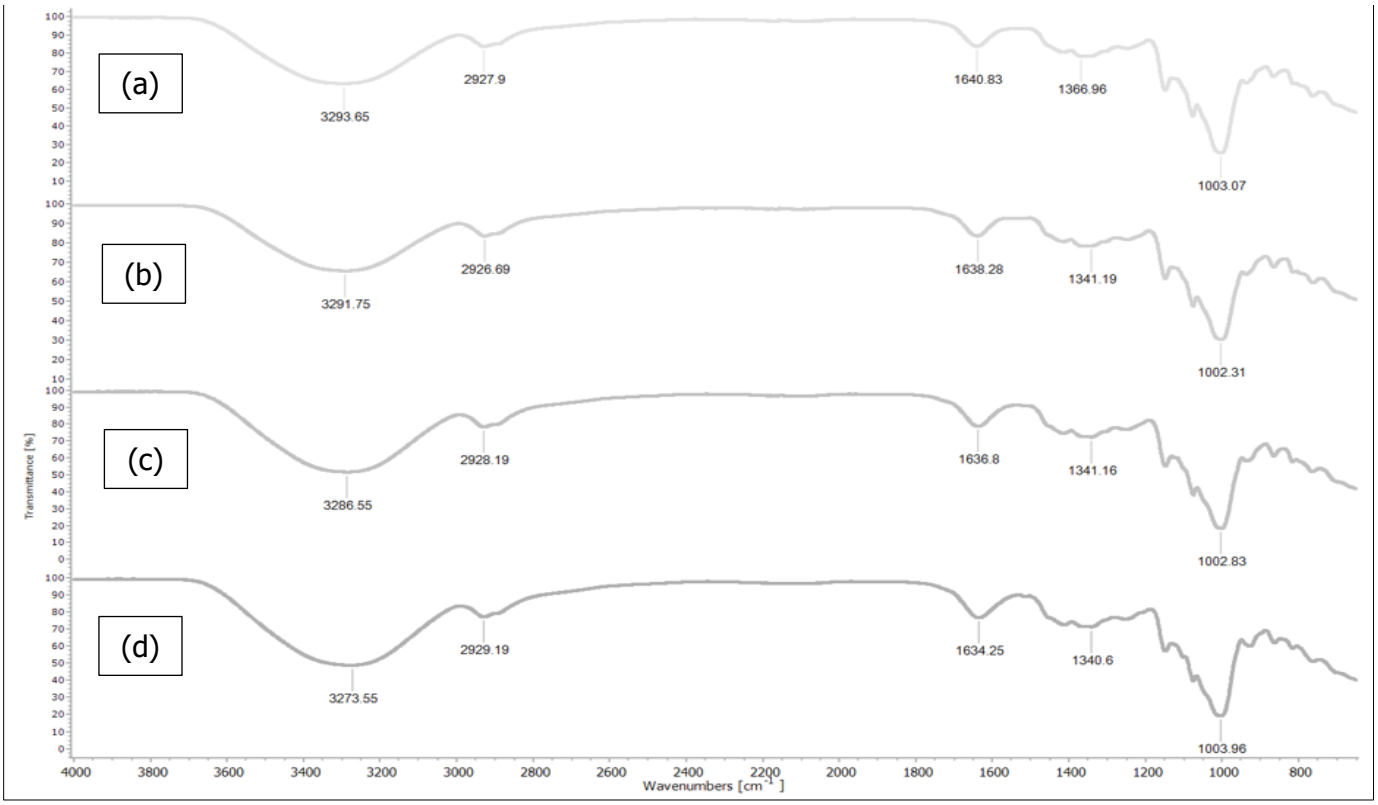

Figure 2. FTIR spectra of (a) BA, (b) BAO 0.5%, (c) BAO 1% and (d) BAO 2% Biofilm

Besides, the peaks at 1002 cm^{-1} is commonly associated with the stretching vibrations of the C-O bond and the symmetric stretching of C-O-C glycosidic bond in cellulose and related polysaccharides (Liu *et al.,* 2021). The band with a frequency range of 926.63 to 936.52 cm^{-1} is specifically linked to the configuration of CO, COH, and COC stretching bonds in the starch glycosidic backbone.

3.6 Antimicrobial Properties

Table 4 demonstrates the antimicrobial effectiveness of BA, BAO 0.5%, BAO 1% and BAO 2% films against *E. coli*. BAO 1% and BAO 2% slightly inhibiting the growth of *E. coli* (O157: H7), a common foodborne pathogen. This inhibitory effect is notably absent BA and BAO 0.5% films, underscoring the significance of the extract's concentration in achieving antimicrobial efficacy. The phenolic compounds in the orange peel extract, are recognized for their antimicrobial and antioxidant properties. These compounds use multiple mechanisms to exert their antibacterial effects, such as disrupting microbial cell membranes, inhibiting

essential enzymes, and interfering with critical biological processes. The interaction of polyphenolic compounds with lipid constituents of bacterial cell membranes is particularly noteworthy, as it compromises the integrity and functionality of microbial cells, leading to their eventual destruction. This research aligns with and extends the findings of Viñas-Ospino *et al.* (2023) and Manso *et al.* (2021), who have documented the antimicrobial and antioxidant capabilities of polyphenolic compounds found in natural extracts.

Table 4. Inhibition activity against *E.coli* growth by banana starch and alginate reinforced with different concentrations of orange peel

Biofilm	Inhibition Zone (mm)
BA	0.00 ± 0.00^{b}
BAO 0.5%	0.00 ± 0.00^{b}
BAO 1%	0.01 ± 0.01^{ab}
BAO 2%	0.01 ± 0.00^{a}

3.7 Application of Biofilm Solution on Lady Finger Banana

The post-harvest shelf life of bananas is notably diminished by oxygen and moisture in the ambient environment, resulting in rapid spoilage (Dora *et al.,* 2020). The emergence of brown spots commonly appear on banana peels within 5 days at room temperature at shown in Figure 3.

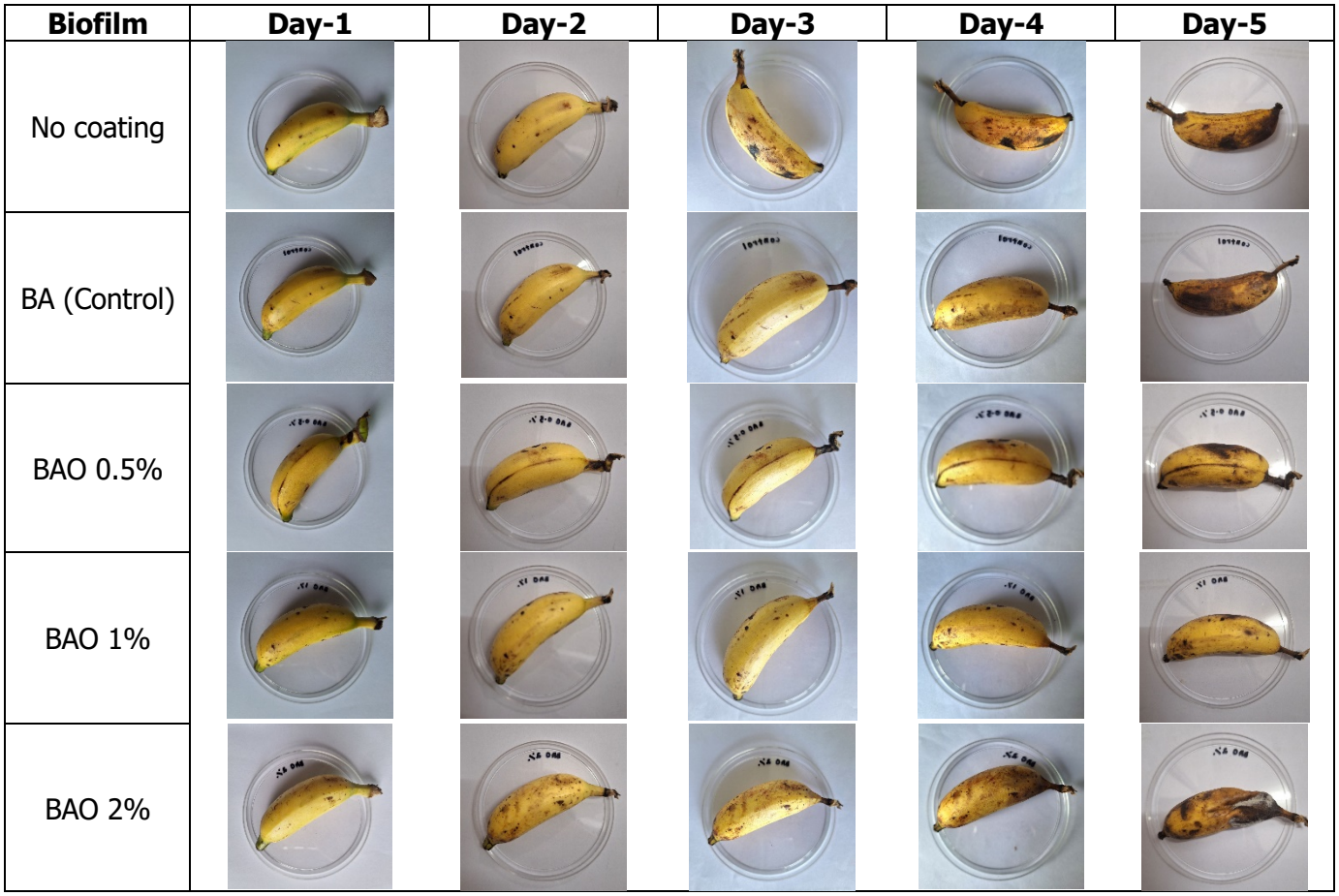

Figure 3. Lady finger banana coated in a solution made from a mixture of banana starch and alginate, combined with orange peel extract at 0.5,1, and 2 percentages.

Remarkably, there is no discernible distinction in the appearance of the bananas on the second day, with only minimal occurrences of brown patches and discolouration. By the third day, uncoated bananas show significant peel alterations, while those treated with BA, BAO 0.5%, and BAO 1% remain unaffected. However, BAO 2% film noticeably increases the prevalence of brown patches, with the final observation day revealing both black and brown patches on the peels. This contrasted with bananas coated with BAO 1%, which showed a notable resistance to such discolouration, highlighting its effectiveness. The adverse effects at higher concentrations may be due to the complex interaction of antioxidants and phenolic compounds in the orange peel extract. While these compounds extend shelf life by neutralizing reactive species and reducing microbial infections, excess amounts can disrupt the fruit's microbial balance, leading to spoilage (Hernández *et al.,* 2021).

Moreover, Othman *et al.* (2021) highlighted that bananas are both producers and responders to ethylene, a key hormone in fruit ripening, which necessitates careful consideration of storage and handling practices to manage ethylene exposure and its ripening implications. This is further complicated by the susceptibility of bananas to enzymatic browning when damaged, as elucidated by Rahman *et al.* (2019), who note that bruised bananas absorb ethylene more readily, accelerating ripening and subsequent browning.

Conclusion

The utilization of orange peel extract in the film formulation led to improvements in thickness, density, moisture content, and water solubility compared to films comprised solely of banana and alginate starch. Furthermore, incorporating orange peel extracts augmented the film's antioxidant properties, as evidenced by the direct correlation between the concentration of orange peel extract and the trapping of DPPH radicals. FESEM research findings indicate that incorporating orange peel extracts enhances banana starch particles and alginate structure. An inverse relationship was observed between the quantity of orange peel extracts added and the surface porosity, coupled with increased cross-film flatness. Furthermore, FTIR analysis revealed that the composite film derived from banana starch and alginate, fortified with orange peel powder, exhibits many functional groups that play a pivotal role in forming hydrogen bonds and overall film density. However, the antimicrobial properties of the film revealed a less inhibition against *E. coli*. When employed as a coating for bananas, the formulated film solution has the potential to prolong the shelf life of the fruit and retard its maturation process. Consequently, the resulting film can extend the bananas' shelf life. Future research could explore the optimization of orange peel extract concentrations in the film matrix to maximize antimicrobial efficacy while ensuring the physical properties of the films remain conducive for food packaging applications.

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